INSTITUTO POLITÉCNICO NACIONAL

CENTRO DE BIOTECNOLOGÍA GENÓMICA

Isolation and Genomic Characterization of *Stenotrophomonas spp* in Mexico*"*

"Aislamiento y caracterización genómica de Stenotrophomonas spp en México"

Submitted as a fulfillment for obtaining

Doctor en Ciencias en Biotecnología

BY

ELUFISAN TEMIDAYO OLUYOMI

***B. Tech* (Microbiology), LAUTECH, Ogbomosho, Nigeria**

***MSc*. (Pharmaceutical Microbiology), University of Ibadan, Nigeria**

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SECRETARÍA DE INVESTIGACIÓN Y POSGRADO

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ACTA DE REVISIÓN DE TESIS

En la Ciudad de Reynosa, Tamps. siendo las 16:00 horas del día 06 del mes de Mayo del 2019 se reunieron los miembros de la Comisión Revisora de la Tesis, designada por el Colegio de Profesores de Estudios de Posgrado e Investigación de CBG para examinar la tesis titulada:

Isolation and genomic characterization of *Stenotrophomonas spp* from Mexico

Presentada por el alumno:

Elufisan		Temidayo Oluyomi
Apellido paterno	Apellido materno	Nombre(s)

Con registro:

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aspirante de:

Doctorado en Ciencias en Biotecnología

Después de intercambiar opiniones los miembros de la Comisión manifestaron ***APROBAR LA TESIS***, en virtud de que satisface los requisitos señalados por las disposiciones reglamentarias vigentes.

LA COMISIÓN REVISORA

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INSTITUTO POLITECNICO NACIONAL
CENTRO DE BIOTECNOLOGIA GENOMICA

DEDICATION

I dedicate this work to the God Almighty for providing me the privilege to complete the doctoral dissertation in good health. Also, to my late uncles (Mr. Tunde Adufe Oyesanmi and Mr. Patrice Babatunde Adewoyin) who provided the stair on which I rode to this academic ladder and finally to all my family and friends who contributed immensely to the success of this work.

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Unto the only wise and immortal God be glory forever

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Resumen

Las *Stenotrophomonas* pertenecen a la familia gama proteobacteria, estas son bacterias Gram negativas. Su habilidad intrínseca de resistir a muchos agentes antimicrobianos y el ser halladas en pacientes las hacen de importancia clínica ya que son patógenos oportunistas. Las *Stenotrophomonas* poseen capacidades metabólicas vastas, lo cual mejora su supervivencia en muchos hábitats. Pueden crecer en condiciones muy adversas incluyendo agentes antimicrobianos. Las habilidades de *Stenotrophomonas* han sido explotadas en biorremediación, decoloración de colorantes y mejora del crecimiento de plantas. Aunque las *Stenotrophomonas* pueden causar infecciones en pacientes inmunocomprometidos, sus aplicaciones benéficas no deben de ser ignoradas. El aislamiento y caracterización de nuevos aislados pueden llevar al descubrimiento de nuevas especies con más beneficios y brindar posibles maneras de controlar la resistencia a antibióticos. Como tal, este estudio se enfoca en el aislamiento y la caracterización genómica de especies de *Stenotrophomonas* de diferentes ambientes en México para así entender su diversidad, evolución y sus aplicaciones potenciales.

Varias especies de *Stenotrophomonas* fueron aisladas de diferentes partes de México utilizando tanto plaqueo de agar de doble capa como medio selectivo (agar StenoVIA). Los aislados fueron identificados utilizando técnicas convencionales, espectrometría de Maldi-TOF y secuenciación parcial de 16S ribosomal. La evolución y la diversidad de los aislados fue inferida por análisis filogenético utilizando los genes secuenciados, REP-PCR, y la técnica de huella digital ERIC, respectivamente. El patrón de susceptibilidad antimicrobiana de los aislados fue evaluado utilizando difusión de disco y estudios de susceptibilidad basados en MIC, de acuerdo al estándar CLSI. Un análisis más profundo basado en el fenotipo de *Stenotrophomonas* fue el ensayo de degradación de hidrocarburos, donde los aislados fueron evaluados en medio mínimo con HAP (hidrocarburos aromáticos policíclicos) como fuente de carbono. La secuencia de los genomas reveló los genes que están envueltos en la degradación de HAP, en la degradación de colorantes, y en la resistencia a antibióticos,

Los resultados mostraron que 54 aislados de *Stenotrophomonas* fueron recuperados de aproximadamente 300 muestras de suelos, drenajes, y muestras clínicas. Los aislados presentan características bioquímicas diversas. Algunas cepas de *Stenotrophomonas maltophilia* prefieren arabinosa y manitol como sus únicas fuentes de carbono, lo cual es contrario al rasgo característico de estas especies. Las cepas recuperadas de sitios contaminados con petróleo crudo y desechos textiles degradaron exitosamente hidrocarburos aromáticos policíclicos (HAP) y decoloraron colorantes textiles, respectivamente, en ensayos de degradación y decoloración. Análisis de sus metabolitos por UPLC-MS y GC-MS mostraron que degradan completamente HAP y degradan colorantes textiles, respectivamente. La degradación de HAP por *Stenotrophomonas* produjo catecol (peso molecular, 110.03) como el producto determinado por espectrometría de UPLC. Los aislados de *Stenotrophomonas* fueron resistentes a la mayoría de los antibióticos probados. Mostraron un 100% de resistencia a ampicilina, doxiciclina y amoxicilina, pero fueron susceptibles a fluoroquinolonas. También fueron resistentes a Trimetoprim-Sulfametoxazole (80%) la cual es primera elección en el tratamiento de infecciones de *Stenotrophomonas maltophilia*. El índice de resistencia a múltiples antibióticos (IRMA) mostro que las cepas clínicas son más resistentes a los antibióticos con un índice IRMA de 0.8. Los aislados de drenaje y suelo tuvieron un índice IRMA de 0.65 y 0.7, respectivamente. Cinco genomas fueron secuenciados ya que mostraron características especiales, tales como la degradación de HAP (PEMSOL, ASS1 y SVIA2), decoloración de colorante textil (TEPEL), posible aumento del crecimiento de plantas (ATCM1_4). El ensamble de la secuencia resultó en 1 contig y 1 scaffold en PEMSOL, ASS1 y SVIA2 mientras que ATCM1_4 y TEPEL tienen 8 contigs y 6 contigs, respectivamente. El número de proteínas codificadas en los genomas identificados es de 3905, 4108, 4028, 3681 y 3905, respectivamente. El tamaño del genoma varia entre 4.2 y 4.5 Mb. Cada genoma tiene entre 19 y 27 islas genómicas. Un análisis más avanzado de los genomas mostro que

fueron encontrados algunos genes asociados con los fenotipos que portan las cepas secuenciadas. Los genes que codifican para la alcohol deshidrogenasa, la catecol-2,3-deoxigenasa, y la lactoilglutation liasa, son importantes para la degradación de HAPs. Tirosinasa y AZO-R1 oxidoreductasa son requeridos para la degradación de colorantes azo y colorantes textiles. Por otro lado, también identificamos algunos genes en los genomas que pueden estar relacionados con la resistencia a agentes antimicrobiales, como los genes ampC, transportador de flujo multi fármacos gnl|TC-DB|Q4LDT6|2. A.6.2.34 RND.

Stenotrophomonas es un género bacteriano de importancia económica. Sus habilidades pueden ser mejor explotadas y aplicadas para su uso a gran escala si entendemos los mecanismos relacionados con su supervivencia. El análisis del genoma de algunas cepas en este estudio permitió identificar algunos de los genes importantes que les ayudan a sobrevivir y utilizar sustancias toxicas para su crecimiento. La identificación de genes relacionados con la biorremediación y resistencia a antibióticos en *Stenotrophomonas* permitirá ver como estos pueden ser manipulados para detener esta amenaza de resistencia y entonces ser utilizada con propósitos de biorremediación.

En conclusión, las *Stenotrophomonas* son ampliamente diversas y sus propiedades fenotípicas pueden ser seleccionadas evolutivamente por el ambiente. Por ejemplo, aislados de sitios contaminados con petróleo crudo poseen la habilidad para utilizar HAP para su crecimiento. Asimismo, la resistencia de los aislados bajo el impacto del ambiente fue caracterizada en este estudio. Un resultado importante de este estudio indica la necesidad de mejor higiene publica pues el ambiente puede ser reservorio para bacterias drogorresistentes, entre ella cepas de *Stenotrophomonas*. También hay la necesidad de revisar el uso de fármacos para el tratamiento de infección por *Stenotrophomonas* ya que hay una ocurrencia alta por cepas resistentes a SXT, el cual es la primera opción de tratamiento

Abstract

Stenotrophomonas belongs to the family gamma-proteobacteria, which are Gram-negative bacteria. Their recovery from patients and inherent ability to resist many antimicrobial agents make them of clinical importance though they are opportunistic pathogens. *Stenotrophomonas* possess large metabolic capacities, which enhances its survival in many habitats. They can grow in harsh conditions, including antimicrobial agents. *Stenotrophomonas*' abilities to use various substances for growth have been to use in bioremediation, dye decolorization and plant growth promotion. Although *Stenotrophomonas* can cause infections in patients with underlying disease conditions, their useful applications should not be ignored. Isolation and characterization of new isolates can lead to the discovery of novel species with more benefits and also provide possible ways for controlling antibiotic-resistant behaviors. This study, therefore, focuses on the isolation and genomic characterization of *Stenotrophomonas* species from different environments in Mexico to understand their diversity, evolution and the potential for application.

Stenotrophomonas species were isolated from different parts of Mexico using both double layer agar-plating technique and selective medium (StenoVIA agar). Isolates were identified with bacterial biochemical feature using the conventional methods, MALDI-TOF spectrometry, and the partial16S rRNA sequence. Evolution and diversity of isolates were inferred by phylogenetic analysis using the sequenced genes and REP-PCR & ERIC finger printing technique respectively. The antimicrobial susceptibility pattern of the isolates was evaluated using both disc-diffusion and MIC based susceptibility studies according to the CLSI standard. Further analysis based on Stenotrophomonas' phenotype such as degradation assay was assessed in minimal medium with PAH as a carbon source. The sequencing of the genomes revealed the genes that are involved in the degradation of PAH, dye decolorization and antibiotic resistance.

The results showed that 54 *Stenotrophomonas* isolates were recovered from soil, sewage and clinical specimens from about 300 samples. Varied biochemical characteristics were observed in the isolates as some *Stenotrophomonas maltophilia* strains preferred arabinose and mannitol as their unique carbon source, in contrast to the trait for this species. The strains recovered from crude oil-contaminated sites and textile effluent successfully degraded polycylic aromatic hydrocarbon (PAH) and decolorized textile dyes respectively in degradation and decolorization experiments. The UPLC-MS and GC-MS analysis of their metabolites showed that they completely degraded PAH and decolorized textile dye respectively. The degradation of PAH by the *Stenotrophomonas* produced catechol (molecular weight, 110.03) as the metabolite determined from UPLC spectrometry. *Stenotrophomonas* isolates were resistant to most antibiotics tested. They showed 100% resistance to ampicillin, doxycycline, and amoxycillin but were susceptible to fluoroquinolones. They (100) were also resistant to Trimethoprim-Sulfamethoxazole, which

is the first drug of choice for the treatment of *Stenotrophomonas maltophilia'* infections. The multiple antibiotic resistant indexes (MARI) showed that the clinical strains were more resistant to the tested antibiotic agents with an average MARI index of 0.8. Isolates from sewage and soil had average MARI index of 0.65 and 0.7 respectively. Five genomes were sequenced because they showed unique characteristics such as the degradation of PAH (PEMSOL, ASS1, and SVIA2), Textile dye decolorization (TEPEL), possible plant growth promotion (ATCM1_4). The sequence assemblage resulted in 1 contig and one scaffold in PEMSOL, ASS1, and SVIA2, in contrast to ATCM1_4 and TEPEL, which have eight contigs and six contigs, respectively. The coding proteins in the genomes identified are 3905, 4108, 4028, 3681 and 3905, respectively. The size of the genome is between 4.2 Mb and 4.5 Mb. Each genome has between 19 and 27 genomic islands.

Further analysis showed that some genes associated with their phenotypes were also found on the genomes. Alcohol dehydrogenase encoding gene, catechol 2, 3 dioxygenases, lactoylgluthatione lyase are important for the degradation of PAHs. Tyrosinase and Azo R1 oxidoreductase required for the degradation of Azo dyes and textile dye were found in the genome of *Stenotrophomonas* sp. TEPEL. We also identified some genes in the genome of all the sequenced strain that often confer resistance on bacteria to antimicrobial agents such as the ampC genes.

Stenotrophomonas is a genus of economically essential bacteria. Their benefit can be better exploited and applied for large scale use if we understand the mechanisms involved in their survival. Genome analysis of some strains in the study gave insight to some of the essential genes which assist them to survive and use toxic substances for growth. The identification of the genes involved in bioremediation and antibiotic resistance in *Stenotrophomonas* will give insight to how they can be manipulated to halt the resistant menace and hence, can be applied for bioremediation purposes

In conclusion, *Stenotrophomonas* are highly diverse, and their phenotypic properties can be stimulated by their environment. For example, isolates from crude oil contaminated sites possess the ability to use PAH for growth. Also, the resistant properties of the strains revealed that environmental strains can acquire antimicrobial resistance from the environment. A significant result from this study indicates the need for better policies and practices of public hygiene because the environment could be a reservoir for drug resistant bacteria among *Stenotrophomonas* strains. There is also a need to review the drug use for the treatment of *Stenotrophomonas* infection because of the high occurrence of resistant strains to SXT, which is the primary drug of choice.

CHAPTER ONE

1.1 Introduction

1.1.1 The Dual Nature of Bacteria

Bacteria exist in different phases of life, but of paramount importance is their ability to live as either free-living saprophytes, pathogens, or opportunistic pathogens. Depending on the mode of being a bacterium could either be a "threat or a friend." Bacteria are therefore generally grouped either as pathogens that is those that can cause an infection in animal or plants or non-pathogens (These are free-living saprophytes). However, some bacteria could be free-living and opportunistic pathogens, which generally would not cause infection in either plant or animal but can do so in an immune-compromised case. Most non- pathogenic bacteria possess features that can be employed for beneficial purposes such as Plant growth promotion, Bioremediation, and Biotechnology. However, such promising bacteria have not been used or could not be used for such purposes because they can also initiate opportunistic infections. Opportunistic pathogens have continued to receive more attention in recent time because of the role they play in nosocomial infections and other disease conditions associated with compromised immunity. The ability to resist a large number of antimicrobial agents is another reason why opportunistic pathogenic bacteria have become popular recently (WHO 2014).

The role of bacteria in the maintenance of the natural ecosystem has been reiterated many times; bacteria can maintain an effective working ecosystem by causing diseases, degradation of recalcitrants, and promotion of plant growth, among otheres. Owing to the characteristics mentioned above many bacteria have been used for various beneficial purposes such as the cleaning of the Exxon Valdez oil spills (J. Y. Lee *et al*. 2001) and promotion of plant growth (Berg and Smalla 2009) and the synthesis of biomolecules such as biopolymer, bioelectricity and biofuel (Logan and Rabaey 2012; Peralta-Yahya et al. 2012; Steinbüchel 2001). Non-fermenting gram-negative bacteria (NFGNB) are an essential group of bacteria in this category. Several NFGNB bacteria with beneficial ability have been isolated from different ecological niche, possessing unique adaptation mechanisms which enhance their survival in such environments. The optimization of various processes in NFGNB for application purpose on a large scale had been hampered by their potential ability to initiate disease condition in individuals with weak immunity and their intrinsic resistant behavior. Many NFGNB like *Pseudomonas*, *Stenotrophomonas*, *Burkholderia* and *Acinetobacter* have been isolated from extreme

environments such as crude oil contaminated soils, hot sulfur springs, acidic lakes with the potentials for being used as agents for biotechnological (Das et al. 2015; Jung, Baek, and Park 2010; Mukherjee and Roy 2016; O'Sullivan and Mahenthiralingam 2005). However, large-scale studies on many of them have not received full endorsement because dominant species in these genera with such beneficial are also capable of initiating disease conditions. Even when they are harmless, the high rate of resistance that they showed to antimicrobial agents limits their large-scale application. *Stenotrophomonas* is an excellent example of NFGNB with the above-mentioned characteristics.

1.2 History and Significance of *Stenotrophomonas*

The word *Stenotrophomonas* is refers to a genus of a group non-fermentative gram-negative bacteria which is phylogenetically placed in the class Gamma-proteobacteria following several taxonomic revisions (Ryan *et al*. 2009). The first member of the genus was isolated as *Bacterium bookeri* by Edwards in 1943, from the Pleural fluid of a patient with oral carcinoma (Denton and Kerr 1998a; HUGH and LEIFSON 1963). The isolation of the typed strain in 1958 and the analysis of 5 other strains that were previously identified as *Pseudomonas alcaligenes* resulted in the renaming of *Bacterium booker* and some members of the *Pseudomonas alcaligenes* as *Pseudomonas maltophilia* (HUGH and LEIFSON 1963). In 1963, Komagata, *et al.*, reviewed the classification of four *Pseudomonas melanogena* in relation to the described characteristics for *Pseudomonas maltophilia* and discovered that the bacteria which were initially classified as *Pseudomonas melanogena* are *Pseudomonas maltophilia. Pseudomonas melanogena* were reclassified as *Pseudomonas maltophilia* because they showed deoxyribonuclease and nucleoside phosphotransferase activities as reported in *Pseudomonas maltophilia* (Hugh and Leifson 1963). Similarly, some bacteria group previously classified as Alcaligenes faecalis by Urlic and Needham, were reclassified as *Pseudomonas maltophilia* (Hugh & Ryschenkow, 1961, Urlic and Needham, 1953).

In 1973 Palleroni *et al*., showed that the genus *Pseudomonas* has five different rRNA homology groups using DNA-rRNA hybridization technique. The 16s rRNA cistron of the typed strains of *Pseudomonas maltophilia* ATCC 13637 showed closer resemblance to three strains in the genus *Xanthomonas* (Palleroni, Kunisawa, Contopoulou, & Doudoroff, 1973). Vos, Vos, and Ley, 1983 used DNA-rRNA hybridization technique, the guanine-plus cytosine (G/C) content (P. *maltophilia,* 63 to 67.5%; Xanthomonas,63 to 70%), and other important characteristics to

regroup *Pseudomonas maltophilia* as a member of the genus Xanthomonas Ley (Vos, Vos, and Ley 1983). The other methods they employed for their analysis include whole-cell proteins patterns analysis and isoelectric focusing studies of outer membrane esterase. However, the proposal did not receive much acceptance because their method was controversial. The uncertainty in the classification of *P. maltophilia* thus remained unresolved. Similarly, the taxonomic proposal by Swing *et al*., 1983 for *P. maltophilia* as *Xanthomonas maltophilia* was rejected following an extensive DNA-rRNA hybridization which gave different melting points for the 27 strains of *Pseudomonas* and Xanthomonas used in their study (SWINGS *et al*. 2009). On the other hand Yang *et al*., (P. Yang *et al*. 1993) showed that *Pseudomonas maltophilia* differs from other members of the Xanthomonas based on the composition of their cellular fatty acids and Polyamines.

The inability to successfully place *P. maltophilia* into a taxonomic class following the amplification of the 16s rRNA region (Maes 1993) necessitates the need for their reclassification. In 1993, Palleroni and Bradbury proposed a new genus for *P. maltophilia* as *Stenotrophomonas maltophilia* with S. *maltophilia* as the only member of the genus at the time of classification (Palleroni and Bradbury 1993). The experiment of Nesme *et al*., authenticated the reclassification (Nesme et al. 1995). They used the restriction mapping of PCR amplified 16s rRNA to distinguish *Xanthomonas* and *S. maltophilia*. The name *Stenotrophomonas* is a description of their feeding pattern according to the report of Stanier *et al*., (Stanier, Palleroni, and Doudoroff 2009). Thus, the word *Stenotrophomonas* can splitted into Steno. tro.pho.mo'nas. Greek adjective. stenus, narrow; Greek noun. Trophus, one who feeds Greek noun. Monas, a unit, monad; i e., *Stenotrophomonas*, a unit organism feeding on a few substrates. Owing to the presence of *S. maltophilia* as the only member of the genus at the time of classification, it was believed that they are mainly associated with pathogenicity or diseases conditions, however, several other studies have led to the isolation of other species from different environments (K. Wang, Mao, and Li 2018). As a result of further isolation and characterization, the genus presently has 18 species and 16 validated species on the list of prokaryotic names with standing nomenclature (http://www.bacterio.net/-classification.html) as shown in the Table below;

Table 1 Brief Description of the known species that form the member of the genus *Stenotrophomonas*

Species	Year of First Isolation	First Isolation source	Characterization	Country/Continent of isolation	Reference,
Stenotrophomonas maltophilia	1943	Human	*S. maltophilia*, a new bacterial genus for X. *maltophilia*, is first identified from a specimen of pleural fluid	England/Europe	(Palleroni and Bradbury 1993) : Hugh and Ryschenkow, 1961
Stenotrophomonas pictorum	1928	Soil	Soil bacteria that decompose certain aromatic compounds	Scotland/England	Gray and Thornton, 1928; (Ouattara et al. 2017)
Stenotrophomonas panacihumi	2009	Ginseng Field/Soil	*Stenotrophomonas panacihumi* sp. nov., Isolated from Soil of a Ginseng Field	Korea/Asia	(Yi, Srinivasan, and Kim 2010)
Stenotrophomonas pavanii	2011	Sugar cane Stem	Screening for endophytic nitrogen-fixing bacteria in Brazilian sugar cane varieties used in organic farming and description of *Stenotrophomonas pavanii* sp. nov.	Brazil/South America	(Ramos et al. 2011)
Stenotrophomonas nitritireducens	2000	biofilters	Characterization of N_2O-producing *Xanthomonas*-like isolates from biofilters as *Stenotrophomonas nitritireducens*	Germany/Europe	(Finkmann et al. 2000)
Stenotrophomonas acidaminiphila	2002	anaerobic sludge blanket (UASB) reactor	*Stenotrophomonas acidaminiphila* sp. nov., a strictly aerobic bacterium isolated from an upflow anaerobic sludge blanket (UASB) reactor	Mexico/North America	(Labat et al. 2002)
Stenotrophomonas	2002	Soil	*Stenotrophomonas rhizophila* sp. nov., a novel plant-associated bacterium with antifungal	Germany/Europe	(Wolf et al.

rhizophila			properties		2002)
Stenotrophomonas lactitubi	2017	Milking Machine	*Stenotrophomonas lactitubi* sp. nov. and *Stenotrophomonas indicatrix* sp. nov., isolated from surfaces with food contact	Germany/Europe	(Weber et al. 2018)
Stenotrophomonas indicatrix	2017	Dirty dishes	*Stenotrophomonas lactitubi* sp. nov. and *Stenotrophomonas indicatrix* sp. nov., isolated from surfaces with food contact	Germany/Europe	Weber, *et al*., 2018
Stenotrophomonas bentonitica	2017	Environment	*Stenotrophomonas bentonitica* sp. nov., isolated from bentonite formations	Spain/Europe	(Sánchez-Castro et al. 2017)
Stenotrophomonas chelatiphaga					
Stenotrophomonas koreensis	2006	Compost	*Stenotrophomonas koreensis* sp. nov., isolated from compost in South Korea	Korea/Asia	(H. C. Yang et al. 2006)
Stenotrophomonas daejeonensis	2011	Sewage	*Stenotrophomonas daejeonensis* sp. nov., isolated from sewage	South Korea/Asia	(M. Lee et al. 2011a)
Stenotrophomonas ginsengisoli	2010	Soil	*Stenotrophomonas ginsengisoli* sp. nov., isolated from a ginseng field	South Korea/Asia	(Kim et al. 2010)
Stenotrophomonas terrae	2007	Soil	*Stenotrophomonas terrae* sp. nov. and *Stenotrophomonas humi* sp. nov., two nitrate-reducing bacteria isolated from soil	Belgium/Europe	(Heylen et al. 2007)
Stenotrophomonas humi	2007	Soil	*Stenotrophomonas terrae* sp. nov. and *Stenotrophomonas humi* sp. nov., two nitrate-reducing bacteria isolated from soil	Belgium/Europe	Heylen, et al., 2007
Stenotrophomonas Africana	1997	Human	*Stenotrophomonas africana sp. nov., an opportunistic human pathogen in Africa*	Rwanda/Africa	(Coenye 2004)
Stenotrophomonas tumulicola	2016	Stone Chamber	*Stenotrophomonas tumulicola sp. nov., a primary contaminant of the stone chamber interior in the Takamatsuzuka Tumulus*	Japan/Asia	(Handa et al. 2016)

The genus has undergone various taxonomic revision after its recognition as a distinct genus among which is the transfer of *Stenotrophomonas dokdonensis* to the family *Pseudoxanthomonas dokdonensis* and the identification of *S. Africana* as a later synonym of the *Stenotrophomonas maltophilia* (Coenye 2004; M. Lee et al. 2011b). Patil *et al*., (Patil et al. 2016) proposed a review of the genus following a pan genomic analysis of 19 sequenced genome. They suggested that some complexes in *Stenotrophomonas maltophilia* such as *Pseudomonas beteli* ought to be separate species.

Stenotrophomonas are ubiquitous and have been isolated from different sources including human, animal and even insects (Brooke 2012; Hughes et al. 2016). Although *Stenotrophomonas spp* are ubiquitous they are majorly found in the soil and in association with the plants at the rhizosphere level (Alavi et al. 2014; Ryan et al. 2009). *Stenotrophomonas* have also, been isolated from extreme environments such as the soda lake, Hot sulfur spring; organophosphate contaminated soil, crude oil contaminated soil (Iyer, Iken, and Leon 2016; Ma et al. 2004; Memory Tekere 2011; Ryan et al. 2009).

The beneficial role of *Stenotrophomonas* species is associated with their capability to use many complex metabolites for growth. These characteristics informed their application in many biotechnological processes. The biotechnological applications include bioremediation of PAH, long chain alkane, crude oil contaminated oil soil and xenobiotics, phytoremediation, plant growth promotion and biocontrol (Ryan et al. 2009). The degradation of PAH and other xenobiotic compounds is one of the means through which *Stenotrophomonas* spp ensures bioremediation. Several factors have been identified to be responsible for *Stenotrophomonas'* ability to degrade PAH and xenobiotics.

Urszula *et al*., (Urszula et al. 2009) reported the involvement of different dioxygenases in the *Stenotrophomonas* spp., for the degradation of monocyclic hydrocarbon. Similarly, Tebyanian *et al*., 2013 demonstrated the use of *S. maltophilia* for the degradation of hexadecane via bio-emulsification and the action of alkane hydroxylase gene (Tebyanian, Hassanshahian, and Kariminik 2013). Iyer *et al*., 2016 and Ozdal *et al.,* 2017 demonstrated the capabilities of *Stenotrophomonas maltophilia* for the degradation of organophosphates (Iyer, Iken, and Leon 2016; Ozdal et al. 2017). The biotechnological application of *Stenotrophomonas* sp. have been tested in the degradation of selenium compounds, nitrophenol, benzene, toluene, 4 chlorophenol

fluoranthene, anthracene, biphenyl and DDT (Boonchan, Britz, and Stanley 1998; Dungan, Yates, and Frankenberger 2003; D. Y. Lee et al. 2002; Liu, Yang, and Qiao 2007; Pan et al. 2016). Mangwani, *et al*., 2014 demonstrated that *S. acidaminiphila* NCW-702 biofilm has capacity to degrade phenanthrene in a culture dependent assay (Mangwani et al. 2014). In another related study, *S. acidaminiphila* was employed for the degradation of fipronil, an insecticide commonly used in agriculture (Uniyal et al. 2016). This insecticide sometime persists in the soil or surrounding aquatic environment thereby constituting harm to other untargeted free-living invertebrates.

The biotechnological application of *Stenotrophomonas* also includes their use as biocontrol agents in plants. Messiha *et al*., 2007 (Messiha et al. 2007) used *S. maltophilia* strain isolated from soils in Egypt to control *Ralstonia solanacearum* which causes tomatoes' brown root infection in an *In vitro* study. Similarly, Dunne *et al*. (1997) (Dunne et al. 2000) used *S. maltophilia strain* W81 to control the fungi *Pythium ultimum*, which is responsible for the damping off disease in sugar beet. The production of an extracellular protease accounted for the activity of *S. maltophilia* W81 in the bio-control of *P. ultimum*. Jankiewicz & Saks, 2012 showed that *S. maltophilia* strain MUJ can prevent the pathogenic activities of a fungal phyto-pathogen via the production of a chitinase enzyme from the family 18 of the glycosyl hydroxylase (Jankiewicz, Brzezinska, and Saks 2012).

Stenotrophomonas spp can also function as agents for the promotion of plant growth displaying different capabilities for ensuring the adequate growth of various categories of plants. One of the environments from which *Stenotrophomonas are* frequently recovered is the soil rhizosphere. Due to their association with the soil environment, *Stenotrophomonas* spp. have not only learn to survive in the plant rhizosphere but have significantly help in the survival of their host plant. Alavi *et al*., 2013 showed how *S. rhizophila* protects plant root against osmotic stress via the production of spermidine and production & excretion of glucosylglycerol in a transcriptomic study. Similarly, Singh and Jha 2017 demonstrated the *S. maltophilia* SBP-9 enhances the promotion of plant growth via the production of ACC deaminase (ACCD), gibberellic acid, indole acetic acid (IAA), siderophore, and inorganic phosphate solubilization (Singh and Jha 2017). This strain was able to promote the growth of wheat under saline stress condition. The inoculation of this strain into the root of wheat resulted in the production of defense enzymes like

β-1, 3 glucanases, phenylalanine ammonia lyase (PAL), peroxidase (PO), and polyphenol oxidase (PPO), which can protect plants from the infection of pathogens (Singh and Jha 2017). *Stenotrophomonas maltophilia* MTP42 was used as plant growth promoting rhizobacteria and agent for the control of disease by Patel and Saref (Patel and Saraf 2017).

The genus *Stenotrophomonas* has many beneficial species, but their broad application is limited by their intrinsic nature to resist a wide range of antimicrobial agents and the lower frequency of recovering other species but *Stenotrophomonas maltophilia* from the environment. *S. maltophilia*, the most frequently encountered species of the genus are not only beneficial but could also be associated with infection in sick patients. S. *maltophilia* have been recovered from various diseases condition such as cystic fibrosis, bacteremia, endocarditis, respiratory tract infections, meningitis, urinary tract infections, skin and soft tissue infections, mastoiditis, bone and joint infections, peritonitis, typhlitis, and biliary sepsis, wound infections, and central venous catheter (CVC)-related infections (Al-Anazi and Al-Jasser 2014; Brooke 2012; Caylan et al. 2004; Denton and Kerr 1998b; Goss et al. 2004; Micozzi et al. 2002; Muder et al. 1996; Safdar and Rolston 2007; Tada et al. 2013). *S. maltophilia* infections could result in septic shock, respiratory failure, pulmonary hemorrhage, metastatic cellulitis, tissue necrosis that may be extensive, septic thrombophlebitis, disseminated disease, and death (Araoka, Baba, and Yoneyama 2010; Micozzi et al. 2002; Safdar and Rolston 2007; Tada et al. 2013). Although *S. maltophilia* has not been singly isolated from any infection, there are still debates on their roles as a pathogen in the cause of many infections. This is because the presence of *S. maltophilia* in various diseases conditions has exacerbated the underlying diseases conditions (Berdah et al. 2018). The role of S. *maltophilia* in the initiation of new infections in human is uncertain, but certain disease conditions in some lower vertebrates have been linked to them though there were underlying disease conditions. They have been isolated as the only bacteria in some associated infections in the underlying disease cases (Abraham et al. 2016; W.-S. Wang et al. 2004).

S. maltophilia had remained the only member of the genus capable of causing opportunistic infections, until recently when a multi-resistant strain of *S. acidaminiphila* was recovered from the bile of a cholangiocarcinoma patient with obstructive jaundice and cholangitis (Taiwan) (Y. T. Huang et al. 2018). The recovery of *S. acidaminiphila* from a disease condition may be opening another biology of the genus. Similarly, they may support their possibility of evolving a

new mechanism of survival as was reported in the literature. Berg and Martinez, 2015 posit that it is possible for bacteria to learn pathogenicity as they continuously interact with their hosts. It is also possible for bacteria to evolve pathogenic characteristics if they find themselves in an unknown environment.

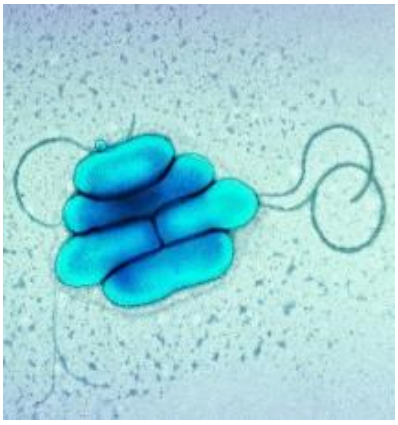
Stenotrophomonas has thus become bacteria of vital importance because of their vast ability to resist a wide range of substances and the inability to cause diseases in the plant. Hence, the need to minimize *Stenotrophomonas* infection to be able to apply them for beneficial purposes. These reasons emphasize the continuous studies of the genus important.

1.3 The Microbiology of *Stenotrophomonas* species

The genus *Stenotrophomonas* comprises gram-negative bacteria which have been isolated from different environmental sources, human and other animals (Furushita et al. 2005; Svensson-Stadler, Mihaylova, and Moore 2012). *Stenotrophomonas* are gram negative rods which move using two or more flagella (Figure 1), (Ryan *et al*., 2009; Brooke *et al*., 2012). However, some species which are non-motile have been reported (Kim et al. 2010; H. C. Yang et al. 2006). Bacteria in the genus *Stenotrophomonas* ranges in size between 0.2 µm and 0.8 µm in diameter and between 0.8 µm and 3.0 µm in length. It is also possible for them to form smaller colony variants especially when the condition is unfavorable. Certain smaller colonies of S. *maltophilia* have been recovered from limited nutrient environments (Brooke, 2012). *Stenotrophomonas* species are usually found existing freely in nature, but there are species which cannot grow in a culture medium. Corsaro and collaborators reported in 2013 that *S. maltophilia* which behave as an endoparasite in Naegleria and Acanthamoeba spp (Corsaro, Müller, and Michel 2013). These strains were unculturable in standard bacteriological growth medium but grew in Acanthamoeba formulated medium. The growth of *Stenotrophomonas* species vary with different medium and clonal diversity may be seen as colour variation in various growth media. S. *maltophilia* for example, when inoculated in Columbia blood agar, forms small greyish, slightly mucous colonies on blood agar (Goncalves-Vidigal et al. 2011) However, some strains may cause a brownish discoloration in clear media, most likely due to secondary chemical reactions among extracellular products (Vidigal et al. 2014). *Stenotrophomonas* species are aerobic bacteria, but some can use nitrogen for their growth when oxygen is absent or present at a low concentration (Ramos et al. 2011). The growth characteristics for members of the genus *Stenotrophomonas* is in Table 2. Although most members of this genus are oxidase negative, a few species such as *S.*

acidaminiphila, S. daejeonensis, Stenotrophomonas ginsengisoli, S. terrae, S. humi, S. africana are oxidase positive. Also, Some *S. maltophilia* complexes have been reported to be oxidase positive and lactose positive. It may therefore no longer enough to rely on a few biochemical characteristics for the identification of *Stenotrophomonas* species. There have been cases of *Stenotrophomonas* species misidentification for other bacteria as a result varied biochemical property. Burdge and collaborators (Burdge et al. 1995) noted 9 % of misidentification of *S. maltophilia* for *Burkholderia cepacia* from the sputum culture of cystic fibrosis patients. Three out of the 32 isolates identified as *B. cepacia* in a laboratory sputum culture were later discovered to be *Stenotrophomonas maltophilia*. Similarly, in a study conducted to determine a rapid method for the identification of *S. maltophilia,* it was noted that API, VITEK, and BIOLOG were only able to identify correctly 33%, 73%, and 67% respectively as *S. maltophilia* (Pinot et al. 2011).

A) B)



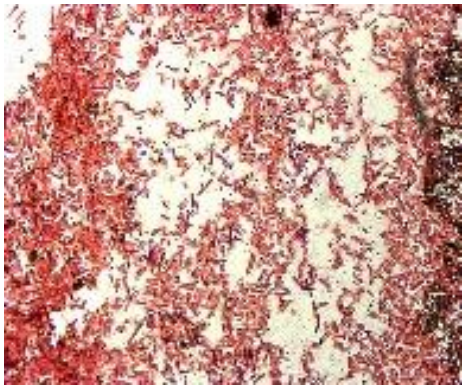


Figure 1a: An image of flagellated *Stenotrophomonas* species

Figure 1b: Image from the gram reaction of *a Stenotrophomonas* species

The role of *S. maltophilia* in nosocomial infections and the frequency of co-isolation with other bacteria such as *Pseudomonas aeruginosa, Acinetobacter baumanii, Burkholderia and Staphylococcus aureus*, (Brooke 2012) makes the quick and accurate identification important for a member of the genus *Stenotrophomonas*. In a bid towards improving the rapid detection of *Stenotrophomonas* species, several media have been developed to isolate *Stenotrophomonas* in a polymicrobial environment selectively. Denton *et al.,* 1999, produced the VIA medium, which

consists of a mannitol agar base with a bromothymol blue (BTB) indicator, 5 mg/liter vancomycin, 32 mg/liter imipenem, and 4 mg/liter amphotericin B. This medium when compared with previously formulated medium containing imipenem on Bacitracin-Chocolate agar, proved to be more effective. VIA agar proof to be more useful for the isolation of *S. maltophilia* particularly in situations where there are low colonies forming unit (Brooke, 2012). Although VIA agar was targeted at isolating *S. maltophilia*, it has also demonstrated the tendency to isolate other members of the genus *Stenotrophomonas* such as *S. rhizophila, S. acidaminiphila,* and *S. terrae* (Pinot *et al*., 2012). The need for quick identification of *Stenotrophomonas* has led to various researches focusing on different molecular techniques for the identification of *Stenotrophomonas* species. Pinot and collaborators (2011) employed SS-PCR technique which amplifies the 23S rRNA region of the *S. maltophilia* DNA and Multiplex PCR technique which amplifies the *ggpS* and the *smeD* gene for the differentiation of *S. maltophilia* and *S. rhizophila*. The amplification of the *smeD* gene was able to identify *S. maltophilia* effectively but suffers from the limitation that this region was absent in other species except *S. rhizophila* tested at the time of their study. The result implies that the method may only be efficient for the rapid detection of *S. maltophilia* and no other species. However, the amplification of the *smeD* region *in Stenotrophomonas* species may be a useful method for the rapid delineation of *Stenotrophomonas* species. Rapid techniques for bacterial identification such as matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS) are now being used for identifying *Stenotrophomonas* species. Vasileuskaya-Schulz and collaborators (Vasileuskaya-Schulz et al. 2011) successfully delineate all the nine available species of *Stenotrophomonas* species at the time of their studies using MALDI-TOF. In a similar situation, sogawa demonstrated the rapid ability of MALDI-TOF MS in the discovery of bacteria identity including some selected member of the genus *Stenotrophomonas*.

The use of MALDI-TOF MS for the identification of bacteria has remained promising but suffers from a limitation that it is software database dependent and as such might not be able to identify new strains or species which are not included in the database. 16s rRNA therefore remain the gold standard for the identification of bacteria. Hence due to the rapidly expanding nature of the genus *Stenotrophomonas* the proper identification procedure for member of the genus is the PCR amplification and sequencing of the 16S rRNA fragment of their genome.

Table 2: General Characteristics of *Stenotrophomonas* species

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Catalase	+	+	+	+	-	+			+	-	+	+	-	-	-	+	+
Optimum growth temp	35 °C	35 ° C	35 ° C	35	-37° C	30-35° C	ND	ND	28 ° C	35 ° C	30 ° C	30 ° C	30° C	37	37	37	37
	No growth at 4°C or 41°C	ND	ND	Can grow at 4 °C and 42 ^{0}C	ND	No growth at 4 and 42 ° C	ND	ND	No growth at 4 ° C	No growth at 4 ° C	No growth at 4 ° C	No growth at 4 and 42 ° C	No	No	No growth at 4 and 42° C	No growth at 42 ° C	No growth at 4 and 42 ° C
Survival at refrigeration temp	Yes	Yes	Yes	Yes	ND	No	ND	ND	No	No	No	No	No	No	No	Yes	Yes
Motility	+	+/-	+	-	ND	-			-	+	-	+	-	+	+	+	+
Nitrate reduction,	Yes, but nitrate is not used as nitrogen source	Yes	No	ND	Yes	Yes			Yes	Yes	No	No	ND	Yes, but does not convert nitrite to nitrate	Yes, but does not convert nitrite to nitrate	No	No
Indole	−	ND	ND	ND	-	-			-	+	ND	-	-	ND	ND	-	-
Citrate	v	-	+	+	ND	-			ND	+	-	+	-	ND	ND	ND	ND
Carbohydrate Utilization																	

Acid production from maltose	+	+	-	-	ND	-			+	+	-	+	-	ND	ND	ND	W
Acid production from glucose	−	+	-	-	ND	-			+	+	-	+	-	ND	ND	ND	+
Carbon source for Growth																	
Arabinose	−	ND	-	-	ND	-			-	+	ND	+	W	ND	ND	ND	ND
Cellobiose	V	+	ND	-	ND	-	-	-	+	ND	-	ND	-	-	-	ND	ND
Glucose	+	+	+	-	ND	-	W	W	+	+	-	+	+	+	+	ND	-
Fructose	v	+	ND	-	ND	+	W	W	-	+	ND	+	-	+	+	+	+
Lactose	+	-	-	-	ND	-	-	-	-	ND	ND	+	ND	-	-	+	+
Maltose	+	+	-	-	ND	+			+	+	-	+	+	+	+	+	+
Mannitol	−	ND	-	-	ND	-			-	ND	ND	+	+	ND	ND	ND	-
Mannose	v	+	+	-	ND	+			+	+	-	+	-	+	+	ND	+
Rhamnose	−	ND	+	-	ND	-			+	ND	-	-	+	ND	ND	ND	-
	+/−	+	ND	-	ND	-			+	+	ND	-	+	-	-	ND	ND

Trehalose																	
Aesculin hydrolysis	+	+	-	+	-	-			+	+	-	+	-	W	-	ND	ND
Gelatin liquefaction	+	-	W	+	ND	-			+	+	+	+	-	+	+	ND	+
Tween 80 hydrolysis	+	+	ND	+	+	+			+	-	ND	+	ND	+	-	+	+
Starch hydrolysis	−	V	ND	-	ND	-			+	+	-	-	ND	ND	ND	ND	-
Urea hydrolysis	−	-	-	-	ND	-			-	-	-	-	-	ND	ND	ND	+
Oxidase	+/-	-	-	-	-	+			+	-	-	+	+	+	+	-	-

Note: [1]*Stenotrophomonas maltophilia* [2]*Stenotrophomonas pictorum* [3]*Stenotrophomonas panacihumi* [4]*Stenotrophomonas pavanii* [5]*Stenotrophomonas nitritireducens* [6]*Stenotrophomonas acidaminiphila* [7]*Stenotrophomonas lactitubi* [8]*Stenotrophomonas indicatrix* [9]*Stenotrophomonas bentonitica* [10]*Stenotrophomonas chelatiphaga* [11]*Stenotrophomonas koreensis* [12]*Stenotrophomonas daejeonensis* [13]*Stenotrophomonas ginsengisoli* [14]*Stenotrophomonas terrae* [15]*Stenotrophomonas humi* [16]*Stenotrophomonas Africana* [17]*Stenotrophomonas tumulicola*

1.3.1. The Pathogenic nature of the genus Stenotrophomonas species

Until recently, *S. maltophilia* is the only recognized member of the genus *Stenotrophomonas* that is associated with disease or infectious condition. However, a recent study reported the recovery of *S. acidaminiphilia* from the bile of cholangiocarcinoma patient with obstructive jaundice and cholangitis (Huang, *et al.*, 2018). This implies that other species of *Stenotrophomonas* which were regarded as non-pathogens may become pathogenic as they interact in their environment since the mechanism involved in host-pathogen interaction is like those involve in beneficial association, it is possible for a non-virulent strain of bacteria to become virulent in a bid for survival.

There are several factors in *Stenotrophomonas* which contribute or can contribute to their pathogenicity. They include, extracellular enzymes, lipopolysaccharides, adherence and motility factors, as well as immune-stimulatory effects, quorum sensing and biofilm formation (Thomas *et al*., 2014; Ugalde, 2000; Ryan *et al*., 2009).

1.3.1.1 Extracellular Enzymes

Stenotrophomonas produces several extracellular enzymes which contribute to their pathogenicity in the infected host. Several extracellular enzymes such as Proteinases, Lipases, play vital role in the infective nature of *S. maltophilia* (Thomas *et al*., 2014). Proteinases plays an important role in invasiveness, host tissue damage, and evading host-defense (Travis, Potempa, & Maeda, 1995). Extracellular lipases help bacteria to thrive in a carbohydrate restricted environment where lipids are the sole carbon source and enhances their adhering to host tissue (Miskin *et al*., 1997). Graham and collaborators, 2009 were able to show the production of lecithinase enzyme by some species of the *S. maltophilia* in their study. Lecithinase enzyme is known to modulate the host immune system in cell-to-cell spread of bacteria as seen in *Listeria monocytogenes* pathogenicity (Vazquez-Boland, *et al*., 1992; Graham et al. 2009). Hyaluronidase encourages tissue invasion (Cheng *et al*., 1995) Thomas and colleagues, reported the presence of hyaluronidase in some S. *maltophilia* recovered from infected patient in the hospital. (Thomas *et al*., 2014). With the help of the DNase bacteria can evade the host immune defense (Brinkmann, *et al*., 2004). Melanin production can protect bacteria from host immune system (Nosanchuk, J. D., & Casadevall, 2003). Melanin production was reported in *S. maltophilia* by several studies (Thomas *et al*., 2014; Liaw, Lee, & Hsueh, 2010). Extracellular enzymes production is therefore important in the pathogenicity of *S.*

maltophilia. Some environmental *Stenotrophomonas* species also produce extracellular enzymes which encourage their interaction with the plant host (Ryan *et al*., 2009).

1.3.1.2 Lipopolysaccharide

Lipopolysaccharide (LPS) is an important constituent of the gram-negative bacteria outer-membrane (Ugalde *et al*., 2000). LPS has three domains: lipid A, the core oligosaccharide, and the O antigen or O side chain. McKay and coworkers (Mckay, et al., 2003) showed that LPS is an important determinant of virulence in *Stenotrophomonas* and, SpgM plays an important role in maintaining the virulence-dependent LPS structure. The alteration in the genes essential for the formation of the O antigen or O side chain (*SpgM* and *PGM*) resulted in the reduction in the virulence of the *Stenotrophomonas* strains tested in their study. This observation confirmed O antigen of the lipopolysaccharide as an important virulence factor in *Stenotrophomonas* species.

1.3.1.3 Adherence and Motility factors

Motility factors such as the flagella, fimbriae, pili and capsules play significant role in bacterial pathogenesis (Josenhans and Suerbaum 2002). These motility factors are also involved in the adherence of bacteria to surfaces and biofilm formation (Zhang et al. 2008). Flagella for example played an important role in the adhesion of a bacterium to its host and may also assist in their attachment to surfaces during colonization (Josenhans and Suerbaum 2002). In some cases, the bacterial flagella also assist in the export of virulent proteins in bacteria (Young, Schmiel, and Miller 1999). Pompilio *and colleagues* (Pompilio *et al*., 2009) demonstrated the importance of flagella in bacterial adherence to cell monolayer and biofilm formation in *S. maltophilia* IB3-1 isolated from a cystic fibrosis patient. Using the scanning electron and confocal microscope, they showed that the loss of flagella significantly decreased bacterial adhesiveness ($P < 0.001$), when compared to that of their parental flagellated strains.

Pili and fimbriae in *S. maltophilia* often enhance their ability to adhere to surfaces (De Oliveira- Garcia *et al*., 2003) and formation of complex biofilms. De Oliveira- Garcia and collaborators characterized SMF1 fimbriae from *S. maltophilia* strains SMDP92 and ATCC 13637. SMF1 fimbriae is composed of a 17 kDa fimbrin subunit that shares significant amino- terminal amino acid sequence similarity to the CupA fimbriae of *Pseudomonas aeruginosa* and several fimbriae from pathogenic *Escherichia coli*. All the clinical *S. maltophilia* isolates tested produced the 17 kDa fimbrin. The genomes of *S. maltophilia* strains K279a and R551- 3 have genes that encode type I pili (Sterm_0582- 85, Sterm_1304- 09, and Sterm_2358- 66; based on R551- 3 genome annotation) and Type IV pili. Type I pili is

associated with adhesion, and early stages of biofilm formation, while type IV pili (Sterm_1417- 22 and Sterm_3223–26), is involved in adherence, auto- aggregation, twitching motility and biofilm formation. The clusters of the genes are distributed throughout each genome of the sequenced strains (K279a and R551- 3) in a similar manner, which may imply that there are some similarities in the plant and animal colonization strategies (Ryan *et al*., 2009). *Stenotrophomonas* species possess many organelles that are associated with adherence which not only enhance pathogenicity but also assist them in plant colonization.

1.3.1.4 Immuno-stimulatory effects

Another property contributing to infectious manifestations is immune-stimulant effect, particularly the induction of IL-8 and TNF-α, both of which are anti-inflammatory cytokines activating neutrophils and macrophages. The long-term activation of these cytokines can disrupt pulmonary functions and result in the development of pneumonia (Miller *et al*. 2005). *S. maltophilia* isolates were observed to be highly immune-stimulatory by inducing significant airway epithelial cells expression of interleukin-8 and the expression of tumor necrosis factor alpha (TNF-α) by macrophages. TNF- α signaling appears to be important in the pathogenesis of *S. maltophilia* infection as less than 20% of TNFR1 null mice (compared with 100% of wild-type mice) developed pneumonia and bacteremia following intranasal inoculation as reported by Waters and colleagues, 2007 (Waters et al. 2007). *Stenotrophomonas* can initiate infection because of their immune-stimulatory effect on their host.

1.3.2 Quorum Sensing and biofilm formation

A process of cell to cell communication in bacteria which ensures the sharing of information among bacteria and regulation of gene expression is known as the quorum sensing. It is a cooperative interactional process in bacteria which involve the production, detection, and response of an extracellular signaling molecule known as Auto-inducer (AI), (Rutherford and Bassler 2012). Many bacteria can control several processes such as bioluminescence, sporulation, competence, antibiotic production, biofilm formation, and virulence factor secretion by quorum sensing (Ng and Bassler 2009; Novick and Geisinger 2008; Williams and Cámara 2009). Some clinically isolated bacteria have been reported to use QS for the regulation of some processes that are associated with virulence (Kleerebezem et al. 1997).

The *S. maltophilia* quorum sensing system depends on fatty acid signal diffusible signal factor (cis-11-methyl-2- dodecenoic acid) (Alavi et al. 2014b; T. P. Huang and Lee Wong 2007; Martínez et al. 2015). This mode of cellular communication was first identified in *Xanthomonas*

campestris (a phytopathogen), as a mechanism for regulating the synthesis of virulence factors (Barber et al. 1997). The regulation of *Stenotrophomonas* motility, the formation of biofilm, resistance to antibiotic and virulence has been associated with the presence of the QS in *Stenotrophomonas* (Huang, & Wong, 2007; Martínez et al. 2015). Quorum sensing behavior in *Stenotrophomonas* depends on two different regulatory pathogenicities (*rpf*F) clusters which differ in the region encoding the N-terminus of both the synthase RpfF and the sensor RpfC (Martínez et al. 2015). The virulence behavior in *S. maltophilia* was linked to the two *rpfF* alleles (*rpfF*-1 and *rpfF*-2). Martinez and colleagues (Martinez, *et al*., 2015) showed that the DSF encoding gene *rpf-1* is a major determinant in the virulent behavior of both the clinical and environmental *S. maltophilia* strains. On the other hand, the *rpfF-2* gene did not affect virulent activities when suppressed but adequately influence virulence when both genes were simultaneously expressed. These authors suggested that rpfF-2 might be acting as a receptor for the signaling communication in *Stenotrophomonas* as illustrated in Figure 2. QS also contribute to the stimulation of plant growth via an improvement in colonization, cell aggregation, biofilm formation and chemotaxis (Alavi *et al*., 2013).

Biofilm formation in *Stenotrophomonas* and other bacteria is one of the methods employed for survival in an unfavorable condition (Bonaventura *et al*., 2004). Several genes such as *spgM*, *rmlA*, and *rpfF* are involve in the formation of biofilm in *Stenotrophomonas* (Zhou, Zhao & Xiao, 2014). An alteration in the *rpfF* gene resulted in the decrease in biofilm formation in *S. maltophilia*. Thus, QS plays a significant role in the biofilm formation by *Stenotrophomonas*.

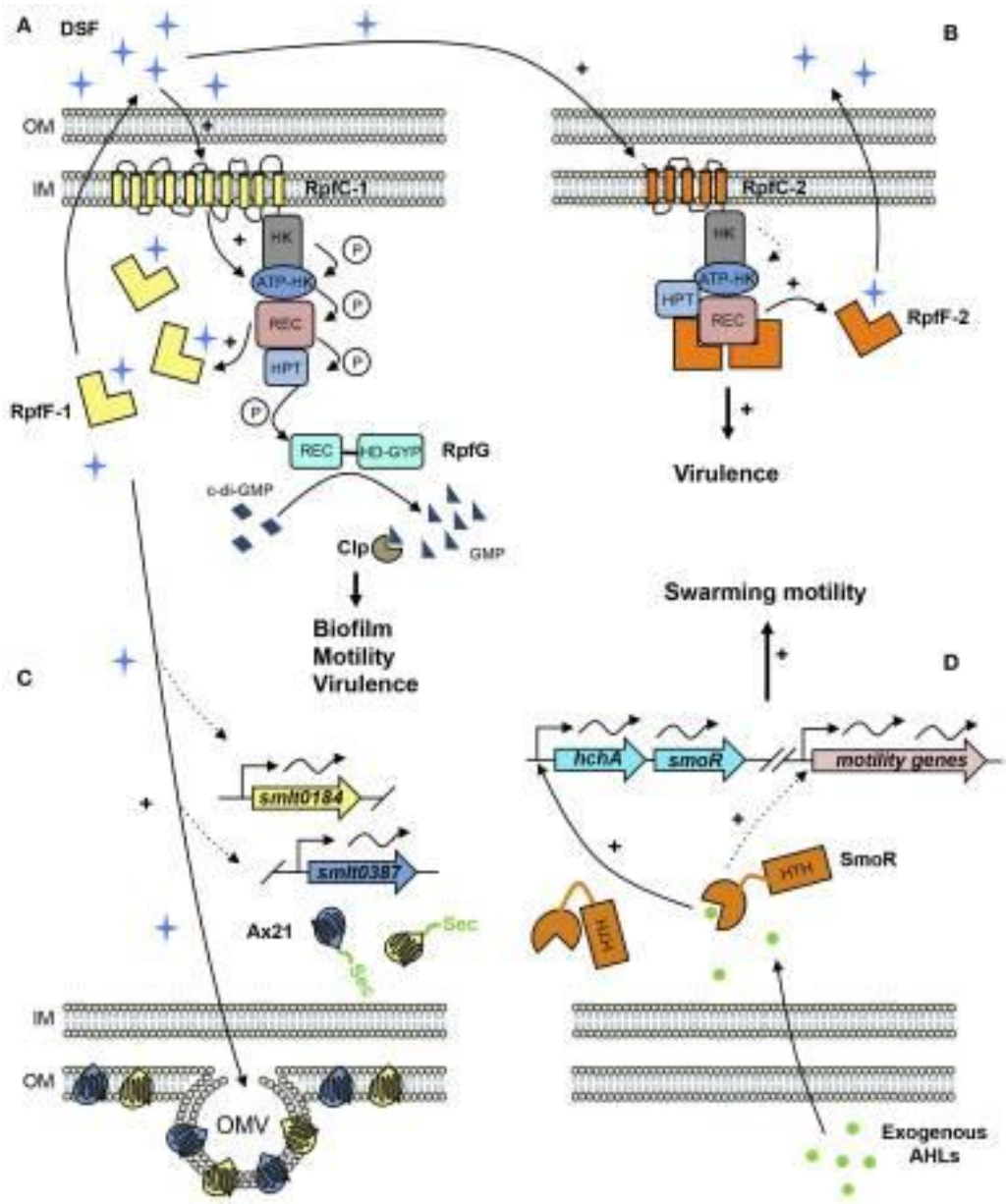


Figure 2: QS signaling network in *Stenotrophomonas* adapted from Huedo *et al*., 2018

A) In rpf-1 strains, RpfC-1 (including 10 TMR) stimulates RpfF-1 basal activity—that increases with cell density—and synthesizes DSF (cis-11-Methyl-2-dodecenoic acid) that accumulates in the extracellular environment. Once DSF concentration reaches a critical threshold, RpfC-1 senses DSF, and induces a phosphorylation cascade throughout its cytoplasmic domains ending in the response regulator RpfG, which degrades cyclic diguanylate monophosphate (c-di-GMP) to GMP activating the transcriptional regulator Clp that stimulates expression of genes involved in biofilm formation, motility, and virulence. (B) In rpf-2 strains, RpfC-2 (5 TMR) permanently represses RpfF-2, resulting in no DSF detection in axenic conditions. DSF produced by neighboring bacteria (e.g., rpf-1 strain) is sensed by RpfC-2 allowing free-active RpfF-2 and subsequent DSF synthesis. (C) DSF also stimulates the production of outer membrane vesicles (OMV) containing high amounts of the two Ax21 proteins (Smlt0184 and Smlt0387). Both Ax21 proteins present a signal peptide that is processed by the general secretory (Sec) system. (D) Exogenous AHL signals, specifically C8-HSL and oxo-C8-HSL, are sensed by the LuxR solo SmoR (Smlt1839), annotated as "LuxR chaperone HchA-associated," activating the transcription of its own operon and promoting swarming motility. Dotted lines represent predicted or supposed interactions based on reported experimental evidences. Protein domains are abbreviated as follows. HK, Histidine kinase domain; REC, Receiver

domain; HPT, Histidine phosphotransferase domain; HD-GYP, Phosphodiesterase domain containing an additional GYP motif; HTH, Helix-Turn-Helix domain

1.3.3 Disruption of Actin Cytoskeletal Structure by Ankyrin-Repeat Protein

Recently, MacDonald and coworkers (Macdonald *et al*. 2016), characterized a putative ankyrin-repeat protein (Smlt3054) unique to clinical *S. maltophilia* isolates which binded F-actin in vitro and co-localizes with actin in transfected HEK293a cells. The Smlt3054 is endogenously expressed and secreted only from clinical *S. maltophilia* isolates and not in the environmental isolate (R551-3) tested. They noted that the *in vitro* binding of Smlt3054 to F-actin led to the thickening of the filaments. They also found out that ectopic expression of Smlt3054−GFP showed strong co-localization with F-actin, with distinct retrograde F-actin waves specifically associated with Smlt3054 in individual cells as well as formation of dense, internal inclusions at the expense of retrograde F-actin waves. This observation showed that there is an interaction between Smlt3054 and F-actin a cytoskeletal protein. The production of Smlt3054 uniquely by clinical strain and its interaction with F-actin might be pointing to the mechanism involved in the emergency of pathogenicity in *S. maltophilia*. Further studies are however required to be able to have a full understanding of the role being played by the ankyrin repeat protein

1.3.4 Antibiotic resistance in Stenotrophomonas spp.

The genus *Stenotrophomonas* contains multi-drug resistant species, which have capacity to resist a wide range of antimicrobial agents and develop resistance to new antibiotics (Valdezate et al. 2001). *S. maltophilia* is the most studied member of the genus *Stenotrophomonas* because it is most frequently encountered from the environments. Several factors contribute to their resistance to antimicrobial agents. Detailed analysis *S. maltophilia* antimicrobial resistance had provided information on some of the factors responsible for their intrinsic and acquired resistance to different antibiotics. These include the possession of genes encoding different multidrug efflux pumps systems, inactivating enzymes, low membrane permeability biofilm formation and other resistance encoding genes borne on plasmid or chromosome (Wang, 2018; Sanchez, 2015).

1.3.4.1 Multi-drug efflux pump (MFP) systems in Stenotrophomonas spp

The genome of *S. maltophilia* and other related *Stenotrophomonas* specie have been found to be littered with plethora of genes encoding multi-drug efflux pumps (Wang *et al*., 2018). These efflux pumps are responsible for varieties of intrinsic and acquired resistance. The MFP that has been reported in *S. maltophilia* include ATP binding cassette (ABC) type multiple efflux pump

(such as SmrA; FuaABC; and MacABCsm) Major Facilitator Super family type (MFS-Type) (such as EmrCABsm and MSHA) and eight Resistance Nodulation cell Division (RND-Type) efflux system. The RND type efflux system that has been reported in *S. maltophilia* can be categorized into two which are the fully characterized and the uncharacterized RND efflux system. The fully characterized RND efflux system in *S. maltophilia* are the SmeABC, SmeDEF, SmeVWX, SmeIJK, SmeYZ, and SmeOP-TolCsm while the uncharacterized efflux pumps are SmeGH and SmeMN (Wang *et al*., 2018). Sometimes the efflux pump encoding genes are present in the genome of *Stenotrophomonas* at a lower level of expression and may not induce resistance in them, however an overexpression of such genes may confer acquired resistance on *Stenotrophomonas* or on the other hand an alteration in a gene regulating expression of these genes can confer antimicrobial resistance on *Stenotrophomonas* species (Li, Zhang, & Poole; Lin, *et al*., 2014).

Stenotrophomonas resistance to major antibiotics has been associated with the SmrA. Al-Hamad and colleagues (2009) reported an increase in resistance to some antibiotics (including fluoroquinolones, tetracycline, doxorubicin and multiple dyes) following the overexpression *of S. maltophilia* SmrA gene in *acrAB* mutant *E. coli* strain SM1411 with an increased rate extrusion of norfloxacin thus reducing the uptake of norfloxacin by the mutant strain. Similarly, the overexpression of FUA (ABC-type,) multidrug efflux pump in *S. maltophilia* K279a confer on them resistance to fusidic acid (Hu, *et al*., 2012). Another type of ABC-Type tripartite efflux pump MacABCsm was found to influence the ejection of macrolides, aminoglycosides and polymyxins and enhanced the formation of biofilm in *S. maltophilia* (Lin *et al*., 2014).

The MFS-type efflux pump conferred resistance on *S. maltophilia* to nalidixic acid and nacrolides. Huang *et al*., 2013 demonstrated the role of EmrCABsm pump in ensuring the discharge of hydrophobic compound from the cell of *S. maltophilia.* A novel MFS-type efflux pump MfsA which is regulated by SoxR has been identified in *S. maltophilia* to be responsible for its resistance to paraquat and aminoglycosides (kanamycin, streptomycin and neomycin), cephalosporins (cefazolin and cefalexin), the macrolide erythromycin, fluoroquinolones (ciprofloxacin, norfloxacin, levofloxacin and ofloxacin), rifampicin, tetracycline and chloramphenicol) (Srijaruskul, *et al*., 2015; Dulyayangkul, *et al*., 2016).

Several RND type multiple efflux has been reported in *Stenotrophomonas* as stated above and linked to *Stenotrophomonas* resistance to different antibiotics including quinolones and

sulfamethoxazole and erythromycin (Wang *et al*., 2018; Alonzo & Martínez, 2000). SmeDEF RND is a membrane fusion protein, resistance nodulation division MDR efflux which was identified by Alonzo and Martínez (2000) as one of the efflux systems responsible for the resistance in *Stenotrophomonas maltophilia* D457R to chloramphenicol, quinolone, tetracycline, trimethoprim-sulfamethoxazole, and macrolides. SmeDEF has since been reported in other *S. maltophilia* strains. SmeDEF has been reported not to be associated with resistance to aminoglycosides (Huang, et al., 2017). The SmeABC and SmeDEF efflux system were identified to be responsible for the extrusion ciprofloxacin and meropenem by *S. maltophilia* (Chang *et al*., 2004). The overexpression of SmeVWX and SmeDEF efflux system was reported as the reason for observed resistance to quinolone in clinical S. maltophilia isolates (Garcia, et al., 2015). Other RND type MDR efflux system has been linked with resistance to other antibiotics, for example SmeYZ contributes to the resistance in *S. maltophilia* to aminoglycoside and trimethoprim-sulfamethoxazole (Lin et al., 2015) while SmeOP-TOlCsm extruded nalidixic acid, doxycycline, amikacin, gentamicin, erythromycin, leucomycin, carbonyl cyanide 3-chlorophenylhydrazone, crystal violet, sodium dodecyl sulfate, and tetrachlorosalicylanilide from the cell of *S. maltophilia* (Lin, et al., 2014). SmeIJK is responsible for the intrinsic resistance of *S. maltophilia* to gentamicin, amikacin, tetracycline, minocycline, ciprofloxacin, and leucomycin. SmeIJK also associated with acquired resistance to levofloxacin, when overexpressed alone or in coordinate hyperproduction with SmeYZ (Crossman *et al*., 2008; Gould, Okazaki, &Avison, 2012; Huang, *et al*., 2014; Wang *et al*., 2015). In addition to MFS system S. maltophilia possess some other mechanisms of resistance to antibiotics, some of which may be specific to the different class of antibiotics. these mechanisms include enzyme inactivation, resistant genes and low cellular permeability among others.

1.3.4.2 Resistance to beta-lactam antibiotics

The beta-lactam is a group of antibiotics which act on a family of enzymes, comprising transpeptidases and carboxpeptidases, that is involved in essential steps in the biosynthesis of the bacterial cell wall and its maturation (Page, 2012). *Stenotrophomonas* have been reported to be intrinsically resistant to many beta-lactam antibiotics owing to the presence of two inducible β-lactamases L1 and L2 encoding genes in their genomes (Crowder, *et al*., 1998; Flores-Trevino, 2014). L1 is a Zn^{+} dependent β-lactamases which ensures that *S. maltophilia* is resistant to cephalosporines, carbapenem, penicillin's, and not monobactam, L2 however is an acid sensitive cephalosporine which confers resistance on *S. maltophilia* to cephalosporines, monobactam and

penicillin (Walsh, MacGowan, & Bennett, 1997; Crowder, *et al*., 1998). The expression of L1/L2 in *S. maltophilia* is regulated simultaneously by the transcription of AmpR which is encoded by ampR that is situated upstream of $blaL_2$ (Okazaki, & Avison, 2008). AmpR act a weak repressor of $blaL_2$ in the presence of a beta-lactam antibiotic and as a weak activator of $blaL_2$ in the absence of beta-lactam antibiotics. These beta-lactamases are not induced when the ampG and ampN operon (which encode a permease transporter) was deleted from the genome *S. maltophilia* (Huang, *et al*., 2010) on the other hand, however the inhibition of the transcription of *mrcA* or *ampDI* (encoding penicillin-binding protein 1a [PBP1a] and a cytoplasmic N-acetyl-muramyl-L-alanine amidase [AmpDI] resulted in the overexpression of L1/L2 β-lactamases (Yang, *et al*., 2009; Lin, *et al*., 2011). Several other genes associated with resistance to beta-lactam antibiotics have been reported in *S. maltophilia*. Some of these genes are located on mobile genetic elements, they include *TEM-2, TEM-116, TEM-127, CTX-M-1, SHV-1*, and *CTX-M*-15 and the globally disseminated metallo-β- lactamase NDM-1 (Avison, *et al*., 2000; al Naiemi *et al*.,2006; Lavigne, *et al*., 2008; Liu, *et al*., 2012; Maravić, *et al*., 2014). Integrons responsible for *S. maltophilia* resistance to beta-lactamase have been isolated from *Stenotrophomonas maltophilia* (Wu et al. 2012). Furlan Pitando-Silva & Stehling, 2018. reported the presence of blaNDM-1 inside an IncA/C plasmid from a *Stenotrophomonas* strain isolated from the soil in Brazil. bla PER has also been detected in an IncA/C Plasmid on another *S. maltophilia* isolated from the soil in Brazil (Furlan, Pitondo-Silva, and Stehling 2018). The recovery of bla PER and and bla NDM-1 from S. maltophilia revealed their ready nature to acquire resistant gene. The requisite of genes in *S. maltophilia* either on the chromosome or as mobile genetic element may be a major reason while most beta-lactam antibiotic are not effective for the treatment of *S. maltophilia* infections.

1.3.4.3 Resistance to Quinolones

Quinolones and fluoroquinolones are synthetic antibiotics which are bactericidal, with a broad-spectrum activity against many clinically important pathogens including gram-negative and gram-positive bacteria (Sharm *et al*.,2009). They act by inhibiting the replication and transcription of bacterial DNA by either disrupting the activity of the DNA gyrase thereby preventing the detachment of the DNA gyrase from the DNA (Cozzarreli, 1980; Schmitz *et al*., 1998; Sharma *et al*.,2009). DNA gyrase is an important adenosine triphosphate-hydrolyzing topoisomerase II enzyme which relaxes supercoiled DNA by breaking both strands of DNA chain, crossing them over, and then resealing them (Schmitz, *et al*., 1998). Fluoroquinolones'

resistance in *Stenotrophomonas* is still limited but there are reported cases of *Stenotrophomonas* resistance to fluoroquinolones.

Low level of resistance has been expected to fluoroquinolone by bacteria and if any, the mechanism of resistance should be restricted to a chromosomal borne gene. Such kind of resistance should not be due to an acquisition of resistant genes via horizontal gene transfer and may likely be due to a mutation in the quinolone targets (DNA gyrase or topoisomerase IV) or the overproduction of multidrug efflux pump (Martinez *et al*., 1998; Hooper, 1999). However, studies have reported plasmid borne resistant gene in bacteria to fluoroquinolones with no mutation in DNA gyrase (Martínez-Martínez *et al*.,1998; Wang *et al*., 2018). Although, *Smqnr* confers resistance on *S. maltophilia* to fluoroquinolones, its activity has been associated only with low level of resistance. Resistance to fluoroquinolones reported in *S. maltophilia* is due majorly to expulsion from the bacterial cell by the SmeDEF and SmeVWX efflux system (Wang *et al*., 2018).

qnrA1 was first identified in *Klebsiella pneumoniae* as a plasmid borne gene conferring resistant on *K. pneumoniae*. *SmQnr* was found as a chromosomally borne gene in the genome of *Stenotrophomonas* conferring on them intrinsic capacity to resist fluoroquinolones (Martínez-Martínez *et al*.,1998). The gene *qnr* confers resistance on bacteria against fluoroquinolones by protecting the DNA isomerase and topoisomerase IV from the activities of fluoroquinolones (Arsène, & Leclercq, 2007; Sánchez *et al*., 2008).

1.3.4.5 Resistance to Aminoglycosides

Aminoglycoside are aminocyclitols that attack bacterial ribosomes thus disrupting protein synthesis (Shakil *et al*., 2008). Aminoglycoside are mostly active against aerobic gram-negative bacteria. *S. maltophilia* are intrinsically resistant to aminoglycosides. Some chromosomal borne gene in *S. maltophilia* has been demonstrated responsible for the intrinsic resistance to aminoglycosides. Li and coworkers (2003) demonstrated the role of gene [*aac(6′)-Iz*] encoding an aminoglycoside-modifying enzyme, AAC (6′)-*Iz* acetyltransferase in *S. maltophilia* resistance to aminoglycoside. The unmarked chromosomal deletions of the *aac (6′)-Iz*gene resulted in an increase susceptibility of previously resistant *S. maltophilia* to amikacin, netilmicin, sisomicin and tobramycin (4- to 32-fold decrease in MICs). Similarly, Okazaki and Avison (Okazaki and Avison 2007) studied the role of chromosomally borne *Aph(3)-IIc* on the resistance of *S. maltophilia.* The disruption of the gene *Aph (3)-IIc* from the genome of *S. maltophilia* K279a resulted in an increase susceptibility to kanamycin, neomycin, butirosin, and paromomycin.

These genes however confer low level of resistance on *S. maltophilia*. The main mechanism of resistance to aminoglycosides is due to the activities of the efflux pumps SmeABC, SmeYZ, SmeOPTolCsm, and MacABCsm.

1.3.4.6 Resistance to Phenicol and Trimethoprim-sulfamethoxazole

Chloramphenicol interferes with bacteria protein to exert its effect on bacteria such that the bacterium becomes unable to synthesize its needed protein (Jardetzky, 1963). Chloramphenicol and florfenicol resistance in *Stenotrophomonas* species have been suggested could be due to the possession of chromosomally encoded novel *floR* gene (He *et al*., 2014). Other mechanisms involved in *Stenotrophomonas*' resistance to chloramphenicol includes the possession of MFS exporter gene *cmlA1* and chloramphenicol acetyltransferase genes *catB2* and *catB8,* which separately reside in a gene cassette of class 1 integron, which confer resistance to chloramphenicol (Wang *et al*., 2018).

Trimethoprim-Sulfamethoxazole (SXT) is the recommended antimicrobial agent for the treatment of *S. maltophilia* infection because of *S. maltophilia*' high rate of susceptibility to SXT. Recently, the rate of *S. maltophilia* to SXT has continued to increase, though the resistance rate varied per geographical location (Vartivarian, *et al*., 2000; Micozzi *et al*., 2000; Flores-Trevino, 2014), the observed resistance has been less than 10% on the average (Chung *et al*., 2013). The analysis of Tolemanand colleagues (2007) showed that *S. maltophil*ia resistance to SXT was due to either *sul1*, as part of a class 1 integron, or *sul2*, which were located on a large plasmid, or due to the presence of some insertion regions (ISCR3, ISCR2s, ISCR9, ISCR10). Thus, the observed resistance was due to the presence of a class 1 integron and an ISCR insertion element linked to *sul2* gene. Another factor that is responsible for this resistance in *S. maltophilia* is the expression of efflux pumps SmeDEF, SmeVWX in their genome. SmeDEF have the potential to extrude antibiotics from the genome of bacteria (Sanchez and Martinez, 2015). In a related observation, *S. acidaminiphila* SUNEO isolated from the bile of a cholangiocarcinoma patient with obstructive jaundice and cholangitis, showed resistance to SXT because of a mutation within the sulfonamide-binding site of folP in SUNEO, which may reduce the binding affinity of sulfamethoxazole (Huang *et al*., 2018)

1.3.4.7 Colistin resistance

Colistin has become the drug of last resort for the treatment of multidrug-resistant (MDR) gram-negative bacteria infections (Stoyanova, *et al*., 2016). Colistin has been used in synergy for the treatment of MDR *S. maltophilia* infection (Giamarellos-Bourboulis *et al*.,2002; Wood *et al*.,

2010; Betts *et al*., 2014). Stoyanova and collaborators (2016) isolated a colistin resistant *S. maltophilia* from a cystic fibrosis patient in Bulgaria. The mechanism associated with *Stenotrophomonas*' resistance to colistin has not be fully explained but may be due to one of the arsenals for resistance possessed by *S. maltophilia*. This thus necessitates the need to study more on the underlying molecular mechanism involved in *S. maltophilia* resistance to colistin.

1.3.4.8. Biofilm formation and the alteration of cellular membrane lipopolysaccharide

S. maltophilia possess the potential to form biofilm on different surfaces to enhance their survival in a nutrient-limited environment. *S. maltophilia* has been recovered from various biofilm associated with the surfaces of aquifers, washed salads, hemodialysis, water and dialysate samples, faucets, tap water, bottled water, contaminated chlorhexidine-cetrimide topical antiseptic hand-washing soap, contact lens solutions, ice machines, and sink drains (Brooke, 2012). Biofilm formation has been employed by *Stenotrophomonas* to resist various antibiotics. Biofilm cause resistance in *Stenotrophomonas* by inhibiting the direct contact of antimicrobial agents with *Stenotrophomonas* (Sanchez, 2015). Several factors have been attributed responsible for the formation of biofilm in *Stenotrophomonas*. Some genes involved in the regulation and expression of biofilm formation in *Stenotrophomonas* are also involved in the biofilm formation. The MacABCsm efflux pump for example has been linked with the formation of biofilm in *S. maltophilia* (Lin *et al*., 2014). In addition, genes required for the formation of flagella, and exopolysaccharide synthesis has been shown to affect the formation of biofilm (Huang *et al.*, 2006; Kang *et al*., 2015).

The *spgM* gene codes for a bifunctional enzyme with both PGM and phosphomannomutase activities. Mutatiom in the *spgM* gene in *S. maltophilia* has resulted in shorter O polysaccharide chains. The alteration in this gene content did not result in a change in lipopolysaccharide chemistry but increased the susceptibility to several antimicrobial agents in mutant strain and were completely avirulent in an animal model of infection (Mckay *et al*., 2003).

The two-component regulatory system PhoPQ is another important factor that is involved in the resistance of numerous Gram-negative bacteria, including *S. maltophilia*, to cationic antimicrobial polypeptides, i.e., polymyxin B. Mutation of *S. maltophilia PhoP* have been shown to increase the susceptibility of *S. maltophilia* to polymyxin B, chloramphenicol, ampicillin, gentamicin, kanamycin, streptomycin, and spectinomycin (96). Similarly, the downregulation of the SmeZ efflux transporter expressed by a *PhoP* mutant contributes to increased drug susceptibility, particularly to aminoglycosides (Wang *et al*., 2018).

Antibiotic resistance is a common phenomenon with *Stenotrophomonas* species such that the medium often employ for their isolation usually requires the incorporation of some antibiotic. This is to selectively isolate them in the means of other bacteria. The antibiotic resistance phenotype of *Stenotrophomonas* species could be intrinsic or acquired. In either case, factors responsible for the development of resistance could either phenotypical such as biofilm formation or molecular involving various genes born on plasmid, chromosome or mobile genetic elements.

1.4 The beneficial nature of Stenotrophomonas

Stenotrophomonas species are highly promising bacteria which can be exploited for beneficial purposes. Several studies have reported their use for different beneficial purpose in different experimental scales. The benefits of *Stenotrophomonas* lie on their diverse metabolic capacity. These include their tendency to tolerate heavy metals, production of substances that can promote plant growth and production of stress tolerant and osmotolerant substances (Ryan, *et al*., 2009).

The beneficial effect of *Stenotrophomonas* species have been tested in various capacity. This include promotion of plant growth, phyto-pathogen control, bioremediation of Polycyclic Aromatic Hydrocarbon (PAH) and metal remediation and other biotechnological uses.

1.4.1 Plant growth promotion and Protection

Stenotrophomonas has been used for the promotion of plant growth because of their ability to produce substances which enhances plant growth. *S. rhizophila* isolated from the soil successfully improve the growth of wheat, tomato, lettuce, sweet pepper, melon, celery and carrot in the highly salinated soils of Uzbekistan (Berg *et al*., 2010). The production of compatible solutes (such as trehalose and glucosylglycerol) which annihilate the effect of high concentration of salt contributes to their role in the promotion of plant growth in highly salinated soil (Ryan, *et al* 2009; Berg *et al*., 2010). This also protect the plant host from the osmotic shock that may result from the high salt concentration. *S. maltophilia* produces indole acetic acid (IAA) a plant growth promoting hormone, they also assist in the fixation of nitrogen in plants and help in the oxidation of elemental sulfur making them available for Plant use (Ryan *et al*., 2009). The successful use of *S. maltophilia* for the promotion of plant growth also rely on their ability to resist attack from their preys which are mostly the soil protozoans. *S. maltophilia* R551-3 possesses genes which encode refractile inclusion bodies, known as R bodies, which are toxic to sensitive species of Paramecium (Heruth *et al*., 1994; Ryan *et al*., 2009). This might be important for their escape from protozoan's attack. The ability to alter leave surface and increase water

availability to the plant thereby increasing plant health is another mechanism through which *S. maltophilia* promotes plant growth (Schreiber *et al*., 2005). In addition to plant growth promotion, Stenotrophomonas species also help in the protection of plant against phytopathogens. Berg and colleagues (1994,) demonstrated in a greenhouse experiment analyzed the use of *S. maltophilia* in the control of *Verticillium dahliae* kleb, a fungus that causes Oil seed rape wilt. Dunne and coworkers (2000) purified an extracellular enzyme, and successfully use this enzyme in the control of *Pythium* mediated damping off diseases in sugar beet. *Stenotrophomonas* spp. have been used in the control of Ralstonia *solanacearum* (Mesihha *et al*., 2007; Elhalag, 2015). The role of *Stenotrophomonas* in the control of phytopathogen has been linked to many factors including the production of antifungal substances such as maltophilin, xanthobaccin and volatile organic compounds (VOC) (Ryan *et al.*, 2009). Its antibacterial activity has been linked with the production of an alkaline serine protease enzyme (Elhalag *et al*., 2016).

1.4.2 Biotechnological application of Stenotrophomonas

The biotechnological application of *Stenotrophomonas* includes their use as plant growth promoters, control of phytopathogens, and bioremediatory agents. Different *Stenotrophomonas* species have been tested for their role in the degradation and remediation of pollutants including Feathers (Jeong *et al*., 2010). Table 3 shows examples where *Stenotrophomonas* has been used for bioremediation purposes. Molecular and fhenotypic mechanisms have been used to explain the *Stenotrophomonas*' capability to degrade a wide range of substances including xenobiotics. Some Stenotrophomonas species have capacity to intrinsically to resist various heavy metals. *S. maltophilia* strains Sm777 and D457R have been demonstrated to tolerate various toxic metals, such as cadmium, lead, cobalt, zinc, mercury and silver (Ryan *et al*., 2009). Molecular analyses have revealed the presence of genes associated with heavy metal tolerance in *Stenotrophomonas*. genes coding for copper and mercury resistance located on genomic island was found *S. maltophilia* K279a but not in the *S. maltophilia* R551- 3 genome, on the other hand, loci coding for arsenic resistance (ars, Stemr_2020- 2024) and two tellurium resistance proteins (Stemr_2893- 94) were identified in the *S. maltophilia* R551- 3 genome but not found in *S. maltophilia* K279a (Ryan, *et al*., 2009).

Stenotrophomonas spp. are important multifunctional bacteria which can be applied for various beneficial purposes, but their application has not been fully exploited because they could initiate opportunistic infection or be vehicles for the transmission of resistant genes. Successful

application of *Stenotrophomonas* species can better by achieved by the detail understanding of their complete metabolic capacity. Complete genome sequence can give a better insight to the understanding of *Stenotrophomonas* metabolic behavior.

Table 3: Example of biodegradation Potential of *Stenotrophomonas* species

Application of *Stenotrophomonas sp* for remediation	References
Degradation of hexahydro-1, 3, 5-trinitro-1, 3, 5-triazine (RDX) by *Stenotrophomonas maltophilia* PB1.	Binks *et al*., 1995
Microbial degradation and detoxification of high molecular weight polycyclic aromatic hydrocarbons by *Stenotrophomonas maltophilia* strain VUN 10,003	Juhasz , Stanley, and Britz, 2000
Keratin degradation: a cooperative action of two enzymes from *Stenotrophomonas* sp	Yamamura *et al*., 2002
Degradation Characteristics of Toluene, Benzene, Ethylbenzene, and Xylene by *Stenotrophomonas maltophilia* T3-c	Lee *et al*., 2002
Isolation and characterization of a novel strain of Stenotrophomonas maltophilia possessing various dioxygenases for monocyclic hydrocarbon degradation	Urszula, *et al*., 2009
Degradation of methomyl by the novel bacterial strain Stenotrophomonas maltophilia M	Mohamed, M. S. 2009
Multiple degradation pathways of phenanthrene by Stenotrophomonas maltophilia C6	Gao *et al*., 2006
Degradation of 4-nitroaniline by Stenotrophomonas strain HPC 135	Qureshi, *et al*., 2007
Simultaneous Cr (VI) reduction and phenol degradation using Stenotrophomonas sp. isolated from tannery effluent contaminated soil	Gunasundari, & Muthukumar, 2013

Hexadecane-degradation by Teskumurella and Stenotrophomonas strains isolated from hydrocarbon contaminated soils	Hassanshahian, Tebyanian, & Kariminik, A. 2013

1.5 Genome sequencing and analysis of Stenotrophomonas

The sequencing of bacterial genome has been used to elucidate the contents of their genetic makeup and how genetic properties can affect phenotypes. Genome sequencing is a process which try to splice the bacteria genome and then reassemble it, so as to understand the relationship between the genotype and phenotype of the bacteria. The sequencing and assembling of a bacteria genome can result in the production of a complete genome sequence or a draft genome. A complete genome sequence represents a finished product in which the order and accuracy of every base pair have been verified while the draft genome is a collection of contigs of various sizes, with unknown order and orientation that contain sequencing errors and possible mis-assemblies (Fraser *et al*., 1995). The complete genome or draft genome sequence analyses have been used to explain many phenomena in bacteria and other organism. In a bid towards understanding the characteristics of *Stenotrophomonas* particularly with respect to successful delineation and application, several studies have used the analyzes of the complete genome sequence, (Ryan *et al*., 2009, Patil *et al*., 2016). The first genome of *Stenotrophomonas* sequenced was sequenced in 2008 by Crossman and coworkers. Due to the reduction in sequencing cost, many other studies have employed the use of genome sequencing and transcriptomic analysis to decipher several metabolic processes taking place in *Stenotrophomonas*. Presently there over 130 whole genome sequences for the different species of *Stenotrophomonas* available on National Center for Biotechnology information (NCBI) database. However, there are only 21 completely sequenced genomes while the others were whole genome sequence short guns (Table 4). Bacteria in the genus *Stenotrophomonas* have high metabolic diversity and complete genome sequence analysis will remain a good tool for detail understanding their physiology and linking their phenotype with the genotype.

Table 4: List complete genome sequence available for *Stenotrophomonas maltophilia* and other members of the genus.

Organism name	Strain	Clade ID	Biosample	BioProject	Assembly	Level	Size
Stenotrophomonas maltophilia K279a	K279a	19485	SAMEA1705934	PRJNA30351	GCA_000072485.1	Complete Genome	4.85113

Stenotrophomonas maltophilia R551-3	R551-3	19485	SAMN00623065	PRJNA17107	GCA_000020665.1	Complete Genome	4.57397
Stenotrophomonas maltophilia JV3	JV3	19485	SAMN02261377	PRJNA53943	GCA_000223885.1	Complete Genome	4.54448
Stenotrophomonas maltophilia D457	D457	19485	SAMEA2272378	PRJEA89665	GCA_000284595.1	Complete Genome	4.76916
Stenotrophomonas maltophilia	ISMMS3	19485	SAMN03389650	PRJNA277366	GCA_001274595.1	Complete Genome	4.804
Stenotrophomonas maltophilia	ISMMS2	19485	SAMN03389647	PRJNA277366	GCA_001274655.1	Complete Genome	4.50972
Stenotrophomonas maltophilia	ISMMS2R	19485	SAMN03389649	PRJNA277366	GCA_001274675.1	Complete Genome	4.50972
Stenotrophomonas maltophilia	AA1	19485	SAMN06130959	PRJNA357031	GCA_002025605.1	Complete Genome	4.66334
Stenotrophomonas maltophilia	OUC_Est10	19485	SAMN04992827	PRJNA321363	GCA_002138415.1	Complete Genome	4.66874
Stenotrophomonas maltophilia	AB550	19485	SAMN06678536	PRJNA381518	GCA_002189545.2	Complete Genome	4.94343
Stenotrophomonas maltophilia	FDAARGOS_325	19485	SAMN06173338	PRJNA231221	GCA_002208885.2	Complete Genome	4.85151
Stenotrophomonas maltophilia	CSM2	19485	SAMN08161503	PRJNA421960	GCA_002847385.1	Complete Genome	4.73905
Stenotrophomonas maltophilia	FDAARGOS_92	19485	SAMN03996266	PRJNA231221	GCA_002951115.1	Complete Genome	4.82022
Stenotrophomonas maltophilia	SJTH1	19485	SAMN08643285	PRJNA437214	GCA_003006435.1	Complete Genome	4.93232
Stenotrophomonas maltophilia	W18	19485	SAMN08798397	PRJNA445756	GCA_003030985.1	Complete Genome	4.73843
Stenotrophomonas maltophilia	SJTL3	19485	SAMN09355365	PRJNA474584	GCA_003205835.1	Complete Genome	4.891
Stenotrophomonas maltophilia	NCTC10257	19485	SAMEA4076705	PRJEB6403	GCA_900186865.1	Complete Genome	5.00426
Stenotrophomonas maltophilia	NCTC10258	19485	SAMEA3856672	PRJEB6403	GCA_900475405.1	Complete Genome	4.48112
Stenotrophomonas maltophilia	NCTC10498	19485	SAMEA3956094	PRJEB6403	GCA_900475685.1	Complete Genome	4.66135
Stenotrophomonas	13637	19485	SAMN02874005	PRJNA244350	GCA_000742995.1	Chromosome	4.98931

maltophilia							
Stenotrophomonas maltophilia EPM1	EPM1	19485	SAMN02471395	PRJNA165731	GCA_000344215.1	Chromosome	4.78777
Stenotrophomonas nitritireducens	2001	41447	SAMN05428703	PRJNA330867	GCA_001700965.1	Complete Genome	4.54173
Stenotrophomonas acidaminiphila	ZAC14D2_NAIMI4_2	40634	SAMN04099006	PRJNA296415	GCA_001314305.1	Complete Genome	4.1383
Stenotrophomonas acidaminiphila	SUNEO	40634	SAMN06335323	PRJNA374779	GCA_002951995.1	Complete Genome	3.66086
Stenotrophomonas rhizophila	QL-P4	21195	SAMN05276013	PRJNA326321	GCA_001704155.1	Complete Genome	4.19865
Stenotrophomonas rhizophila	DSM14405	21195	SAMN02727981	PRJNA244760	GCA_000661955.1	Chromosome	4.64898

Many studies have used complete genome sequence to explain the mechanism responsible for some unique characteristics observed in the genus *Stenotrophomonas*. Patil and colleagues (2016) employed pan genome analysis of completely sequence genome to explain the ecology of *Stenotrophomonas*. They also propose the separation of the genus into 18 via complete genome sequence analysis. Also, Huang and collaborators 2018 employed complete genome and antimicrobial resistome analysis of a clinical isolate *S. acidaminiphila* SUNEO to explain the genetic basis of its resistance to Sulfamethoxazole-Trimethoprim. The role of *Stenotrophomonas* K279a in the in opportunistic infection was elaborated by the complete sequence of their genome. (Crossman *et al*., 2008). This also provided the basis for distinguishing between the environmental strain of *S. maltophilia* R551-3 and the clinical strain K279a (Ryan *et al*., 2009; Alavi *et al*., 2014). Whole genome sequence analysis has been employed in the understanding of the mechanisms involved in the promotion of plant growth by *Stenotrophomonas* species (Alavi *et al*., 2014). Many studies have employed complete genome sequencing to explain how *Stenotrophomonas* assist in the promotion of plant growth, and how they displayed resistance to various antimicrobial agents (Lira *et al*., 2012; Zhu *et al*., 2012; Pak *et al*., 2015).

Whole genome is an important molecular technique that can guide on the understanding of bacterial metabolic behavior, the mechanism responsible for the observed behavior and how bacteria can be manipulated to suit desired purposes.

1.6 Justification of the Studies

The genus *Stenotrophomonas* contains gram-negative bacteria, which are intrinsically resistant to many antimicrobial agents and other recalcitrant substances such as heavy metals and xenobiotics. Studies on *Stenotrophomonas* species have shown that these bacteria are important tool for biotechnology in plant growth promotion and bioremediation. However, their intrinsic resistance to antibiotic and possibility for the spreading resistant genes in the community make trouble in many opportunistic infections. The need to control resistance and apply *Stenotrophomonas* species for beneficial purposes necessitates further studies on members of the genus. New strain isolation of *Stenotrophomonas* using analyses based on the physiological characteristics and genome sequencing could help in understanding how to distinguish between the harmful and beneficial groups, identify new species, and give information about the use of *Stenotrophomonas* for favorable purposes on a large scale.

Isolation of *Stenotrophomonas* species from Mexico can assist in obtaining strains that can be effectively used for biotechnological applications in Mexico. Thus, this study is to aim at isolation of *Stenotrophomonas* species from different parts of Mexico, characterizing them, for understanding their evolution and function

1.7 Objective of the Study

The general objective of the study is to understand the diversity, evolution and function of *Stenotrophomonas* spp. in Mexico

specific objectives include:

- Isolation of *Stenotrophomonas* species from soil and sewage in Mexico
- Phenotypic and genetic characterization of isolated *Stenotrophomonas* spp
- Genomic sequencing and comparative analysis of isolated *Stenotrophomonas* spp genome

CHAPTER 2

Material and Method

2.1 Antimicrobial agents

Table 5: Antimicrobial agents

Antibiotics	Company
Augmentin, Ceftriaxone, Nitrofurantoin, Gentamicin, Sulfamethoxazole-Trimethoprim, Ofloxacin, Ampicillin, Ciprofloxacin, Tetracycline and Pefloxacin	Foidisc laboratory, Nigeria
Imipenem	Sigma Aldrich, St. Louis USA
Amphotericin B	Sigma Aldrich, St. Louis USA
Chloramphenicol	Sigma Aldrich, St Louis USA
Doxycycline	Sigma Aldrich, St Louis USA
Levofloxacin	Amsa laboratories Mexico-City, Mexico
Ceftazidime	Pisa Pharmaceutical Gudalajara, Mexico
Vancomycin	Sigma Aldrich, St Louis USA

2.2 Chemical products

Table 6: Chemical products

Chemicals	Company
100bp Ladder, 1 Kb Ladder	Eurofilm, USA

Acetic acid	Caledon Ontario Canada
Agarose	Bioline USA
Ammonium Chloride	Sigma Aldrich, USA
Anthracene	Sigma Aldrich, USA
Anthraquinone	Sigma Aldrich, USA
Biphenyl	Sigma Aldrich, USA
Bromo cresol Purple	Sigma Aldrich, USA
Bromothymol Blue	Sigma Aldrich, USA
Calcium chloride	Caledon, Ontario, Canada
Crystal Violet	Jalmek Cientifica Monterrey, Nuevo Leon, Mexico
Crystal Violet	Jalmek Cientifica Monterrey, Nuevo Leon, Mexico
Dichloromethane	Sigma Aldrich, USA
$DMSO_4$	Sigma Aldrich, USA
EDTA Disodium Dihydrate	Bio Basic Ontario, Canada
FD 375 SVIA media Supplement	Himedia India
Fructose	
Glucose	
KH_2PO_4 Potassium dihydrogen phosphate	Jalmek Cientifica Monterrey, Nuevo Leon, Mexico
$KHPO_4$ Potassium hydrogen phosphate	Jalmek Cientifica Monterrey, Nuevo Leon, Mexico
L Arabinose	Sigma Aldrich, USA

Lactose	
Magnesium Chloride	Sigma Aldrich, USA
Maltose	
Mannitol	
Methionine	Sigma Aldrich
NAH_2PO_4 Sodium dihydrogen phosphate	Jalmek Cientifica Monterrey, Nuevo Leon, Mexico
$NAHPO_4$ Sodium hydrogen phosphate	Jalmek Cientifica Monterrey, Nuevo Leon, Mexico
Naphthalene	Sigma Aldrich, USA
N Hexane	Sigma Aldrich
Peptone	MCD laboratories, Mexico
Phenanthrene	Sigma Aldrich, USA
Phenanthridine	Sigma Aldrich, USA
Resublime Iodine	Jalmek Cientifica Monterrey, Nuevo Leon, Mexico
Safranin Red	Jalmek Cientifica Monterrey, Nuevo Leon, Mexico
Sodium acetate	Bio Basic, Ontario, Canada
Sodium chloride	Sigma Aldrich, USA
Sucrose	
Tris (Hydroxylmethyl) Aminomethane $C_4H_{11}NO_3$	Bio Basic, Ontario, Canada
Tryptone	Biobasic, Ontario, Canada

Tween 80	
Urea	Applchem Duwsidt, Germany
Xylene	Sigma Aldrich, USA

Buffer solutions, solutions and media (solid and liquid)

Buffer	Typical Composition
Phosphate Buffer	
TAE buffer	
Nutrient agar	
Dilute Nutrient Broth agar (DNB)	
Yeast Peptone agar (YP)	
Tryptic Soy agar	
Luria Bertani agar	
Blood agar	
MacConkey agar	
Mannitol Salt agar	
HIMEDIA STENOVIA agar	
MacConkey Broth	
Luria Bertani broth	
Nutrient broth	
Columbia agar with sheep blood	23 g/L special peptone
(Oxoid, Wesel, Germany)	1 g/L starch

5 g/L NaCl

10 g/L agar

pH 7.3 ± 0.2

Commercial Kits

Taq PCR Kit	Promega
Oxidase strip	Sigma Aldrich, USA
Genomic DNA extraction kit	Promega,
PCR product purification Kit	Qiagen
Exosap PCR product purification kit	BioRad

Equipment

Labline 30 ^{O} C shaker Incubator (Thermofisher, Massachusetts, United States)

MaxQ 480R 37 ^{O} C shaker incubator (Thermofisher, Massachusetts, United States)

Shell Lab 37 ^{O} C Incubator (SHELDON MANUFACTURING, INC., USA)

Shell Lab 30 ^{O} C (SHELDON MANUFACTURING, INC., USA)

MALDI Biotyper V.3.1.66 (Brucker, USA)

2.*3 Bacterial Isolation and Cultivation*

2.3.1 Sample collection

Sewage and soil samples were collected from eight states of Mexico (Tabasco (n= 2, soil, (2); sewage, (0)), Tamaulipas (n=100, soil (31) sewage (69)), Tlaxcala (n=24, soil (1), sewage, (23)), Coahuila (n=4 soil(0), sewage (4)), Chihuahua (n= soil (0); sewage (4)), Mexico state, (n= 20, soil (20); sewage (0); Nuevo Leon (n= 1, soil (0), sewage (1)) and Mexico City (n=6, soil (6); sewage (0)), between April 2015 and June 2017 and aseptically transferred to the Laboratorio de Biotecnología Genómica, Centro de Biotecnología Genómica, Instituto Politécnico, Nacional,

Mexico, for further analysis. The rational for the selection was due to the presence of the environmental features of the sewage and the soil. Some of the features include crude oil contamination, Ancient sea larvae, textile effluent, high temperature Agricultural activities and high level of pollution (sanitary and hospital wastes).

2.3.2 Isolation of Stenotrophomonas species

Screening of samples for the presence of *Stenotrophomonas* species was carried out with two different protocols, (1.) Double layer agar plate technique (DLAP) and (2) Isolation with selective medium.

*2.3.2a Isolation of Stenotrophomonas with DLA*P

Corsaro, Müller & Michel, (2013) reported the isolation of ultrastructure endo-parasitic *Stenotrophomonas maltophilia* which was unable to grow independent of the amoeba host from which they were isolated. We therefore employed DLAP technique for the recovery *Stenotrophomonas maltophilia* complexes in this category from sewage samples. DLAP technique was carried out as described by Jurketvich, 2000 and Oyedara *et al*., 2016 with slight modification.

400 µl of *Escherichia coli* washed in phosphate buffer was co-cultured with 100 µl of serially diluted water samples (10^{-2} to 10^{-8}) from sewage channels and incubated at 30^{o}C for 7days. Plates were observed daily for the development of distinct plaque forming units. Plaque formed on plates were cut out aseptically for further analysis.

2.3.2b Isolation with Selective medium Stenotrophomonas Vancomycin Imipenem Amphotericin (SVIA) agar.

Prior to acquiring SVIA agar and its supplement from HIMEDIA (India), we formulated a selective medium in the lab which has Tryptic soy agar as the basal medium into which three antibiotics (vancomycin, Imipenem and Amphotericin B) were incorporated at the concentration; 10mg/L, 64mg/L and 2.5 mg/L respectively. We also included in this medium 0.2mg/ml methionine for the isolation of methionine auxotrophic strains. This medium was use for the recovery of Stenotrophomonas species from the first 30 samples collected in this study. Subsequent bacterial isolations were done with SVIA medium (HIMEDIA, India). A slight modification was carried out by adding 0.2mg/mL to the agar during preparation before sterilizing them. The pH for all medium used for the isolation of *Stenotrophomonas* species was left at 7.4 ± 0.2. By other hand, 100 µL of sewage or 100mg of soil sample were initially inoculated in Luria-Bertani and incubated at 30 ^{o} C shaker incubator (Labline 30 ^{O} C shaker

Incubator, Thermofisher, Massachusetts, United States) at a revolution of 200 rpm for 24 hours. This is to increase the population of the suspected *Stenotrophomonas* species in case they are present in the samples in low proportion. These 24-h old culture was then serially diluted (10^{-2} to 10^{8}) and 100 µL of each dilution was then spread on SVIA agar for the selection of *Stenotrophomonas* species. The plates were incubated for 24-48 hours in a static incubator (SHELDON MANUFACTURING, INC., USA). Colonies which appeared on plate were selected for further analysis.

2.4. Identification of Isolates

The identification of *Stenotrophomonas* species has been very complex because of the high level of intra species and inter species variation which is common in the genus. A combination of many techniques for their identification will therefore give a good premise for determining the identity of the suspected colonies. We employed three methods for the identification of the suspected colonies. Colonies with yellow, orange, or green color on SVIA agar were selected for further identification analysis. The identity of colonies was determined by biochemical testing of isolates, Matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (MALDI TOF), and and also at the molecular level by amplifying certain fragment of *Stenotrophomonas* 16S rRNA region in the suspected colonies.

2.4.1 Identification of isolate by Phenotypic or Biochemical Characteristics

The identity of colonies was determined by biochemical testing of isolates as described in the Bergey's manual of determinative Bacteriological studies (Garrity *et al*., 2005)

2*.4.1a Growth Characteristics*

Optimal temperature and pH for growth were determined in two different experiments using Luria broth, and agars which include; Luria agar, MacConkey, and Tryptic soy agar as described by Lee and coworkers (2011).

2*.4.1a1 Determination of temperature range for bacterial growth*

Bacterial growth was analyzed at different temperature ranges to determine different the optimal temperature at which bacterial isolates can grow. Isolates were cultured in both liquid and on solid media. The liquid media used was Luria broth while the solid media in which growth temperature was checked include Luria agar, Tryptic soy agar and Yeast Peptone agar. Bacteria were inoculated into liquid medium and incubated at different temperature, which are 4^{o} C, 30^{o} C, 37^{o} C and 42^{o} C. Growth was determined through spectrophotometric analysis (Beckmam Coultman, USA) in 3hours interval for 36 hours. The values obtained were then used to construct

a growth curve for the isolates (using spectrophotometer colony forming unit conversion formula to convert spectrophotometer reading to cfu/ml). For solid medium; isolates were streaked on solid medium and incubated at different temperature for 24-48 hours. Growth on plate as colony forming unit were used as measures for bacteria capability to grow at the incubation temperature.

2.4.1a2 Determination of bacterial growth pH

The effect of pH on growth was examined on Luria Bertani agar (LB) using four different buffers to obtain different pH as described by Lee and colleagues (2011). All buffer concentration was 50mM. Sodium acetate buffer was used to determine growth at pH range (5.0-5.5), potassium phosphate buffer for pH (6.0-8.0), Na2CO3/NaHCO3 buffer pH (8.5-10.0) and Na2PO4/NaOH buffer (10.5 11.0). All experimental set ups were incubated at 30^{o}C for 5days and the results recorded. All experiments were done in duplicate.

2.4.2 Assimilation of different sugars as unique carbon source

The use of various sugars as a sole source of carbon was evaluated in liquid medium containing the test sugar as the only source of carbon. Liquid medium contains 2g peptone, 5g, Sodium chloride (NaCl) 0.3g dibasic potassium phosphate (K2HPO4), 3mg bromothymol blue, 10g sugar in 1Liter of Distilled water. Isolates ability to ferment different sugars as a sole carbon source were determined by the color changed in the liquid culture from purple to yellow following incubation for 24-48hr at 35^{o}C. *E. coli* was used as positive control in the experimental set up while an uninoculated sugar medium serves as the negative control.

2.4.3 Other Biochemical tests

Biochemical tests were carried out according to the Bergey's manual of determinative Bacteriological studies. Oxidase tests were performed by using commercially available kit (Sigma Aldrich, USA) Catalase activities were determined by immersing a loopful of isolate in Hydrogen peroxides. Bubble formation/Gas production following immersion was used to determine catalase activities. Bile Aesculin hydrolysis were determined by streaking bacteria on Bile Aesculin plate and incubated at 37^{o}C for 24 hrs. The formation of black coloration on plates indicates Aesculin hydrolysis. Decarboxylase and Deaminase enzyme activity were evaluated by inoculating isolates in basal medium with 1% amino acid and Lactose (Lysine, Serine, and Ornithine) to be tested. Decarboxylase tests were implemented as described by Elston (1971). Change in the color of the broth solution from purple into yellow and back to purple after 48hrs of incubation was considered for decarboxylase activity. Phenylalanine deaminases was evaluated with Phenylalanine Agar and production of green coloration on semi solid agar

following the addition of 0.1N HCl and 10% Ferric chloride is expected to give a positive feedback for Phenylalanine deaminase test. Urease activities was evaluated as described by Brink, 2010. *Proteus mirabilis* CDBB-B-1343(ATCC 21100) was used as positive control while *E* coli DH5α was used as negative control for the analysis Gelatin Hydrolysis was evaluated using gelatin agar plate by inoculating Nutrient plate agar supplemented with 0.8% gelatin with bacteria after which they were incubated at 30ºC for 24hrs. Gelatin hydrolysis were confirmed by flooding the plates with ammonium chloride to confirm gelatin opacity on plates. Hydrolysis of starch was tested on nutrient agar plates supplemented with 1% soluble starch. Starch hydrolysis was detected by flooding the plate with iodine solution (Tindall *et al.,* 2007). Hydrolysis of Tween 80 was analyzed using the method described by Sierra and colleagues (1957).

2.4.4 Species Identification with MALTI-DOF

The species identification was also determined by MALTI-DOF. Strains were prepared for MALDI-TOF mass spectrometry using the manufacturer's recommended direct transfer protocol. A single colony of each strain was inoculated directly onto the MSP 96 target plate spot (Bruker Daltonics, Bremen, Germany). Each spot was overlaid with α-cyano-4-hydroxycinnamic acid matrix (Sigma Aldrich, St. Louis, MO, USA). The target plate was analyzed by the Bruker Microflex LT system (Bruker Daltonics). The protein profile of each spot generated m/z values of 3000–15,000, which was then analyzed by the MALDI Biotyper V.3.1.66 with the most updated spectra library, V.7.0 (7311 spectra). The top 10 identification matches were generated along with confidence scores which ranged from 0.0 to 3.0. A score >2.0 indicated promising species-level identification. If two or more different species were shown within the top 10 matches, the species of the isolate would be considered as indeterminate and repeated (Sogawa *et al.,* 2013).

2.4.5 Molecular identification of Colonies

In the case of isolates recovered from samples by using SVIA agar, a colony was picked from distinct yellow colony which appeared on plates and then transferred into LB broth for further incubation at 30 º C for 24 hours. On the other hand, however, plaque formed on DLAP agar was carefully cut with sterile 1 ml pipette tip and transferred into DNB broth. This was incubated at 30 º C for 10 days to ensure that the prey load has reduced drastically. Genomic DNA was extracted from 1ml of this overnight grown culture using Promega wizard genomic DNA purification kit (Promega, Madison, USA) according to the manufacturer's instruction

The 16s rRNA gene fragment was amplified by PCR using primers steno1 (5' AGG GAA ACT TAC GCT AAT ACC- 3') and steno2 (5' CTC TGT CCC TAC CAT TGT AG-3'), and a pair of reference primers proposed by Pinnot and collaborators (2009). The PCR mix contains 0.5µl, 2.5 U Taq DNA polymerase, 0.5µL of 10mM d -NTP mix, 2.5 µL of 10× PCR buffer, 1 µL (0.5µM) of each primer, 0.75 µL (50mM) MgCl2, 16.75 µL double distilled water and 2 µL DNA (10 ng/µL). PCR reaction mixture was initially denatured at 95 ºC, for 3 min subjected to 25 cycles of denaturation at 95 ºC for 30 s, annealed at 62 ºC for 30 s, extended at 72 ºC for 30 s, finally extended at 72 ºC for 5 min, and allowed to cool to 4 ºC. The amplified PCR product was analyzed using 0.7 % agarose gel electrophoresis. The PCR amplified products were directly sequenced at the Centro de Biotecnología Genómica, Instituto Politécnico Nacional (IPN), Mexico, using Sanger sequencing technology. The 16S rRNA gene sequences obtained were analyzed using the Lasergene program Seqman® software (DNAstar Inc., Madison, Wisconsin, USA), and the Blastn program of the National Center for Biotechnology Institute (NCBI) (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Some of the partial 16S rRNA gene sequences of the *Stenotrophomonas* strains isolated were submitted to the NCBI database with the accession numbers KX470411.1, KX500117.1, KX785139.1 - KX785155.1, KX863509.1, and KY454854.1 - KY454855.1).

2.4.5 Antimicrobial susceptibility testing against all isolates

Antibiotic susceptibility testing for all the isolates retrieved from soil and sewage and other strains from hospitalized patients were analyzed. Kirby-Bauer Disc diffusion susceptibility test Hudzicki, 2009 and microtiter plate broth dilution as described by Wiegand Hilpert & Hancock, 2008 was employed for the analysis of isolates susceptibility to antibiotics. The antibiotics used for the disc diffusion assay include, Tetracycline (30µg), Pefloxacin (5µg), Amoxicillin/Clavulanic acid (30µg), Ceftriaxone (30µg), Nitrofurantoin (200µg), Gentamycin (10µg), Ofloxacin (5µg), Amoxycillin (25µg), Ciprofloxacin (10µg), Cotrimoxazole (25µg). The antibiotics employed for the microtiter plate broth dilution assay include, levofloxacin, ceftazidime, doxycycline, chloramphenicol, ofloxacin, trimethoprim-sulfamethoxazole, ceftriaxone, imipenem and ampicillin. Antibiotic susceptibility tests were conducted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (M100S) (CLSI, 2016). *S. maltophilia* ATCC 13637 reference strain was used for quality assurance purposes. An adjusted suspension of each isolate to the density of 0.5 McFarland standard was plated on Müller-Hinton agar (MHA) in three directions to ensure uniform growth for the Kirby-Bauer disc diffusion

testing. The test strips were applied once the agar surface was completely dry. The plates were incubated for 24 h at 35 °C. The susceptibility was interpreted by the zone of growth inhibition that intersected the test strip. CLSI has established interpretive criteria for the characterization of susceptibility breakpoints of *S. maltophilia*.

2.5 Biofilm formation assay

Biofilm formation assay was carried out as previously described by O'Toole, Kaplan, & Kolter (2000). The optical density (OD) reading was taking on a microtiter plate reader (IMAD plate reader BIO-RAD, USA) at a wavelength of 500 nm. The OD value was used to determine the formation of biofilm by isolates. An uninoculated culture in the plate was used as a negative control. The low cut-off included three standard deviations above the mean OD of control wells (Christensen *et al.*, 1985). The results were subtracted from the negative control (OD_{500} = 0.042) and expressed as means. Strains were classified as follows: no biofilm producer (OD_{500} = 0.042); weak biofilm producer (≤0.0 OD_{500} ≤0.1); average biofilm producer (>0.1 OD_{500} ≤0.58); strong biofilm producer (OD_{500} ≥ 0.59).

2.6 Phylogeny Genotyping and Diversity of Isolates

2.6.1 Phylogeny

Genomic DNA was extracted from 5ml Bacterial culture grown on Luria broth using Promega wizard genomic DNA purification kit (Promega, Madison, USA) according to the manufacturer's instruction. The 16s rRNA genes were amplified by PCR as described in section 2.4.5. Sequences obtained were analyze with Seqman software version 13. Sequences were aligned with related sequences retrieved from NCBI data base using Mega 6.0 (Tamura *et al.*, 2013) and Phylogenetic tree was constructed using Neighbor Joining algorithm and Maximum parsimony algorithm. Reliability of tree topologies was confirmed by bootstrap analysis using 1000 repeat alignment.

2.6.2 Genotyping

All the strains isolated were included in this study. A total of 54 strains from the environment and 7 strains confirmed molecularly were included in the study. Genomic DNA from the isolates were extracted as reported above and used for the studies. ATCC13637 was used to assure quality control.

*2.6.2a Repetitive-Sequence-Based Polymerase Chain Reaction Other Phenotypic features and functions (rep-PCR***)**

Rep-PCR for isolates was carried out as described by PawełTrzciński and collaborators (2011). Amplification of DNA fragments of the selected isolates was carried out with the use of primers

complementary to repetitive sequences in the bacterial genome: REP1R-I (5`-IIIICGICGICATCIGGC-3`) and REP2-I (5`-ICGICTTATCIGGCCTAC-3`). The reaction mixture (25 µl) consisted of 1x buffer for PCR, 0.2 mM $MgCl_2$, 0.50 mM of each nucleotide, 0.5 µM of each primer, 2.5 U of Taq polymerase (Promega, USA) and 10 ng of genomic DNA. The reaction was carried out in multigene optima thermocycler (Labnet, USA). The thermal profile used for the reactions include 42 cycles (94 °C x 1 min, 52 °C x 1 min, 72 °C x 1min). The amplified products were analyzed on 2% agarose gel.

2.6.2b. Enterobacterial Repetitive Intergenic Consensus - Polymerase Chain Reaction (ERIC-PCR)

The genomic DNA was amplified with (5`-ATGTAAGCTCCTGGGGATTC AC-3`) and ERIC2 (5`-AAGTAAGTGACTGGGGTGA GCG-3`) as described by PawełTrzciński and coworkers (2011) The amplified reaction has a final volume of 25 µl. The reaction mix contained 1x buffer for PCR, 0.2 mM $MgCl_2$, 0.50 mM of each nucleotide, 0.5 µM of each primer, 2.5 U of Taq polymerase (Promega, USA) and 10 ng of genomic DNA. The amplification products were analyzed by electrophoresis using 2 % agarose gels. A 1 kbp DNA ladder was used as a molecular size marker. The band pattern was generated with bio-numeric software package with 1% tolerance (Applied Maths, http://www.applied-maths.com/bionumerics). Similarity, the coefficient was generated from a similarity matrix calculated with Dice coefficient. The discriminatory power of ERIC PCR to type two bacteria into two distinct group was determined by using the Simpsons index of diversity at 95% confidence interval.

2.7 *Textile Dye tolerance test*

Textile dye tolerance was evaluated by the decolorizing assay, which was carried out as described by Nachiyah and RajKumar (2003), with little modification. 50mg of each azo dyes (reactive yellow, acidic blue, brilliant blue and reactive black) was added to four different Yeast extract, Glucose Sodium chloride medium and the medium was adjusted to pH 7.0 respectively. Medium was then sterilized in the autoclave at 121°C for 15min after which 100µl of bacterial isolate was inoculated into the medium for decolorization study. Culture was incubated at 35°C in a rotary incubator at rev of 200rpm for 48hours. Control was prepared as medium without bacterial inoculum. Culture was examined after 24 hours and 48 hours for dye decolorization. Change in color was determined by spectrophotometry analysis (600nm) using the Beckman Coulter spectrophotometer (USA). Changing in color was determined by taking the OD value

culture at the point of inoculation and after every other day mentioned above. This was used to determine the percentage rate per decolorization using the formula below:

% Decolorization = $(Initial\ Absorbance - Absorbance\ at\ time\ t)/Initial\ Absorbance$

2.8 Hydrocarbon degradation test

2.8.1 Tolerance test

Stenotrophomonas sp. Pemsol isolated from crude contaminated soil was tested for its ability tolerate varied concentration of six PAHs in this study.

Hydrocarbon tolerance test was carried out using Bushnell Hass (BH) medium supplemented with PAH. 1% and 5% of 100 mg in the case of Naphthalene, Phenanthridine, Anthraquinones, Biphenyl, Phenanthrene and 40mg with respect to Anthracene. All hydrocarbons were dissolved in Dimethyl chloride and the solvent was left to evaporate before introducing the hydrocarbons in experimental set ups. The experimental design has three set up which include the test experiment and two controls. All experimental were in duplicates. 100 µl of overnight grown culture of *Stenotrophomonas sp*ecies washed in phosphate buffer was inoculated in 100ml each BH medium containing either 1% and 5% each of the above-mentioned PAH at a concentration of 100mg/ml in 250ml Erlenmeyer flask while each uninoculated BH medium with Hydrocarbon and BH medium with *Stenotrophomonas* served as controls. All experimental set up were incubated at 30^{o}C in a rotatory incubator at 200 rpm for 7 days following inoculation with test bacteria. *Stenotrophomonas'* tolerance was checked every two days using colony counting method. 100 µl of each serially diluted (10^{-4}) inoculated medium was spread on Luria Bertani agar plates and were incubated at 30^{o}C for 24 hours after which the colony formed were counted. Spectrophotometric analysis was also carried out on culture from all experimental set up to corroborate the observations from colony counting method.

2.8.2 Bio-emulsification Assay

Emulsifying properties of isolates was tested as per the description of Panjiar and collaborators (2015).

2.*8.2a Biodegradation activity of Stenotrophomonas sp. Pemsol*

Stenotrophomonas strains was analyzed for the biodegradation of Naphthalene due to its ability to tolerate and grow in the different hydrocarbons tested.

100 µl of overnight grown *Stenotrophomonas* (strain Pemsol) inoculum (OD =1) was introduced into 100 ml liquid Bushnell Hass medium already supplemented with 1% (100 mg/ml)

Naphthalene v/v and medium without Naphthalene in a 250 ml Erlenmeyer flask while the third tube contained only Naphthalene supplemented Bushnell Hass medium. All set ups were in triplicate and tube were incubated at 30^{o} C in a rotatory checker with a revolution of 200 rpm. OD value of set up was checked three times to determine the growth activity of bacteria. This was done on the 1^{st} day of the experiment, the 10^{th} day and the 30^{th} day which is the last day of the experiment.

2.8.3 Extraction of Hydrocarbon and analysis of degradation products

Naphthalene was extracted twice with equal volume of hexane following a period of incubation. Liquid culture (100 ml) containing *Stenotrophomonas sp*. Pemsol inoculum and hydrocarbon was shaken vigorously three times with equal volume of hexane. The mixture was kept at room temperature for 1 hr for proper separation of the emulsions and then transferred into a separating funnel to separate the emulsion. The hexane phase was collected for the analysis of PAH product.

Identifying Metabolic fingerprints of the intermediate with Fourier-transform infrared spectroscopy (FTIR)8The products obtained from the extraction of hydrocarbon compound was left to dry and about 1mg was checked on Bruker Alpha FT-IR spectrometer (AXS Inc., Madison, WI, USA) to determine the profiles of their plasmon resonance peaks.

2.8.3a Identification of Intermediates from the Degradation of Naphthalene using UPLC-MS/MS and Gas chromatography

UPLC-MS System

Naphthalene was selected to analyze their degradation metabolites after 30 days. Naphthalene were obtained by liquid extraction with hexane (10 mL) three times and the solvent was eliminated for vacuum pressure. After that, 1 ml of dichlorometane was added and 0.1 mL was added to 0.9 mL of 0.1% formic acid in acetonitrile and analyzed by UPLC-MS with an ACQUITY QDa mass detector from Waters (Milford, MA, USA) under the following conditions: column: ACQUITY UPLC® BEH C_{18} 1.7 µm, 2.1 × 100 mm; mobile phase: 0.1% formic acid/acetonitrile; run time: 5 min; flow rate: 0.3 mL/min; injection volume: 0.5 µL; temperature: 40 °C.

2.8.3b GC-MS System

Naphthalene were obtained by liquid extraction with hexane (10 mL) three times and the solvent was eliminated for vacuum pressure. After that, 1 ml of dichlorometane was added and 0.1 mL was added to 0.9 mL of 0.1% formic acid in acetonitrile and analyzed by gas chromatograph (HP

5890 II plus, Hewlett-Packard, Florida, USA) equipped with a flame ionization detector, using an HP-5 capillary column (60 m length, 0.23 mm internal diameter and 2.5 lm film thickness). The oven temperature was set at 45 C for the first 1 min and increased by 5 °C per min to a temperature of 100°C, and then increased by 8° C per min to a final temperature of 320° C and a 5 min hold. The carrier gas (N2) flow rate was set at 1.5 ml /min and the temperature of the injector and the detector were used at 280° and 250° C, respectively. The sample volume injected was in 1:1. External PAHs, crude oil, and TPH standards were used for calibration. External standards were prepared by dissolving PAHs and the crude oil in hexane, and the TPH standard was used (ASTM D5442 C12–C60 quantitative linearity standard, Supelco, Bellefonte, USA).

2.9 Whole genome sequencing and analysis

2.9.1 Sample preparation and genome sequencing

The genomic DNA was extracted as described above using the Promega DNA extraction kit (USA) according to the manufacturer's instruction. The extracted bacterial genomic DNA were then sent out for whole genome sequencing at the Unidad Universitaria de Secuenciación Masiva y Bioinformática at the Instituto de Biotecnología, UNAM. The genome was sequenced using the Illumina MiSeq platform.

2.9.2 Genome assembly

The reads quality was checked with Fastqc (Andrew, 2010) embedded in trim-galore and the adaptors from the raw reads were trimmed with trim-galore version 4.10 which also filtered out reads with poor quality. De novo genome assembly was carried out with a standalone Spades 3.11.1 genome assembler (Center for Algorithmic biotechnology, St. Petersburg State University, Russia) (Bankevich *et al*., 2012). The assembly's quality was checked with the quality assessment tool for genome assembly (QUAST) (Gurevich *et al*., 2013) The assembled contigs were ordered and reduced into a single scaffold with MedusaCombo, an online genome multidraft scaffolder (Bosi *et al*., 2015)

2.9.3 Genome Annotation

The assembled genome was annotated with Prokka annotation pipeline and Prokaryotic Genome Annotation Pipeline (PGAP) (Seeman, 2014; Tatusova, 2016). Further functional genome annotation was done with online genome analysis server WebMGA (http://weizhong-lab.ucsd.edu/metagenomic-analysis) (Wu *et al*., 2011). WebMGA was used to predict the KEGG functions and COG categories present in the genome. PHASTER an online prophage identifier was employed for predicting the phage region in the sequenced genome (Amdt *et al*., 2016). Five

genomes *Stenotrophomonas sp*ecies with one contigs retrieved from NCBI database, in addition to the sequenced genome were used for the analysis of the orthologous gene clusters in OrthoVenn, a web server for genome wide comparison and annotation of orthologous clusters across multiple species (http://www.bioinfogenome.net/OrthoVenn/result.php(Y. Wang et al. 2015). The open reading frame was predicted with Sequence manipulation suite (SMS), www.bioinformatictool/org/sms2/orf_find.html).

2.9.4 Prediction of Genomic Island, Insertion Sequences and Transposon

The genomic island in the sequenced genome was predicted by genomic island viewer 4 (Bertelli *et al*., 2017). The presence of transposon and insertion sequences was predicted with a web-based analysis tool software ISsaga (http://issaga.biotoul.fr/ISsaga2/issaga_index.php) (Varani *et al*., 2011). The annotation of the functional content of the genes associated with genomic island and unique gene predicted for isolates was done with blast2go (Conesa *et al*., 2005)

2.9.5 Comparative genome analysis

The analysis of Pan and core genome analysis was done with Bacterial Pangenome Analysis tool (BPGA) as described by Chaudhari and colleagues (2016). Multiple alignment of conserved genomic sequence with rearrangements (MAUVE) analysis tool (Darling *et al*., 2004) was employed for the synteny analysis of the sequenced genome and other *Stenotrophomonas* species retrieved from NCBI database. Genetic relatedness of isolates and other *Stenotrophomonas sp*ecies were determined by analyzing the average nucleotide identity between *Stenotrophomonas sp*. Pemsol and other fourteen representative species of the other members of the genus on J speciesWS (Richter *et al*., 2015) and Genome-Genome distance hybridization (GGDH) tool (Meier-Kolthoff *et al*., 2018). Further analysis on isolates was carried out on the in the Integrated Microbial Genome (IMG) server (https://img.jgi.doe.gov) and Kbase Platform (https://narrative.kbase.us/narrative/ws.27061.obj.1. Further analysis on the gene structure for the unique genes was carried out using Syntax an online synteny viewer and blast search analysis on NCBI database.

2.9.6 Analysis of resistant genes in the genome of Sequenced Stenotrophomonas species

The genes associated with resistance to tested antimicrobial agents were identified by a blast search against resistance gene identifier (RGI) of the Comprehensive Antibiotic Resistance Database (CARD).

CHAPTER THREE

Results

3.1 Collection and Isolation of *Stenotrophomonas* species

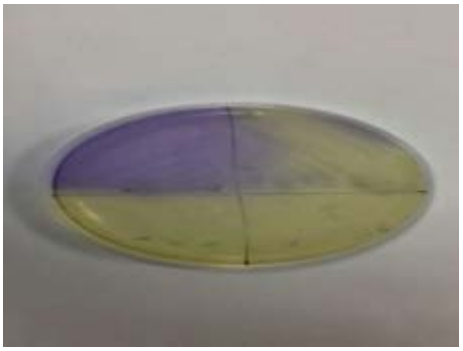
A total of 54 *Stenotrophomonas* species were isolated from over 300 samples collected. Isolates were initially selected to be *Stenotrophomonas* species based on the colors they presented on selective medium Table 7

Table 7: Color characteristics of Isolates and isolation source

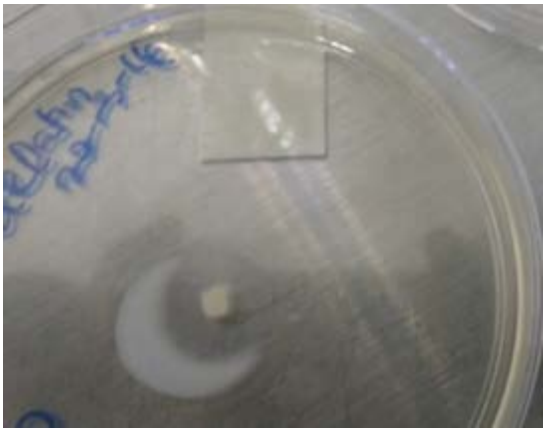
Strain	Isolation Source	Color characteristics on SVIA agar or Plate
1. 15R	Soil	Yellow
2. A915	Sewage	Plaque forming unit
3. ASKF	Soil	Yellow
4. AI	Sewage	Greenish-white
5. ASS1	Soil	Yellow
6. ASS2	Soil	Yellow
7. ATCM1_1	Soil	Green
8. ATCM1_3	Soil	Green
9. ATCM1_4	Soil	Yellow
10. ATCM1_5	Soil	Yellow
11. ATCM2_1	Soil	Yellow
12. ATCM2_2	Soil	Green
13. ATCM2_3	Soil	Yellow
14. B915	Sewage	Plaque forming unit
15. C915	Sewage	Yellow
16. D915	Soil	Yellow
17. DRJ	Sewage	Yellow

18. E915	Sewage	Yellow
19. ETG	Fish	Yellow
20. F915	Sewage	Yellow
21. ETG	Fish	Green
22. FTY	Fish	Yellow
23. FTG	Fish	Green
24. FTO	Fish	Orange
25. J5	Sewage	Yellow
26. JBL	Sewage	Yellow
27. LLD1	Soil	Yellow
28. LLd2_1	Soil	Yellow
29. LLD2_2	Soil	Yellow
30. LLD2_4	Soil	Yellow
31. LLD3_1	Soil	Green
32. LLD3_2	Soil	Green
33. LM2	Sewage	Green
34. Pemsol	Soil	Yellow
35. RS2BY	Soil	Green
36. SDL	Soil	Greenish white
37. SM6	Soil	Green
38. SPM1	Soil	Green
39. SPM3	Soil	Green
40. SVIA1	Soil	Yellow
41. SVIA2	Soil	Yellow
42. Tepegreen	Sewage	Green
43. Tepelarge	Sewage	Yellow
44. Tepesmall	Sewage	Yellow
45. TFLG	Fish	Green

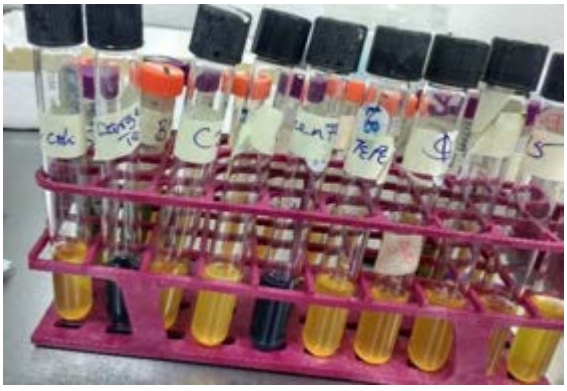
The biochemical characteristics of the isolated strain reflected the potentials of *Stenotrophomonas* species to use different sugars for their growth. The summary of the biochemical behavior of the isolates is shown in table 8. Figure 3 give the image representation of the behavior of isolates to different biochemical test.



a.) Arabinose utilization in some strains isolated



b.) Gelatin hydrolysis test result



c.) Mannitol utilization in Isolates

Figure 3: Selected image for the biochemical characteristics screening of Isolates

3.2 Biochemical Characterization and Isolate identification

The bacterial species isolated were identified using the Biochemical characteristics MALDI-TOF, and 16S rRNA sequencing. 16S rRNA sequencing confirmed all isolates described to be Stenotrophomonas by MALDI_TOF and gave the identity of some strains which MALDI-TOF could not identify. Table 8 & 9

Table 8: Phenotypic and Biochemical Characteristics of *Stenotrophomonas* Isolates.

The characteristics of all environmental isolates and all clinical isolates is shown. Optimal pH growth was 6.5-8.0 for all strains, including environmental and clinical strains. ARA: arabinose utilization; CIT: citrate utilization; DUL: dulcitol utilization; ESC: esculin hydrolysis; FRU: fructose utilization; GAL: galactose utilization; GEL: gelatin hydrolysis; GLU: glucose utilization; LAC: lactose utilization; LYS: lysine decarboxylase activity; MAL: maltose utilization; MAN: mannitol utilization; MNO: mannose utilization; STA: starch hydrolysis; SUC: sucrose utilization; TRE: trehalose utilization; TWE: tween 80 hydrolysis; URE: urease activity; 30°C: Growth at 30°C; 42°C: Growth at 42°C. ND: not determined.

Strains	MAN	ARA	GLU	SUC	TRE	LAC	MAL	MNO	FRU	GAL	DUL	LYS	GEL	TWE	URE	STA	ESC	CIT	30°C	42°C
Environmental strains																				
A915	+	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	-	+	+	+
B915	+	+	+	+	+	-	+	+	+	+	-	-	+	+	-	-	-	+	+	+
C915	+	+	+	+	+	-	+	+	+	+	-	-	+	+	-	-	-	+	+	+
D915	+	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	-	+	+	+
TEPE	+	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	-	-	+	-
TEPEL	-	+	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	-	+	-
SPM1	-	-	-	+	-	-	+	-	+	-	-	-	+	+	-	-	+	-	+	-
SPM3	-	+	-	-	-	-	+	-	-	-	-	-	+	+	-	-	+	-	+	-
ATCM1_1	-	+	+	-	+	-	+	-	+	-	-	-	+	+	-	-	+	-	+	-

ATCM1_3	+	-	-	-	+	-	+	+	+	-	-	+	+	+	-	-	+	-	+	-
ATCM1_4	-	+	+	-	+	-	+	+	+	+	-	+	+	+	-	-	-	+	+	-
ATCM1_5	-	-	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	-	+	-
ATCM2_1	-	+	+	-	-	-	+	+	-	-	-	-	+	+	-	-	+	-	+	-
ATCM2_2	+	-	+	+	+	-	+	-	+	-	-	+	+	+	-	-	+	-	+	-
ATCM2_3	+	+	-	+	+	-	+	+	+	+	-	-	+	+	-	-	+	-	+	-
LLD1	+	+	-	-	-	-	-	+	+	-	-	+	+	+	-	-	+	-	+	-
LLD2_1	-	-	+	+	+	-	+	+	+	-	-	-	+	+	-	-	+	-	+	-
LLD2_2	-	-	+	+	-	-	+	-	+	-	-	-	+	+	-	-	+	-	+	-
LLD2_3	+	-	-	+	-	-	+	-	+	-	-	-	+	+	-	-	+	-	+	-
LLD2_4	-	-	-	+	-	-	-	+	+	-	-	-	+	+	-	-	+	-	+	-
SDL1	+	-	+	-	-	-	+	+	+	-	-	+	+	+	-	-	+	-	+	-
FTY	-	+	-	+	-	-	+	-	+	-	-		+	+	-	-	+	-	+	-
PEMSOL	-	-	-	-	+	-	-	+	+	-	-	+	+	+	-	-	+	-	+	-
TEPEGREEN	+	-	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	-	+	-
LLD3_1	-	-	+	-	-	-	-	-	+	-	-	-	+	-	-	-	+	-	+	-
LLD3_2	-	-	-	-	-	-	-	-	+	-	-	-	+	+	-	-	+	-	+	-
SVIA1	+	+	+	-	+	-	+	+	+	+	-	+	-	+	-	-	-	+	+	-

SVIA2	+	+	+	-	+	-	+	+	+	+	-	-	-	+	-	-	-	+	+	-
ASS1	+	+	+	-	+	-	+	+	+	+	-	+	-	+	-	-	+	+	+	-
ASS2	-	+	+	+	+	-	+	-	+	+	-	+	-	+	-	-	+	+	+	-
J5	+	+	+	-	+	-	+	+	+	+	-	-	-	+	-	-	+	+	+	-
JBL	+	+	+	+	+	-	+	+	+	+	-	-	-	+	-	-	+	+	+	-
SM6	+	+	+	-	+	-	+	+	+	+	-	-	-	+	-	-	+	+	+	-
LM2	+	+	+	+	+	-	+	+	+	+	-	-	-	+	-	-	+	+	+	-
RS2B	+	+	+	-	+	-	+	+	+	+	-	-	-	+	-	-	-	+	+	-
ASKF	+	+	+	+	+	-	+	+	+	+	-	-	-	+	-	-	+	+	+	-
Clinical strains																				
17/1078	+	+	+	-	+	-	+	-	+	-	-	-	-	+	-	-	-	+	+	-
17/1122	-	-	-	-	-	+	+	-	+	-	-	-	-	+	-	-	+	-	+	-
17/1129	-	-	-	+	+	-	+	ND	+	-	ND	+	-	+	-	-	+	+	+	-
17/1174	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	+	+	+	-
17/1177	-	-	+	-	-	-	+	-	-	-	-	+	-	+	-	-	+	-	+	-
17/1178	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	+	+	+	-
17/1184	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	+	+	+	-
17/1193	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	+	-	+	-
17/1259	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	+	+	+	-
17/1293	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	+	-

Table 9: Comparison of Isolates identification by MALDI-TOF and 16S rRNA sequencing

Strains	Identification with MALDI-TOF	16S identification	MALDI-TOF identity score	16S Identity score in percent
ATCM1_1	*Stenotrophomonas maltophilia*	*Stenotrophomonas maltphophilia*	2.465	99
ATCM1_3	*Stenotrophomonas maltophilia*	*Stenotrophomonas maltphophilia*	2.064	97
ATCM1_4	*Stenotrophomonas acidaminiphila*	*Stenotrophomonas acidamiphilia* and *Stenotrophomonas nitritireducen*	2.111	99
ATCM1_5	*Stenotrophomonas maltophilia*	*Stenotrophomonas maltophilia*	2.093	99
ATCM2_1	*Salmonella sp.*	*Stenotrophomonas maltophilia*	2.39	99
ATCM2_3	*Salmonella sp.*	*Stenotrophomonas maltophilia*	2.488	99
AL	*Not determined*	*Stenotrophomonas maltophilia*		99
ASKF	*Stenotrophomonas maltophilia*	*Stenotrophomonas maltophilia*	2.041	99
ASS1	*Stenotrophomonas maltophilia*	*Stenotrophomonas maltophilia*	2.047	99
ASS2	*Stenotrophomonas maltophilia*	*Stenotrophomonas maltophilia*	2.051	99
B915	*Salmonella sp.*	*Stenotrophomonas maltophilia*	2.497	99

C915	*Pseudomonas aeruginosa*	*Stenotrophomonas maltophilia*	2.175	99
D915	*Not determined*	*Stenotrophomonas maltophilia*	Not determined	99
DRJ	*Aeromonas vernii*	*Stenotrophomonas maltophilia*	2.144	99
E915	*Not determined*	*Stenotrophomonas spp*		98
F915	*Not determined*	*Stenotrophomonas spp*		98
J5	*Stenotrophomonas maltophilia*	*Stenotrophomonas maltophilia*	2.144	99
JBL	*Stenotrophomonas maltophilia*	*Stenotrophomonas maltophilia*	2.252	99
LM2	*Vibrio fluvialis*	*Stenotrophomonas pavanii*	2.2	99
LLD2_1	*Salmonella sp.*	*Stenotrophomonas species*	2.39	99
LLD2_2	*Stenotrophomonas maltophilia*	*S. maltophilia*	2.524	99
LLD2_4	*Stenotrophomonas maltophilia*	*S. maltophilia*	2.348	99
LLD3_1	*Stenotrophomonas maltophilia*	*S. maltophilia*	2.095	99
LLD3_2	*Stenotrophomonas maltophilia*	*S. maltophilia*	2.348	99
PEMSOL	*Stenotrophomonas maltophilia*	*S. maltophilia*	2.425	99

FTY	*Stenotrophomonas maltophilia*	*S. nitritireducen*	1.873	99
FTG	*Stenotrophomonas maltophilia*	*S. maltophilia*	2.102	99
RS2BY	*Stenotrophomonas maltophilia*	*S. maltophilia*	2.006	99
SDL	*Salmonella sp.*	*S. maltophilia*	2.446	99
SM6	*Stenotrophomonas maltophilia*	*S. maltophilia*	2.116	99
SPM1	*Pseudomonas aeruginosa*	*S. maltophilia*	2.273	99
SPM3	*Salmonella sp.*	*S. maltophilia*	2.479	99
SVIA1	*Stenotrophomonas maltophilia*	*S. maltophilia*	2.526	99
TEPE	*Salmonella sp.*	*S. maltophilia*	2.479	99
TEPEgreen	*Salmonella sp.*	*S. maltophilia*	2.509	99
TFLG	*Not determined*	*S. maltophilia*		99
TFG	*Not determined*	*S. maltophilia*		99
TY	*Not determined*	*S. maltophilia*		99
15R	*Not determined*	*S. maltophilia*		99
FTO	*Pseudomonas aeruginosa*	*S. maltophilia*	2.196	99
A915	*Salmonella sp.*	*S. maltophilia*	2.389	99

ATCM2_2	*Stenotrophomonas maltophilia*	*S. maltophilia*	2.475	99
SVIA2	*Stenotrophomonas maltophilia*	*S. maltophilia*	2.444	99
TEPEL	*Stenotrophomonas sp.*	*S. maltophilia*	2.425	99
TFG	*Not determined*	*Stenotrophomonas maltophilia*		99
TY	*Not determined*	*Stenotrophomonas maltophilia*		99
17/1078	*Stenotrophomonas maltophilia*	*Stenotrophomonas maltophilia*		99
17/1122	*Stenotrophomonas maltophilia*	*Stenotrophomonas maltophilia*		99
17/1129	*Stenotrophomonas maltophilia*	*Stenotrophomonas maltophilia*		99
17/1174	*Stenotrophomonas maltophilia*	*Stenotrophomonas maltophilia*		99
17/1177	*Stenotrophomonas maltophilia*	*Stenotrophomonas maltophilia*		99
17/1178	*Stenotrophomonas maltophilia*	*Stenotrophomonas maltophilia*		99
17/1184	*Stenotrophomonas maltophilia*	*Stenotrophomonas maltophilia*		99
17/1193	*Stenotrophomonas*	*Stenotrophomonas maltophilia*		99

	maltophilia			
17/1259	*Stenotrophomonas maltophilia*	*Stenotrophomonas maltophilia*		99
17/1293	*Stenotrophomonas maltophilia*	*Stenotrophomonas maltophilia*		99

3.3 Antimicrobial Susceptibility testing in Stenotrophomonas species

Intrinsic resistance is a common characteristic of the genus Stenotrophomonas; the ability of the isolated strains to resist or become susceptible to antibiotics was evaluated using the Kirk Bauer Disc diffusion method and the MIC based antibiotic resistance profiling. Isolates showed complete resistance to the entire beta-lactamase antibiotic tested except Ceftazidime to which isolates were less resistant. High rate of resistance was also noted to Sulfamethoxazole-Trimethoprim (Table 10 and 11).

Table 10: Antibiotic susceptibility pattern result for Isolates

Isolates/antibiotics	SXT	DOXYCYCLINE	CHLORAMPHENICOL	IMIPENEM	CEFTRIAXONE	AMPICILLIN	LEVOFLOXACINE	OFLOXACIN	CEFTAZIDIME
A915	R	R	R	R	R	R	S	S	S
ATCM1_1	R	R	R	R	R	R	S	S	I
ATCM1_3	R	R	R	R	R	R	S	S	S
ATCM1_4	R	R	R	R	S	R	S	S	I
ATCM1_5	R	R	R	R	R	R	S	S	I
ATCM2_1	R	R	R	R	R	R	S	S	R
ATCM2_2	S	R	R	R	R	R	S	S	R
ATCM2_3	R	R	R	R	R	R	S	S	R
ASKF	S	R	S	R	S	R	S	S	S
ASS1	R	R	S	R	R	R	S	S	R
ASS2	R	R	R	R	R	R	S	S	R
B915	S	R	S	R	S	R	S	S	S
C915	S	R	R	R	S	R	S	S	S
D915	R	R	R	R	R	R	S	S	S
DRJ	R	R	R	R	S	R	S	S	S

J5	R	R	S	R	R	R	S	S	S
JBL	R	R	R	R	R	R	S	S	S
LM2	S	R	S	R	S	R	S	S	S
LLD2_1	R	R	S	R	R	R	S	S	R
LLD2_2	R	R	S	R	S	R	S	S	R
LLD2_4	R	R	R	R	S	R	S	S	R
LLD3_1	S	R	S	R	R	R	S	S	R
LLD3_2	R	R	R	R	R	R	S	S	S
PEMSOL	R	R	S	R	R	R	S	S	S
FTY	R	R	S	R	R	R	S	S	I
RS2BY	S	R	S	R	R	R	S	S	S
SDL	ND	R	ND	R	ND	R	S	S	S
SM6	R	R	R	R	I	R	S	S	S
SPM1	R	R	S	R	R	R	S	S	S
SPM3	R	R	R	R	R	R	S	S	S
SVIA1	R	R	S	R	S	R	S	S	S
SVIA2	R	R	R	R	R	R	S	S	I
TEPE G	R	R	S	R	R	R	S	S	S
TEPEY	R	R	R	R	R	R	S	S	S
TEPEL	R	R	S	R	R	R	S	S	S
15R	R	R	S	R	R	R	S	S	S

Key: S= Sensitive

Table 11: Antimicrobial Susceptibility of Stenotrophomonas species. Values corresponding to 100% is from 46 isolates.

Antimicrobial agents	MIC (µg/ml)			Percentages (n)		
	Range	MIC50	MIC90	Susceptible	Intermediate	Resistant
Trimethoprim-sulfamethoxazole	0.125-4	>4	>4	19.6 (9)	0.0 (0)	80.4 (37)
Doxycycline	≤2- >64	>64	>64	0.0 (0)	0.0 (0)	100.0 (46)
Chloramphenicol	≤4 - >64	>16	>32	45.7 (21)	0.0 (0)	54.4 (25)
Imipenem	≤4 ->64	>64	>64	0.0 (0)	0.0 (0)	100.0 (46)
Ceftriaxone	≤8- >16	>64	>64	26.1 (12)	2.2 (1)	71.7 (33)
Ampicillin	≤4- >16	>16	>16	0.0 (0)	0.0 (0)	100.0 (46)
Levofloxacin	≤1- >8	1	2	100.0 (46)	0.0 (0)	0.0 (0)
Ofloxacin	≤1- >8	0.87	2	100.0 (0)	0.0 (0)	0.0 (0)
Ceftazidime	≤4- >16	4	>16	63.0 (29)	11.0 (5)	26.0 (12)

3.4 Phylogeny and Genotyping of Stenotrophomonas species

The Phylogenetic analysis of the isolates revealed that they are member of the genus *Stenotrophomonas* as they cluster closely with bacteria in this genus. The heterogenous nature of the genus was noted in the bacteria isolated as some of them form a different cluster on the phylogenetic tree (Figure 4.) genotyping techniques involving the use of ERIC PCR further confirmed the heterogenous nature of the genus. (Figure 5). ERIC-PCR successfully typed all isolates with a few exceptions (n= nine hospital isolates n=4 soil isolates).

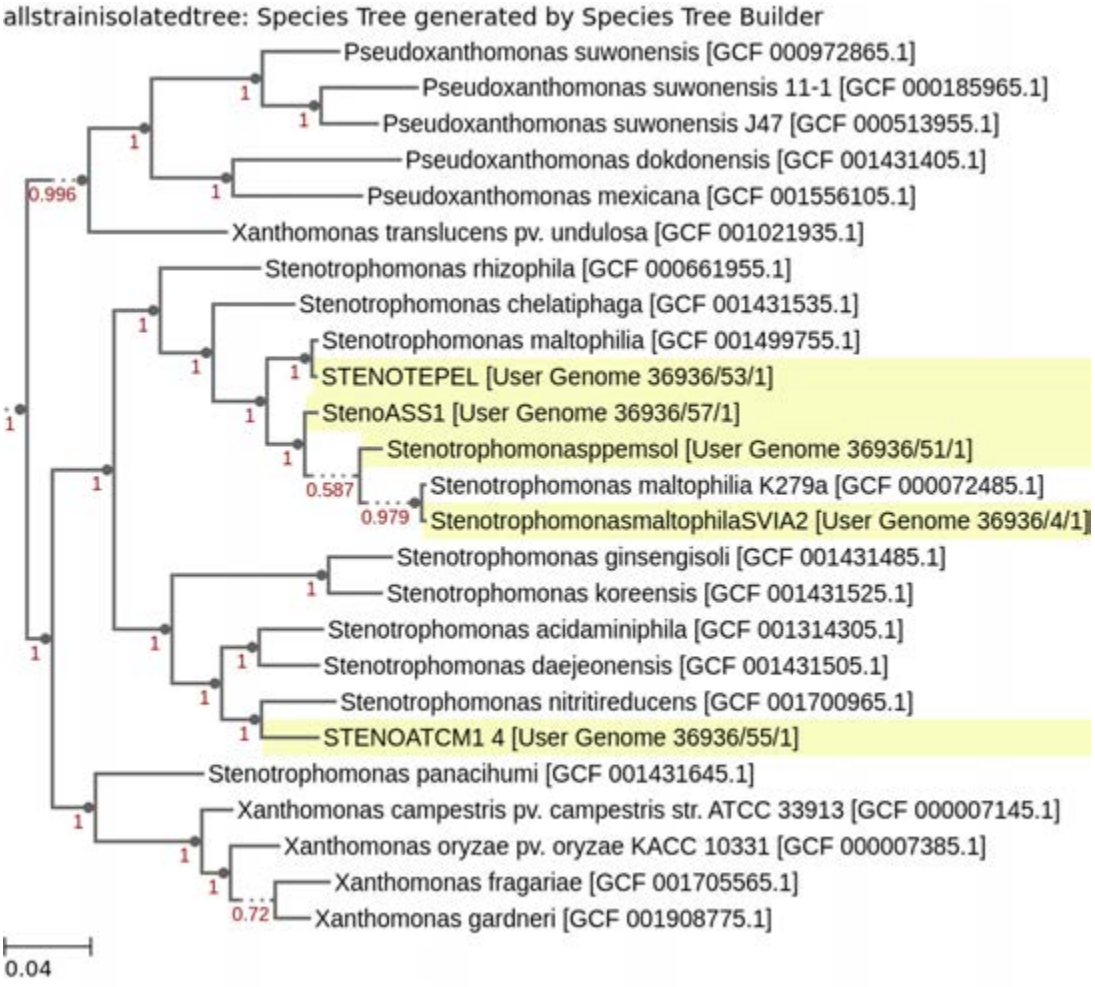


Figure 4: Phylogeny showing the evolutionary history of isolated *Stenotrophomonas* species, which was inferred using the Neighbor-Joining method with bootstrap consensus tree inferred from 1000 replicates. Strains in yellow color are those that have been previously reported here in Mexico, while the isolated strains are in red color.

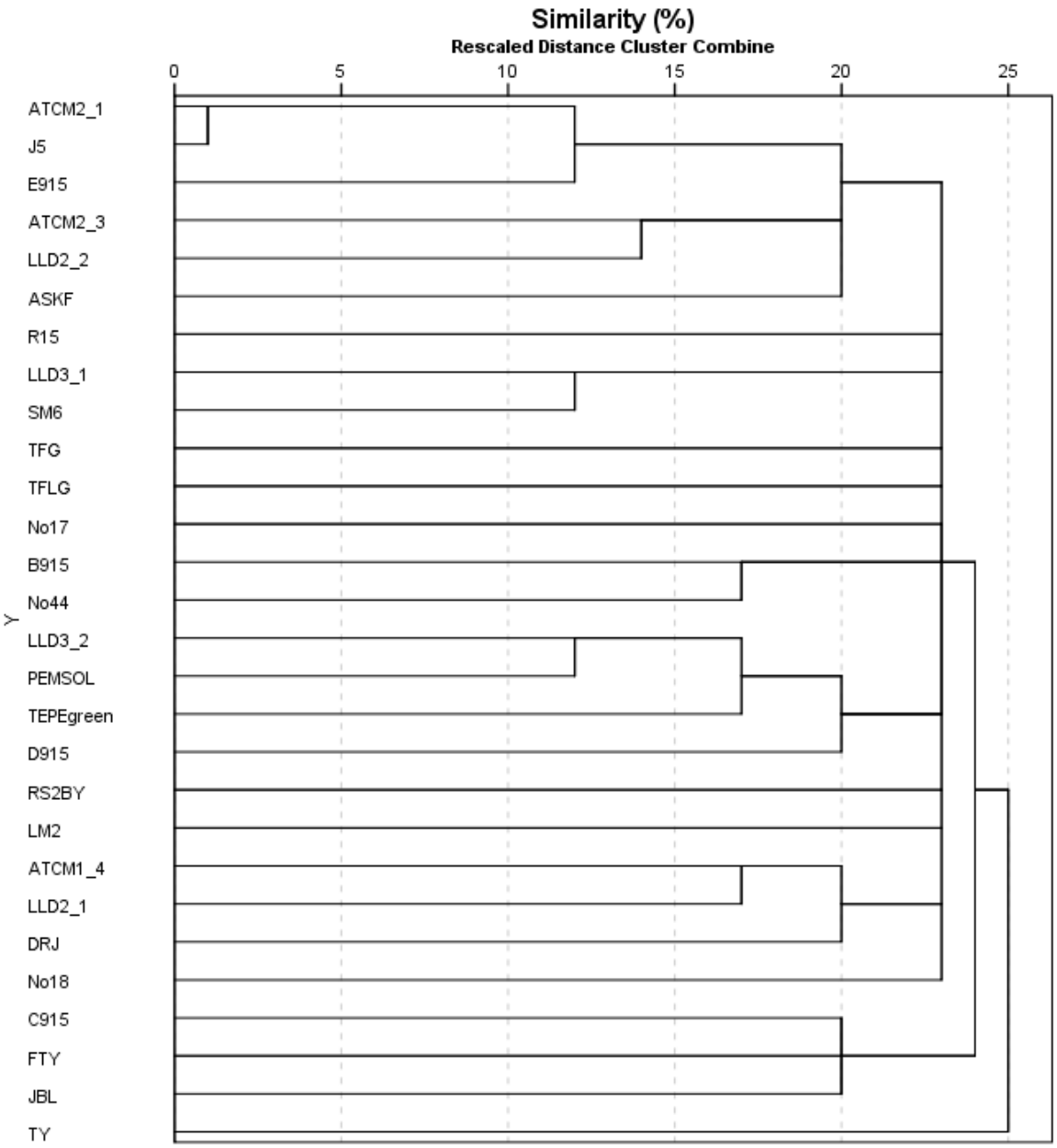


Figure 5: Dendrogram for the ERIC genotyping of Isolated *Stenotrophomonas* species

3.5 Biofilm formation and resistance in Stenotrophomonas isolates

Biofilm formation is one of the mechanisms used by *Stenotrophomonas* species to evade the activities of antimicrobial agents. They also employ this mechanism for the degradation of many xenobiotics. We evaluated the potentials of the isolated *Stenotrophomonas* species for the formation of biofilm. It was observed that majority of the isolates have strong tendencies to form Biofilms (Figure 6).

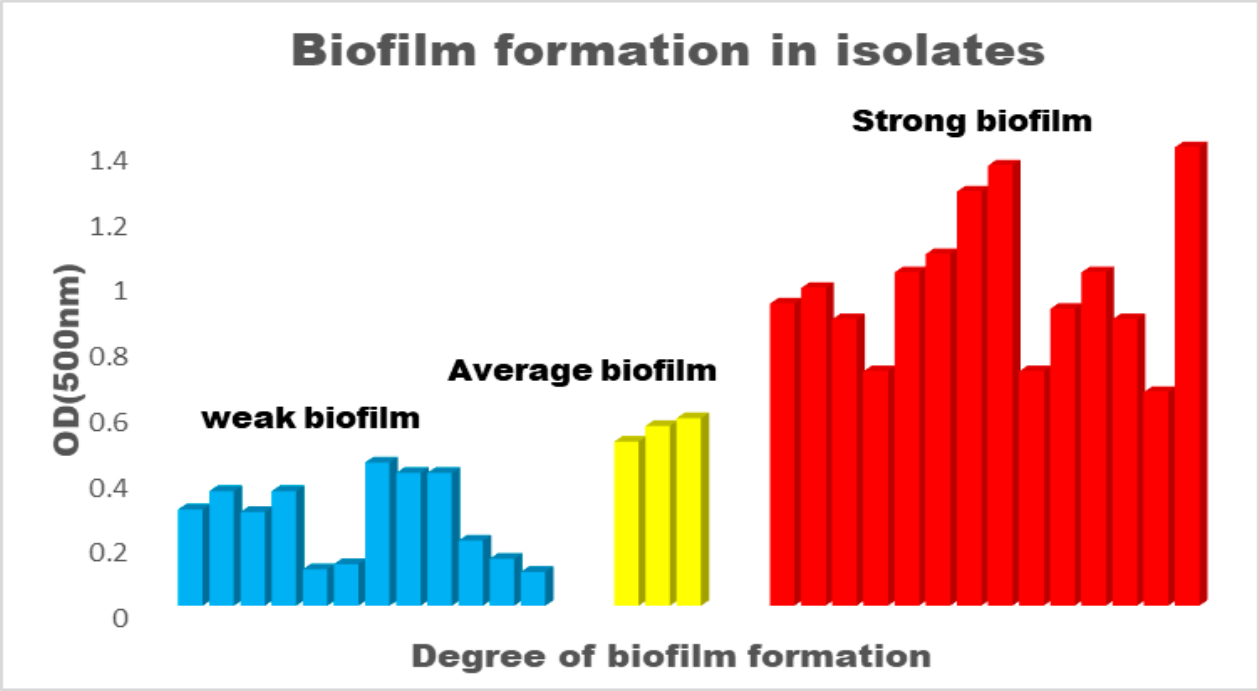


Figure 6: Biofilm formation distribution in Isolates

3.6 Textile Dye decolorization Assay

Two Isolates recovered from textile effluent showed efficient potential for the decolorization of Textile dye in a preliminary assay which utilized four type of dyes (Reactive Black, Brilliant blue, Reactive blue and brilliant yellow).

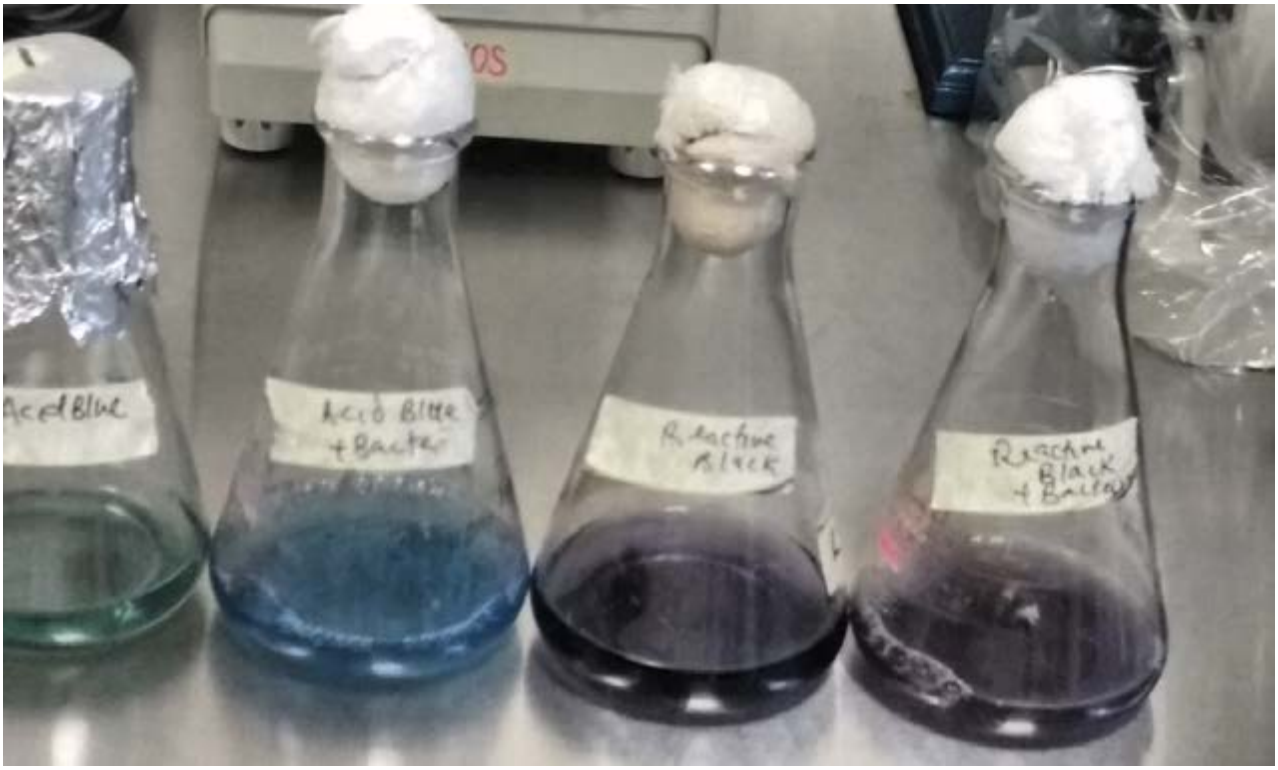


Figure 7: Representative Figure for *Stenotrophomonas* TePeL and TepeS decolorization of azo dyes

3.7 Hydrocarbon tolerance and degradation studies

Stenotrophomonas have been described as versatile bacteria with the ability to use wide range of substance for growth (Ryan et al., 2009). Some of the *Stenotrophomonas* (ASS1, ASS2, SVIA1, SVIA2 and Pemsol) isolates in this study were recovered from oil-contaminated soils. The ability of these strains to grow and survive in oil-contaminated sites was evaluated in a tolerance test varying the concentration of seven different PAHs (anthracene, anthraquinone, biphenyl, naphthalene, phenanthridine, phenanthrene and xylene) in a Bush Nell Hass medium as the only source of carbon. A final concentration of 5mg/ml and 1mg/ml was used to test bacterial growth and survival in PAHs in a growth assay. The survival assay showed that the isolate successfully grew at 1mg/ml of five of the tested PAHs and 0.4mg/ml of anthracene while no growth was found in the medium containing xylene (Figure 8 and 9). Colony count and optical density determination were employed for evaluating *Stenotrophomonas* survival in PAHs (Figure 9)

Isolates' ability to degrade PAH was evaluated in naphthalene degradation assay, which lasted for 30 days. The degradation potential was determined using three different methods which are the analysis of degradation metabolites with Fourier Transform Infra-red FT-IR) spectrometer,

Gas-Column chromatography analysis mass spectrophotometry and Ultra Power liquid chromatography mass spectrophotometry. The metabolites formed from the degradation of naphthalene were analyzed on the 15th day and the 30th day respectively. Figure 11a and 11c showed the introduction of new peaks to the naphthalene peaks on the FTIR spectra of the product formed from the growth of Pemsol in naphthalene containing minimal medium in contrast to the observation with the peaks in 11b. In addition, the UPLC MS spectra in figure 13 revealed the formation of a new compound with molecular mass which corresponds to 109.91 likely to be associated with the formation of catechol after 15th days of degradation assay. This contrast with the peak observed in the control whose molecular mass corresponds to 129 likely to be the naphthalene added to the medium. Pemsol's ability to degrade naphthalene was confirmed by the disappearance of naphthalene peak in figure 14 (experiment assay) contrary to figure 15 (control) in which it persists after 30 days of degradation assay.

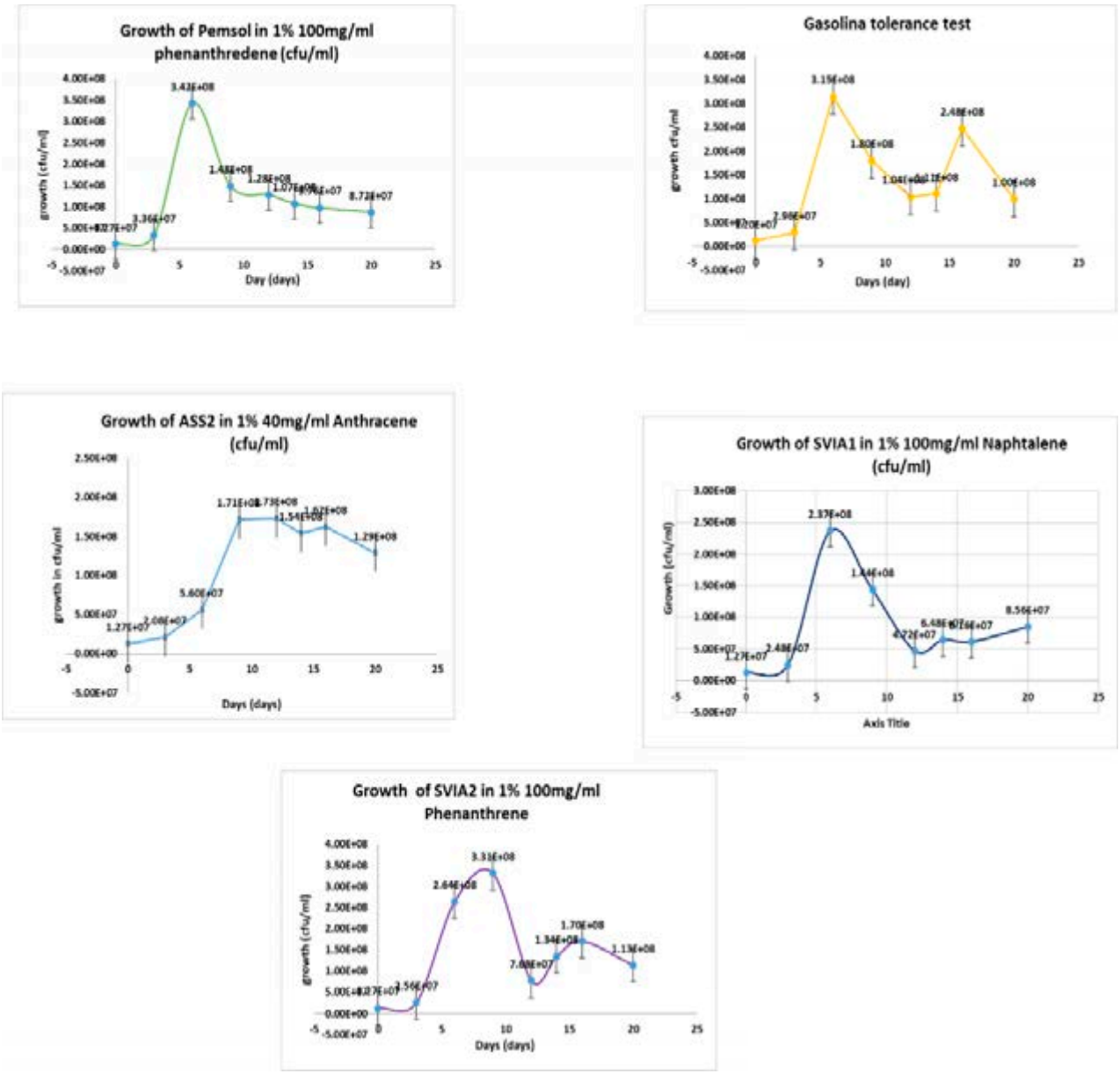


Figure 8: The growth of the five Stenotrophomonas species recovered from PAH contaminated soil in different PAHs at a concentration of 1mg/mL and 5mg/mL hydrocarbons

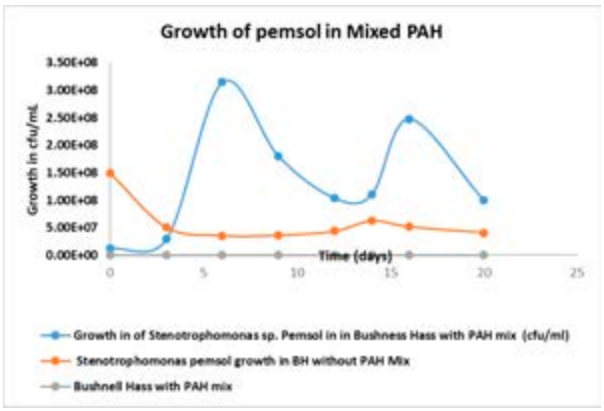
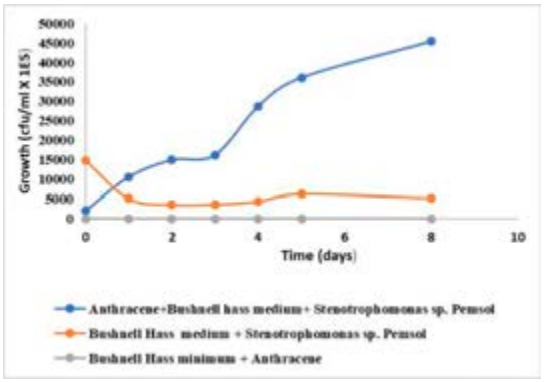
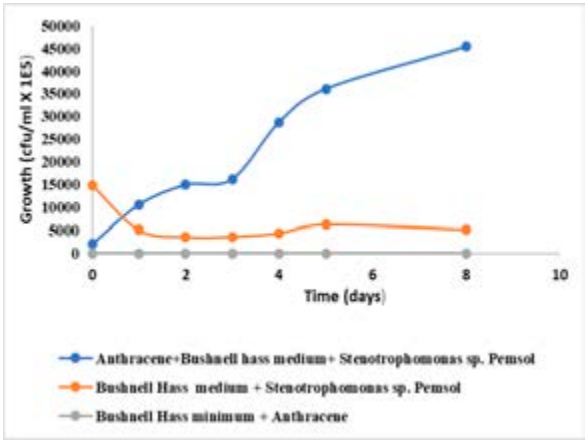
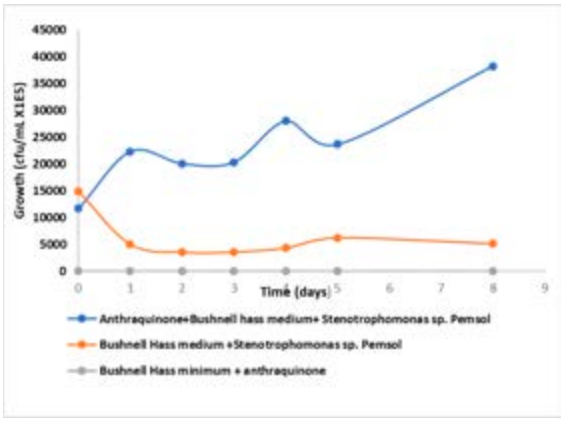
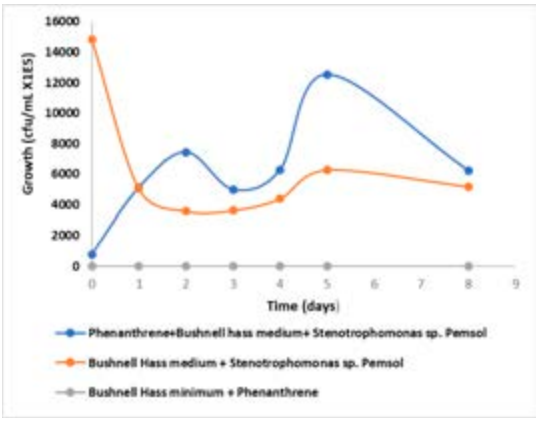


Figure 9: The growth of Stenotrophomonas sp. Pemsol in Mixed PAH at a conc of 1mg/L









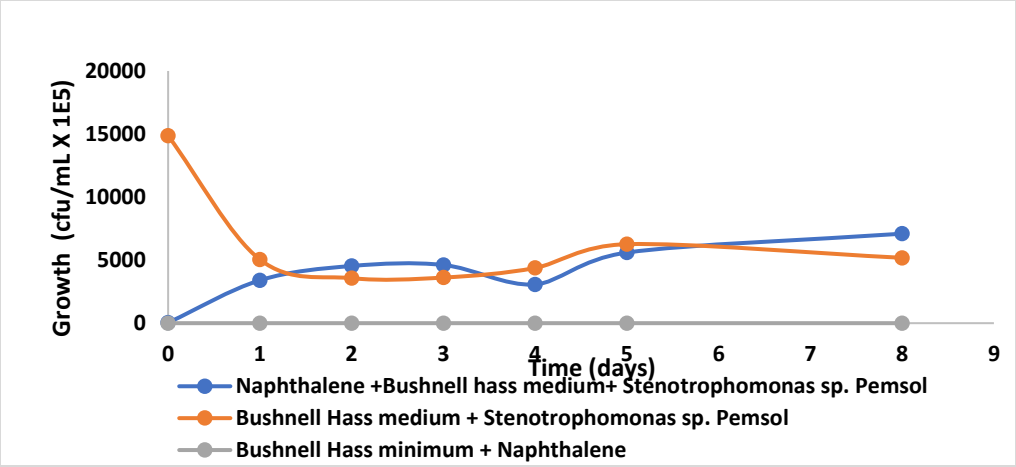


Figure 10: The growth of *Stenotrophomonas* sp. Pemsol in different PAH at 1mg/L

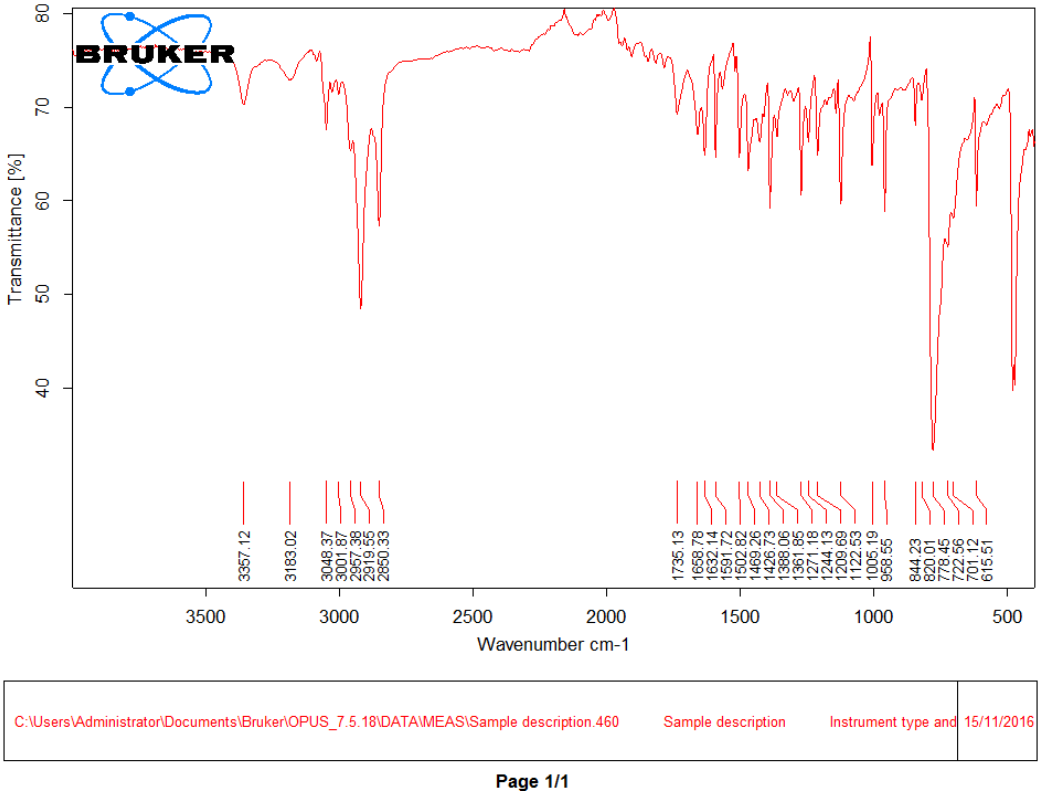


Figure 11a FTIR spectrum for the Metabolite formed from the degradation of Naphthalene by *Stenotrophomonas* sp. Pemsol after 15 days of cultivation

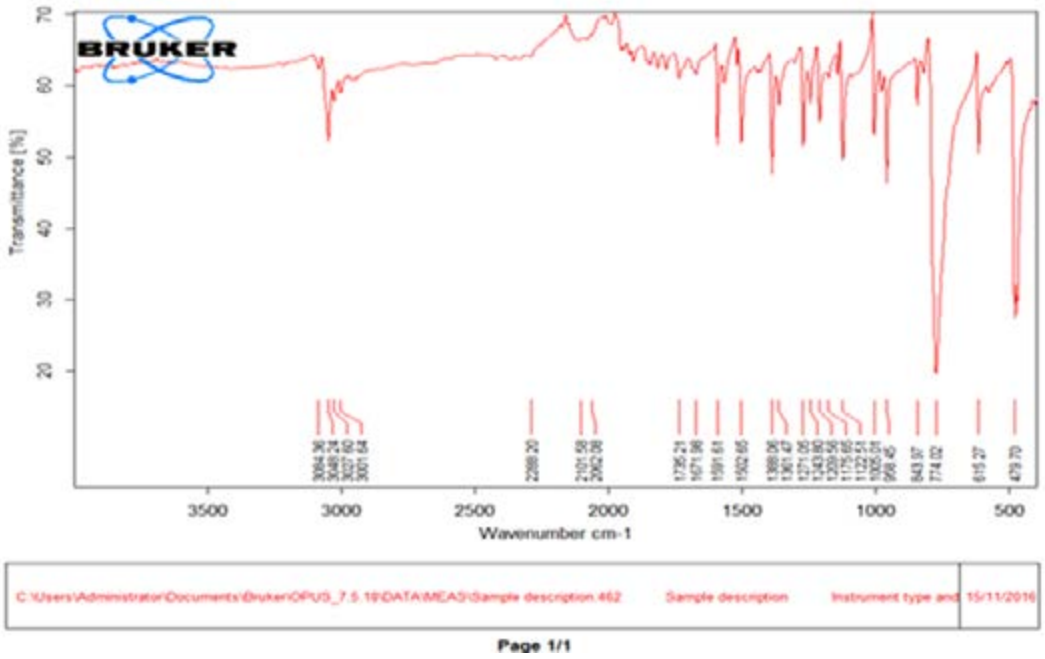


Figure 11b: FTIR spectrum for the Metabolite of Naphthalene after 30days of experiment

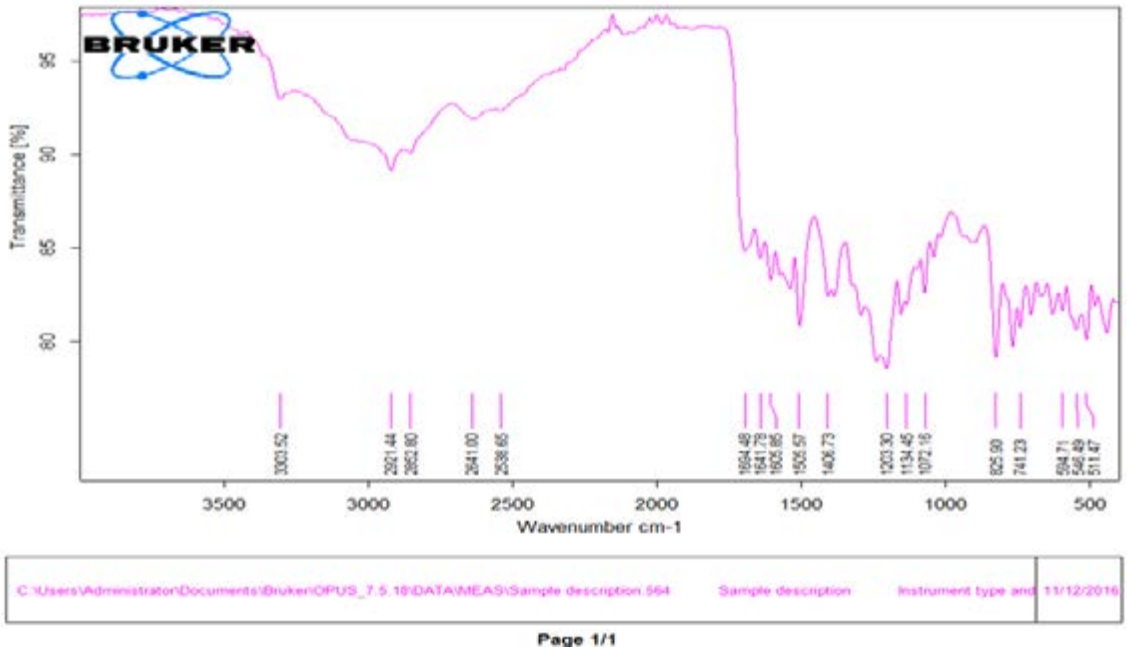


Figure 11c: FTIR spectrum for the Metabolite formed from the degradation of Naphthalene by Stenotrophomonas sp. Pemsol after 30 days of cultivation

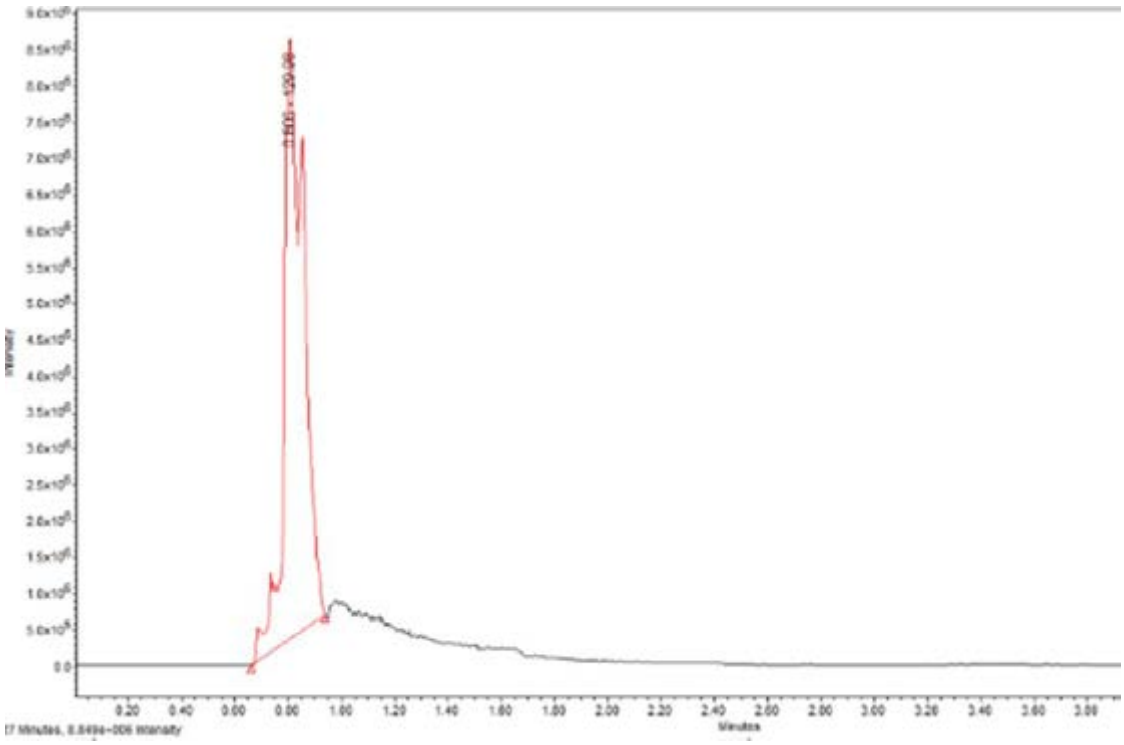


Figure 12: UPLC-MS Spectrum for Naphthalene in the control experiment after 30 days of experimental study on *Stenotrophomonas* species Pemsol's degradation of Naphthalene.

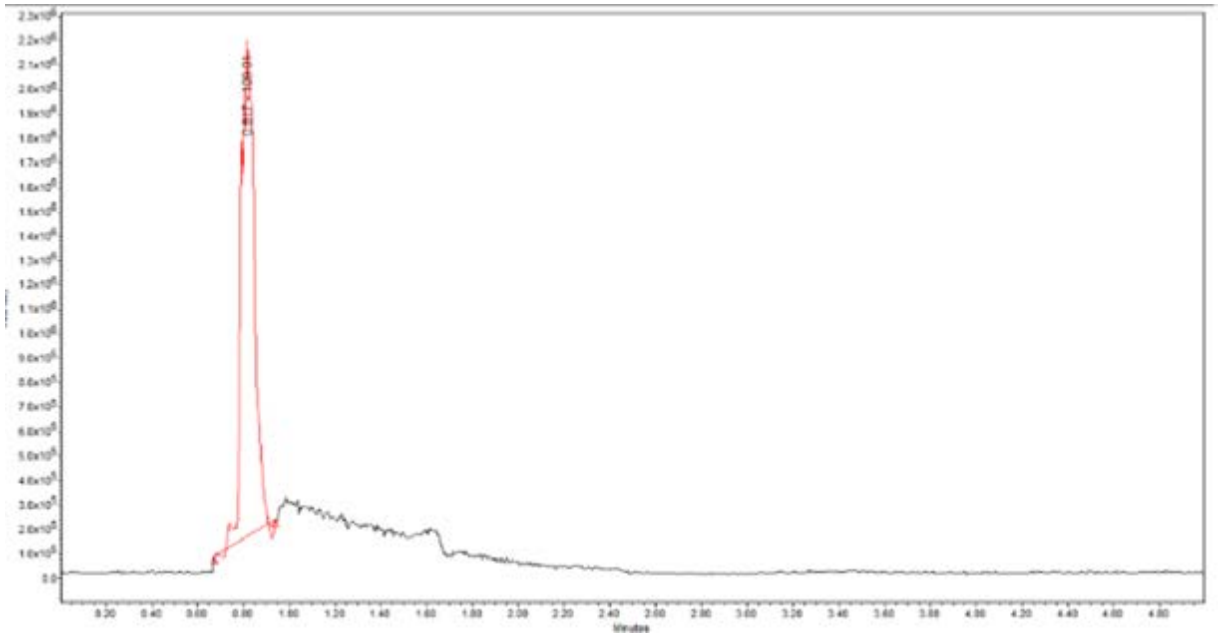


Figure 13: UPLC MS/MS Spectrum for the metabolite formed from the degradation of Naphthalene by *Stenotrophomonas* sp. Pemsol

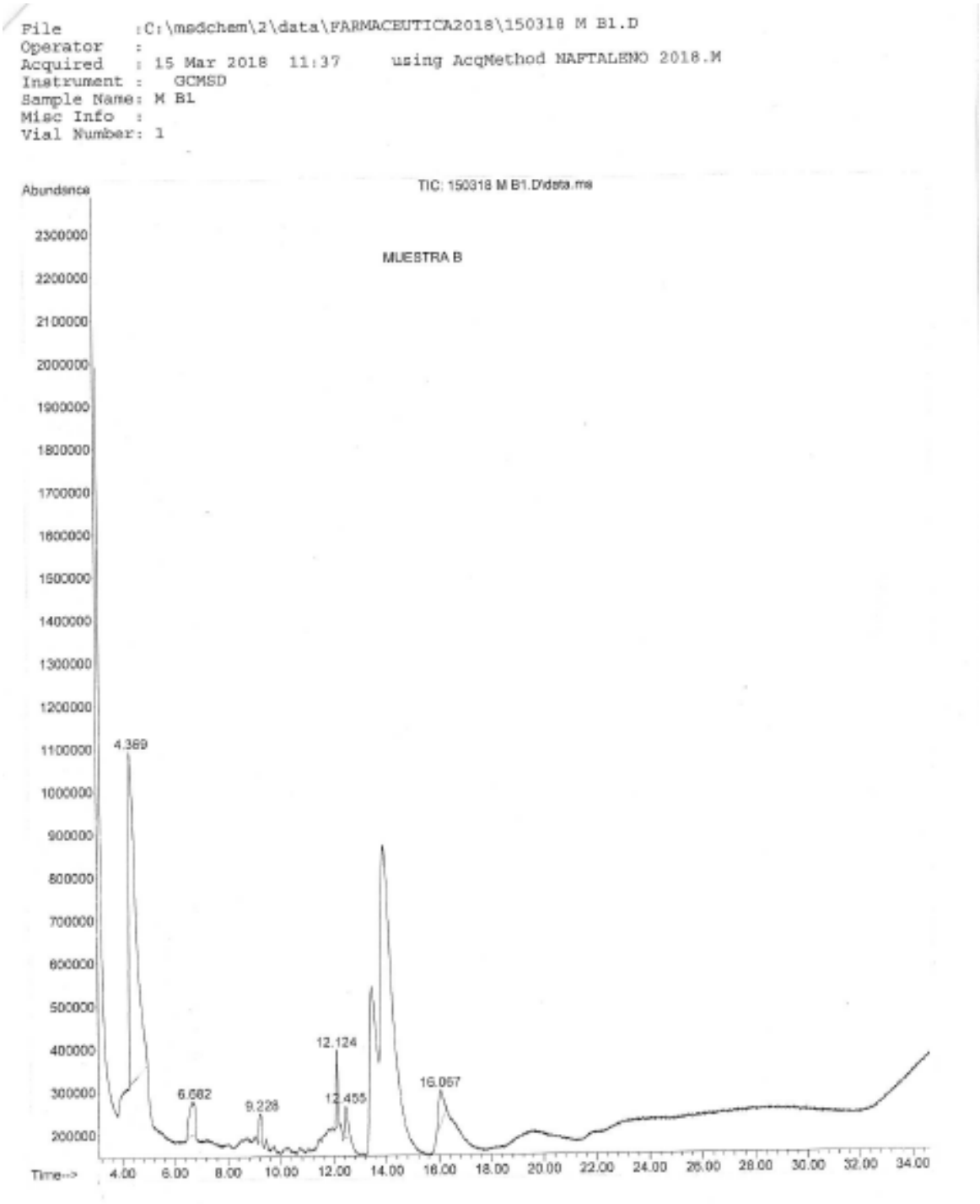


Figure 14: GC-MS spectrum for the metabolites formed by the degradation of Naphthalene by *Stenotrophomonas* sp. Pemsol

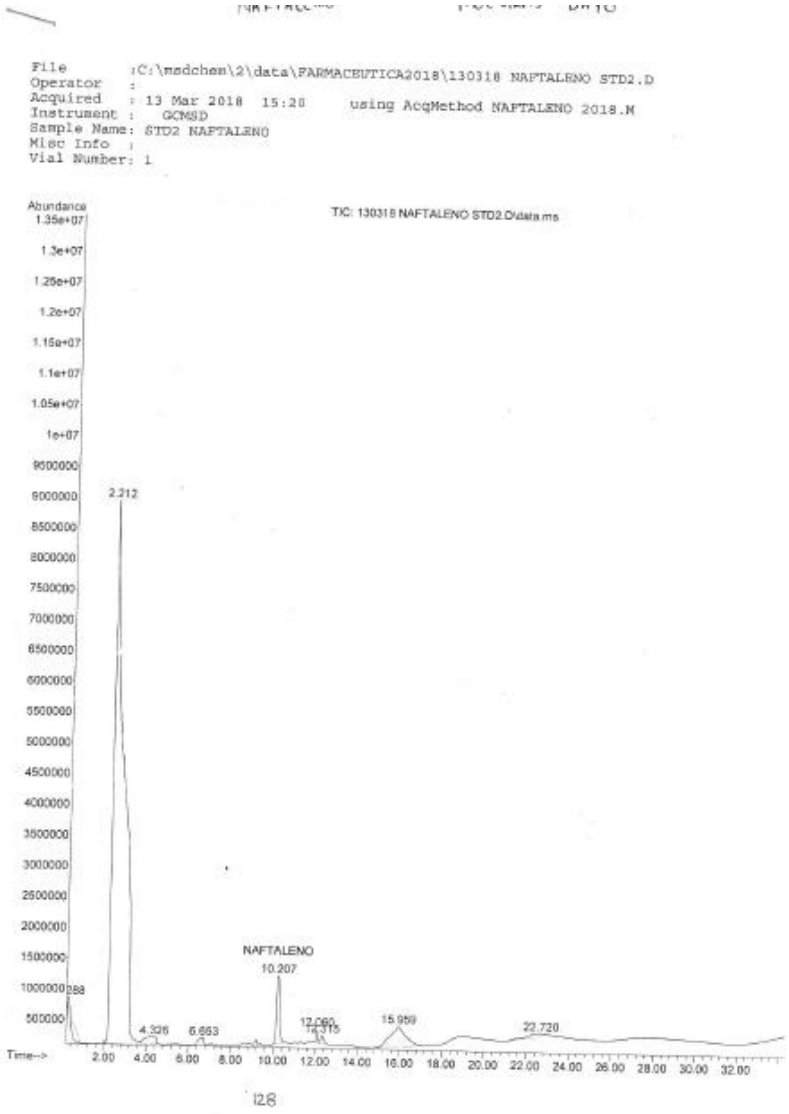


Figure 15: GC-MS spectrum for the of the Naphthalene

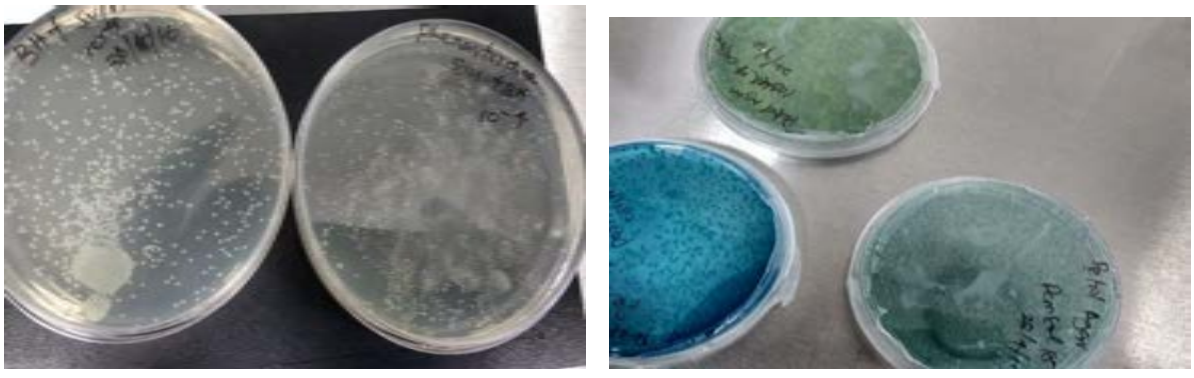


Figure 16. Growth of isolates on BH agar supplemented with PAH

3.8 Biosurfactant and Emulsion formation

One of the mechanisms often employed by hydrocarbon degrading bacteria is the production of bio surfactants, which can dislodge trapped oil molecule in water surfaces for their use. We evaluated the capabilities of the isolated strains to produce biosurfactant via emulsion formation since emulsion has been described as one of the mechanisms through which we can recognize a biosurfactant producing bacteria, only four (SVIA1, SVIA2, ASS1 and ASS2) out of the five bacteria recovered from crude oil contaminated sites have the capability to form bioemulsion (Figure 10). ASS1 displayed the highest prowess for the formation of biofilm; however, SVIA2 displayed the highest emulsion retention capacity (Figure 11). Pemsol could not form bioemulsion and as a result is not in the figure below

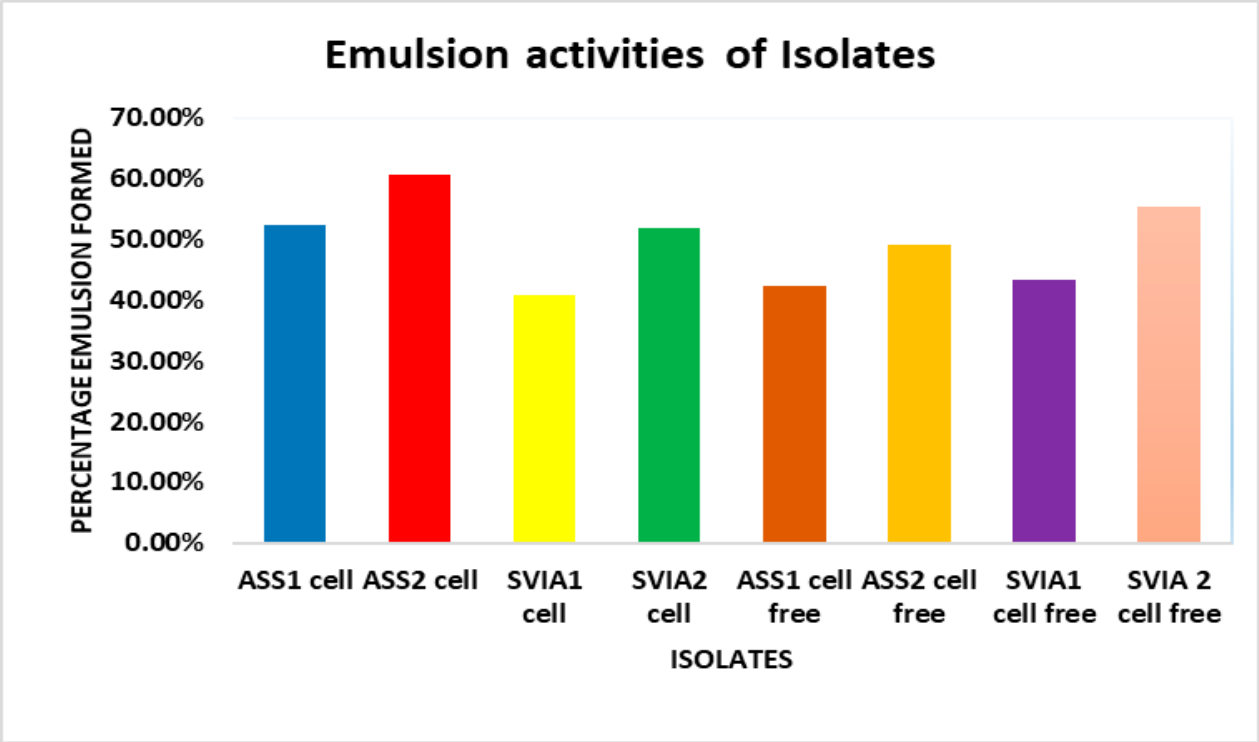


Figure 17: Bio-emulsification of PAH by some of the PAH tolerant strains

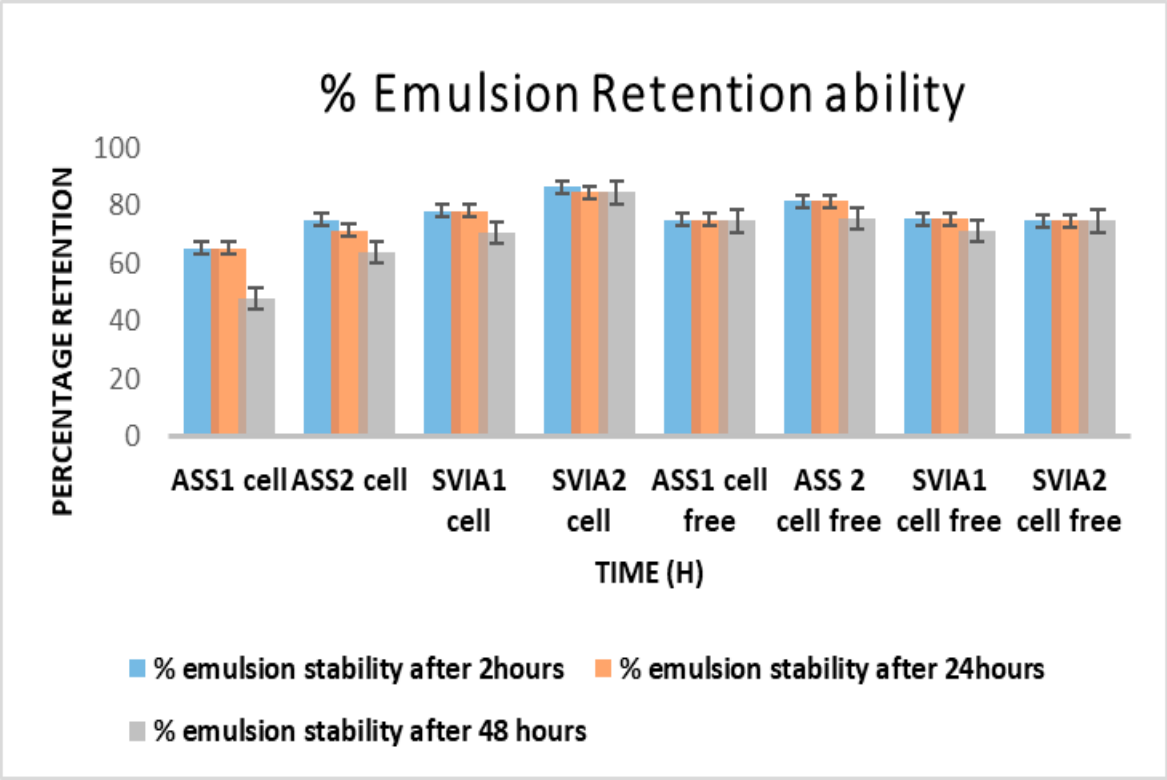


Figure 18: PAH retention capacity in PAH tolerant strains

3.9 Complete Genome Sequence and Analysis

Six isolates (Figure 19) were selected for complete genome sequence because they possess certain metabolic features that could be best elucidated by genome analysis. The strains include ASS1, Pemsol and SVIA2, which were isolated from crude oil wells or crude oil contaminated soil. These strains displayed possible potentials for the degradation of hydrocarbons and PAHs. Others include ATCM1_4, which could be a new species based on 16S rRNA gene sequence analysis, and TepeL, which showed good dye decolorizing potential. Strain B915 was isolated as a plaque forming unit in a coculture assay with *Salmonella*. The general properties of the sequenced genomes are shown in Table 12.

3.9.1 Sequencing and Assembling

Illumina miseq next generation sequencing platform was employed for the sequencing of the genomes. The reads obtained were trimmed with Trim-Galore. The trimmed reads were assembled into contigs with Velvet, Spades and A5 miseq assembling pipeline. Spades yeilded

the best contig size and length when the three sequencing platform results were compared on QUAST. The assembled reads were annotated using Prokka, RAST annotation server and the genomes submitted to NCBI were reanotated with PGAP and the summary of the annotation results are shown in Table 12. The circular genome maps are shown in Figure 19

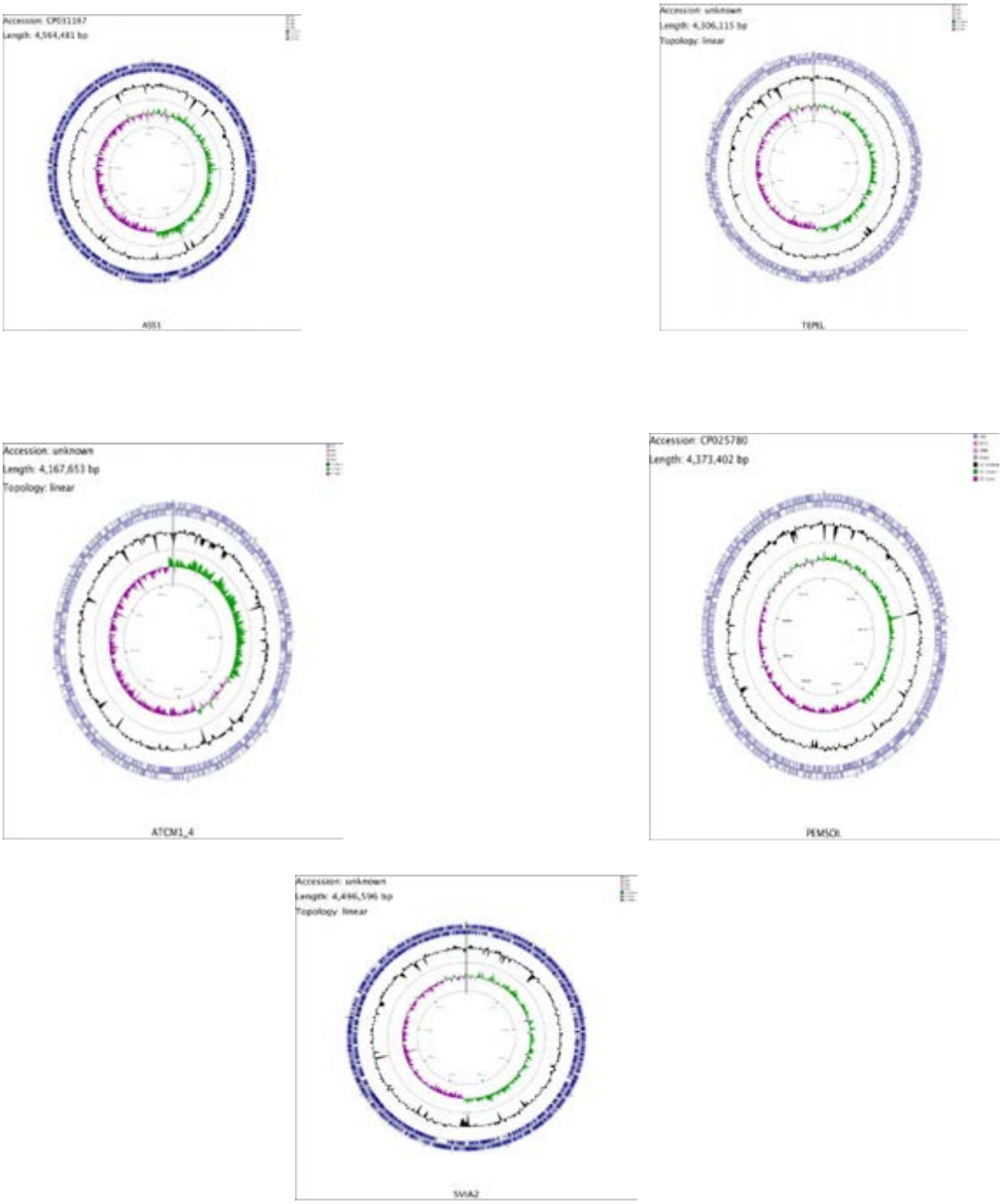


Figure 19 Circular genome maps of the sequenced *Stenotrophomonas* species generated using the CG circular DNA plot. The image gives the GC Skew of the Sequenced genome

Table 12: Genome Characteristics of Sequenced Strains

Name of organism	Phenotype	Bases	CDS	rRNA	tRNA	tmRNA	Number of contigs
SVIA2	Biosurfactant production and hydrocarbon degradation	4497327	4028	5	77	1	1
ASS1	Bio emulsification and degradation of Polycyclic Aromatic hydrocarbon	4613196	4108	7	63	1	1
TEPEL	Decolorization of Textile dyes	4564481	3811	3	66	1	5
PEMSOL	Biodegradation of Polycyclic Aromatic hydrocarbon	4851126	3905	3	69	1	1
ATCM1_4	Novel physiological characteristics; grew as yellow colony on plate	4048492	3681	5	58	1	8

3.9.2 Orthologs gene cluster analysis

Analysis of bacterial orthologs can give insight into how the bacteria evolved or provide information about the functions of the bacteria or genes that it possesses. We analyzed the orthologous gene clusters present in the sequenced strains by using both the Orthovenn web server (Figure 19) and WebMGA (Figure 21), which identified the COG categories present in each strain. OrthoVenn gave a comparison analysis of all the sequenced genomes and *S. maltophilia* K279a as a reference. OrthoVenn analysis showed that all the isolates and the reference shared 2435 clusters of orthologous genes. It also revealed that strain ATCM1_4 may be totally different from the rest as it has the highest number (31) of singletons among all the isolates (Figure 20).

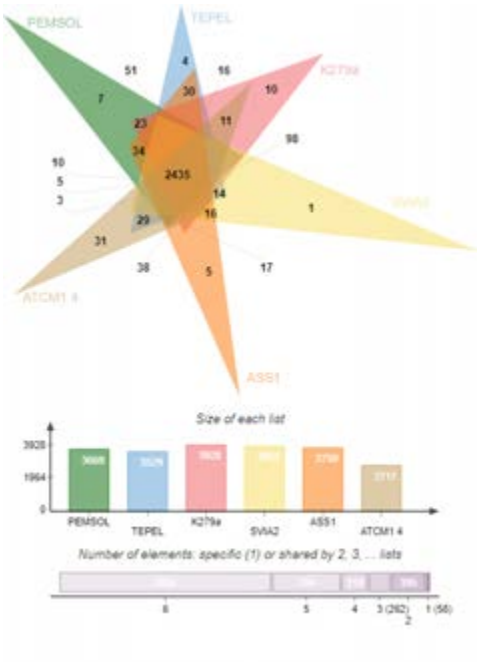


Figure 20. Analysis of the orthologous Clusters among Isolated strains and *Stenotrophomonas maltophilia* K279a. The figure contains information about the clusters of gene orthologs shared by the isolated strains and *S. maltophilia*

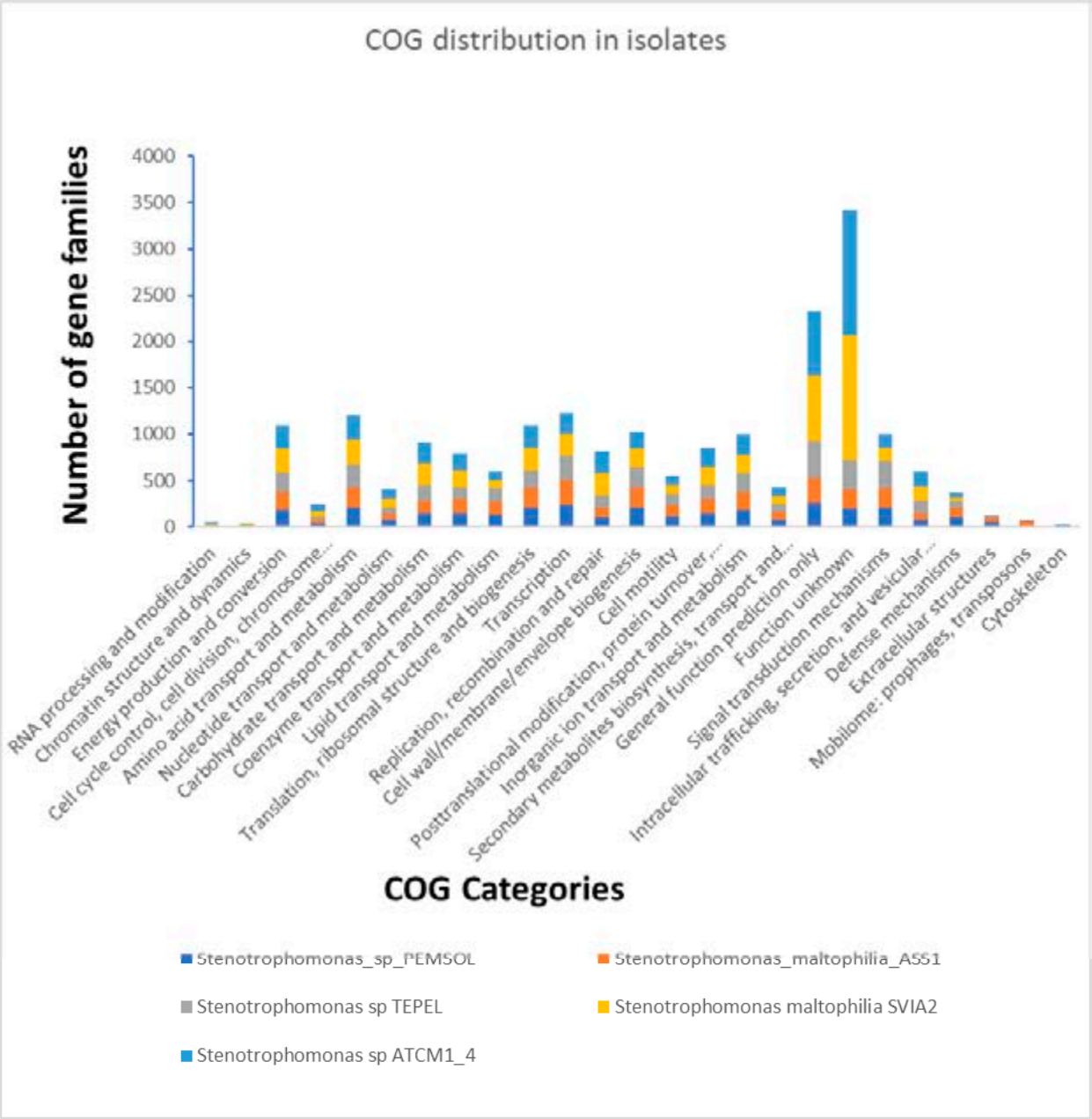
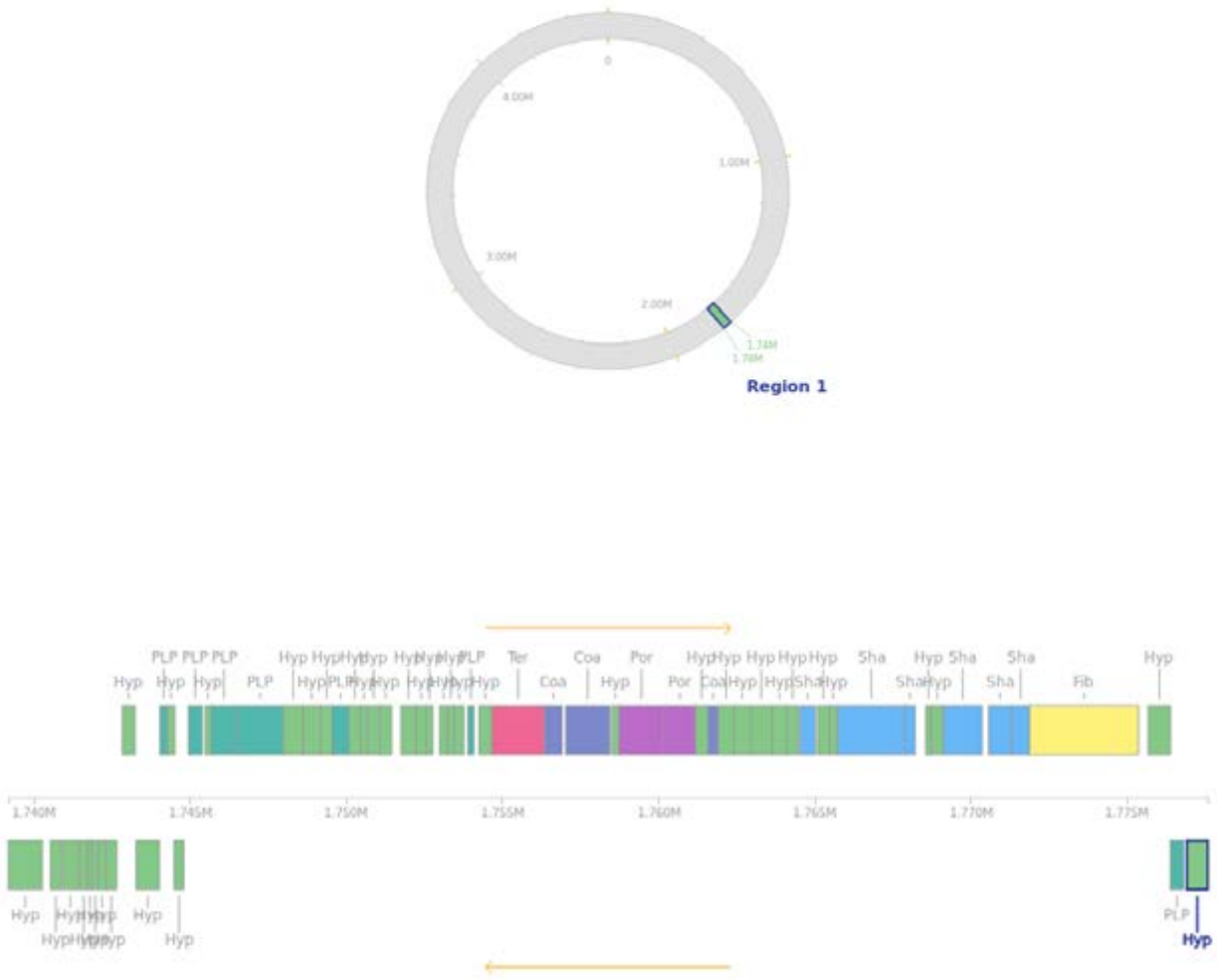


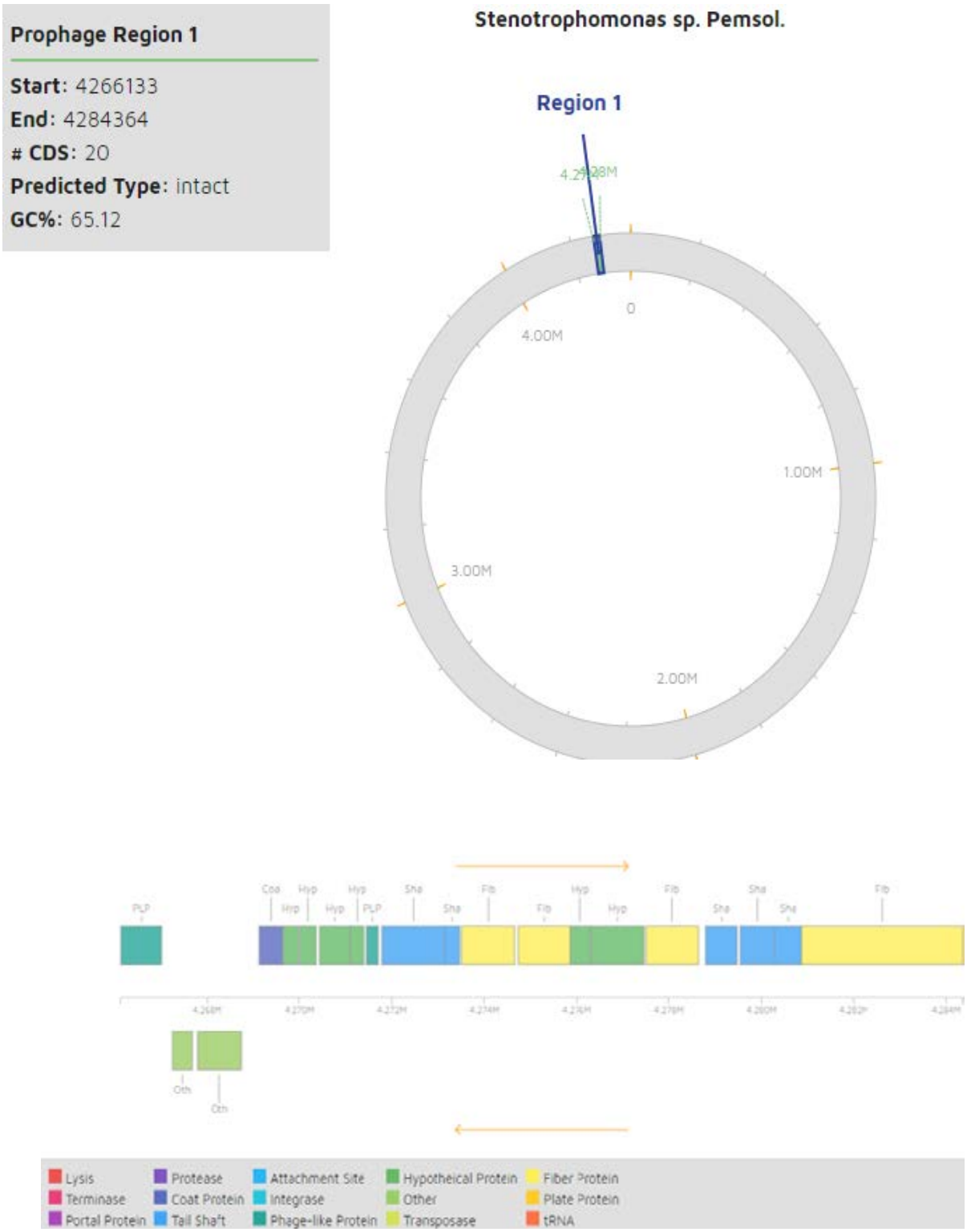
Figure 21 COG comparison among the sequenced genomes generated using orthoMCL. The chart showed the COG categories distribution in each isolate.

3.9.3 Prediction of Prophages in Isolates

Prophages are important constituent of bacterial genomes. Prophages sometimes confer adaptive characteristics on their host. All the genomes sequenced in this study possess prophages with four as the maximum number found in one isolate (ATCM1_4). Strain ASS1 possesses two prophage regions in its genome, SVIA2 has only one prophage, TEPEL has one prophage region in its genome and Pemsol has one prophage (Figure 22a-e)

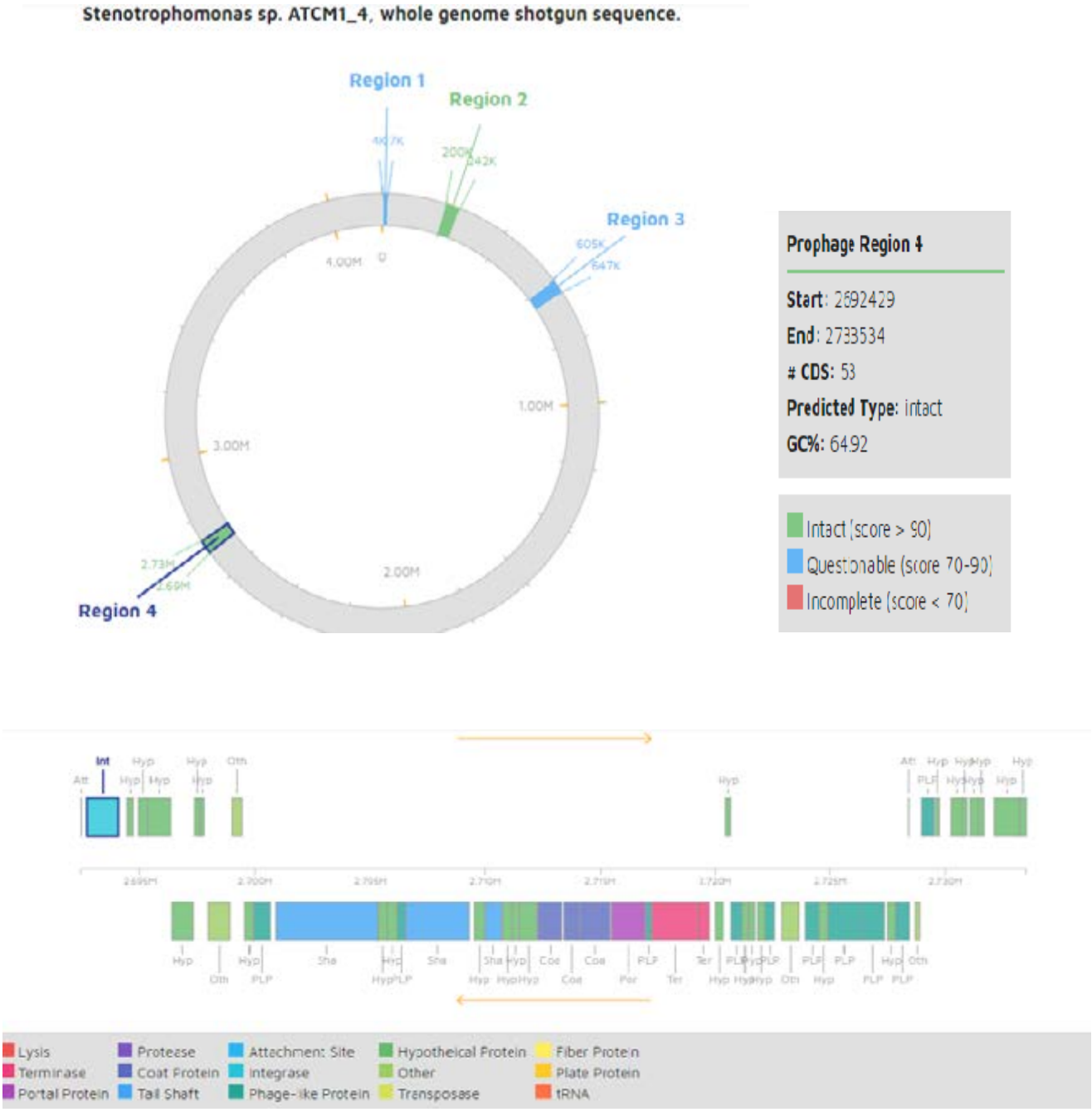


A) ASS1 prophage region

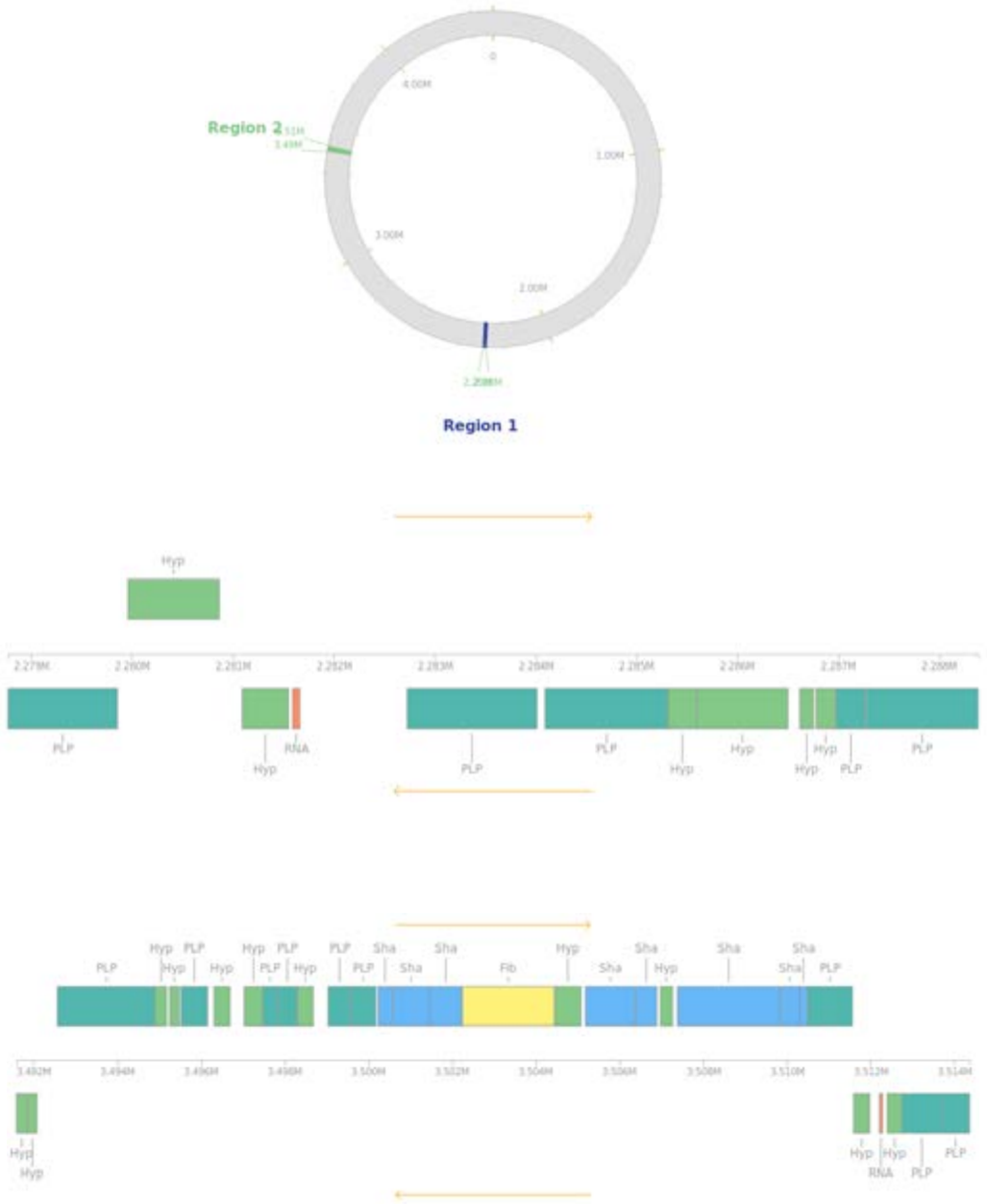


B) *Stenotrophomonas* sp. Pemsol Phage region

C) Prophage region in *Stenotrophomonas* sp. TepeL



D) Prophage region in *Stenotrophomonas* sp. ATCM1_4

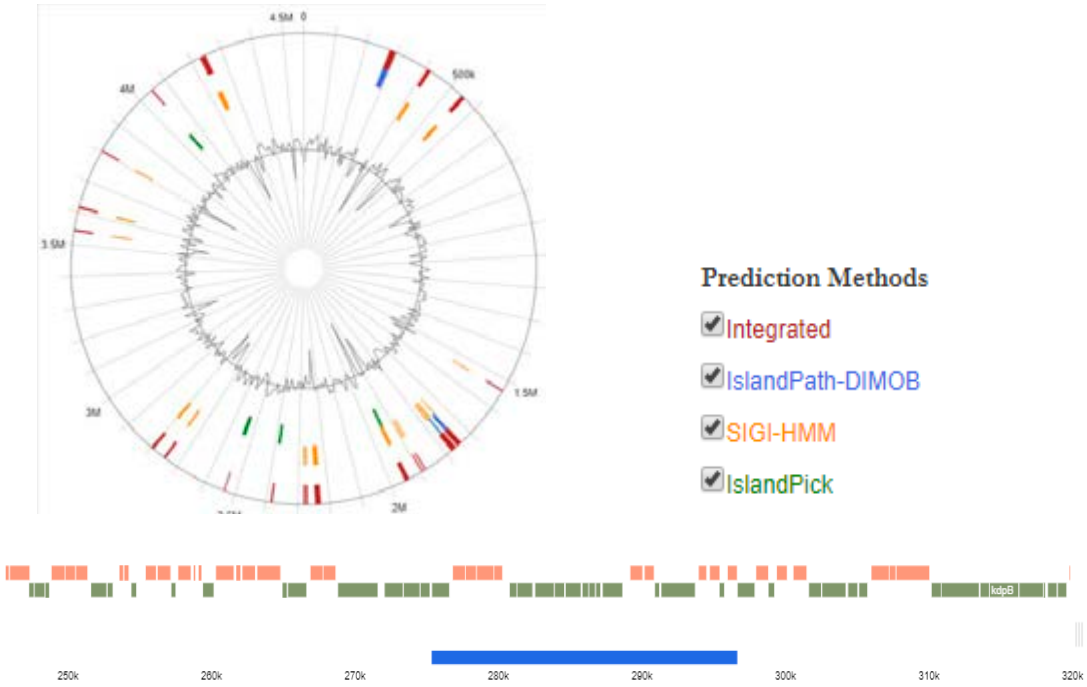


E) Predicted Phage region in *Stenotrophomonas maltophilia*. SVIA2

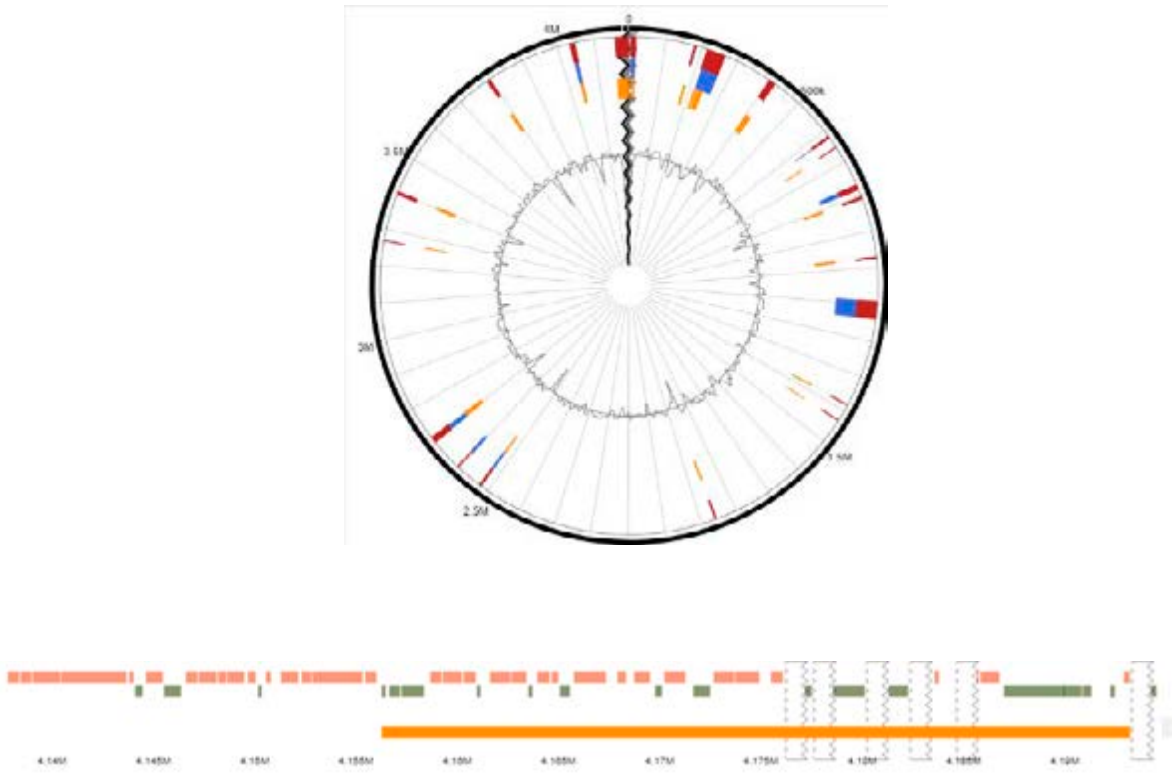
Figure 22. Circular Map of the isolates's genome showing the prophage regions in the isolated strains and linear map of the prophage regions identified in the genomes sequenced.

3.9.4 Prediction of Genomic Islands in Isolates

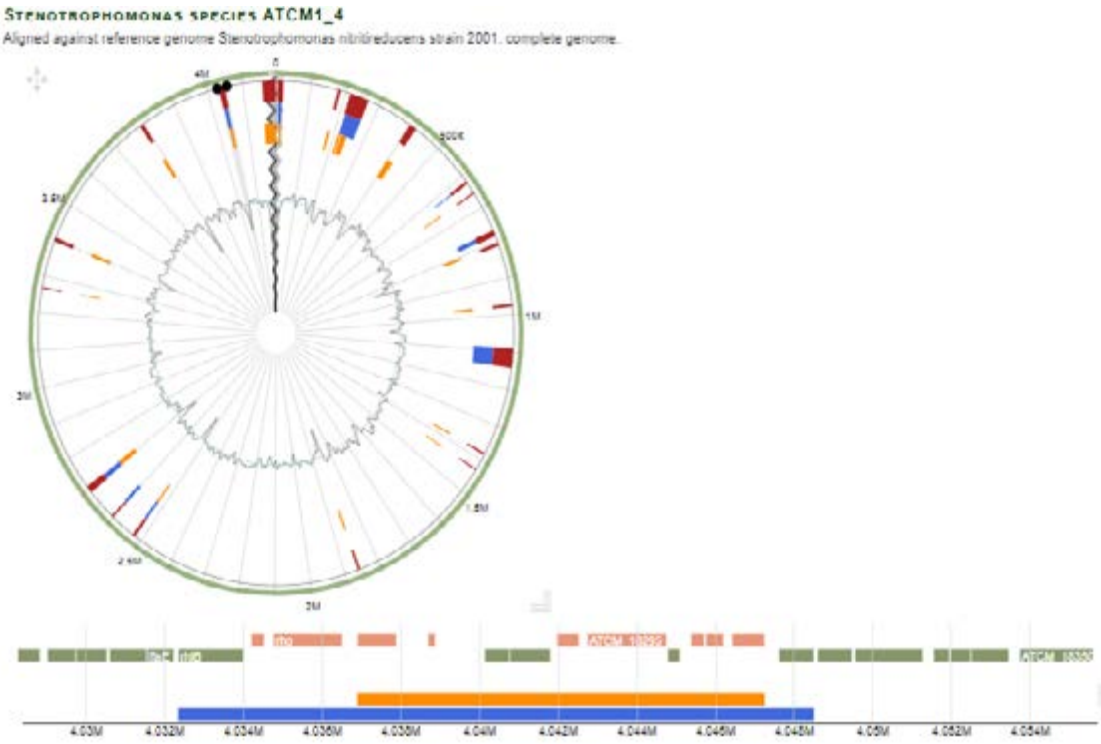
Horizontal or Lateral gene transfer in bacteria usually enhances their genome plasticity, and then further improves bacterial adaptation and evolution (Soares, et al., 2016). Genes acquired horizontally are usually located as a large segment of the bacterial genome known as Genomic Island (GI). The genomic islands in the sequenced genomes were predicted using the web-based Island viewer 4 (Bertelli et al. 2017). ASS1 one harbors 19 genomic islands in its genome, SVIA2 harbors 25 genomic island in its genome, while TEPEL ATCM1_4 and Pemsol has 14, 29, 22 and respectively (Figure 23)



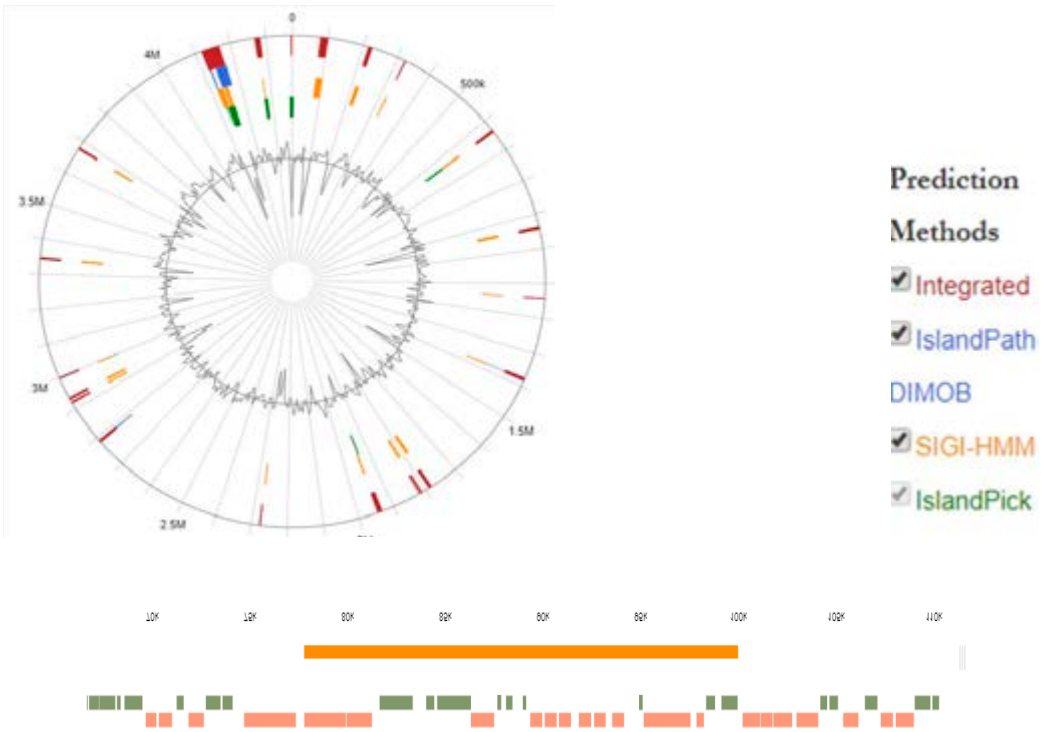
a) Genomic Island Predicted in ASS1 by Island Viewer 4



b) Predicted Genomic Island in *Stenotrophomonas* sp. TEPEL



c) Predicted Genomic Island in ATCM1_4



d) Predicted Genomic Island in Pemsol

Figure 23. A genomic map showing the predicted genomic islands in the isolated genome. The maps were generated using the Island viewer 4 webbase analysis tool. The integrated approach was adopted for identifying the genomic island regions.

3.10. Comparative genome analysis

The comparative analysis of the sequenced *Stenotrophomonas* species in thisstudy and other *Stenotrophomonas* species genomes retrieved from NCBI was carried out as stated earlier. The analysis includes MAUVE alignment (Figure 24), Analysis of the average nucleotide identity among isolates (Figure 25). The Pan Genome analysis was employed for the complete genome comparison of all isolates and other species (Figure 26-30).

The comparative analysis of *Stenotrophomonas* sp. Pemsol and other PAH degrading bacteria was carried out by comparing the abundance of COG categories (Figure 27)

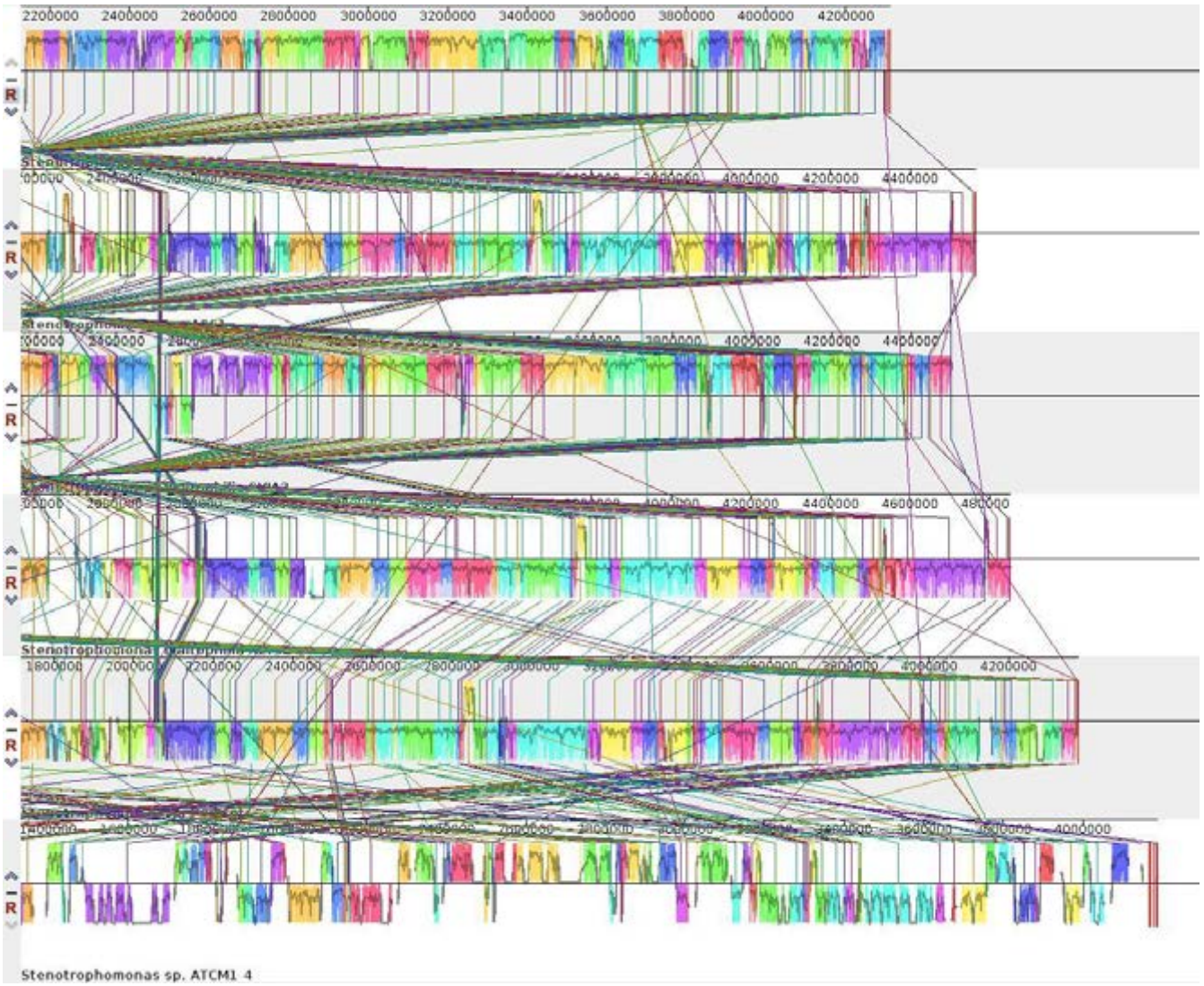


Figure 24. Mauve alignment of the Sequenced *Stenotrophomonas* species showing the orientation of the species in comparison with other isolates.

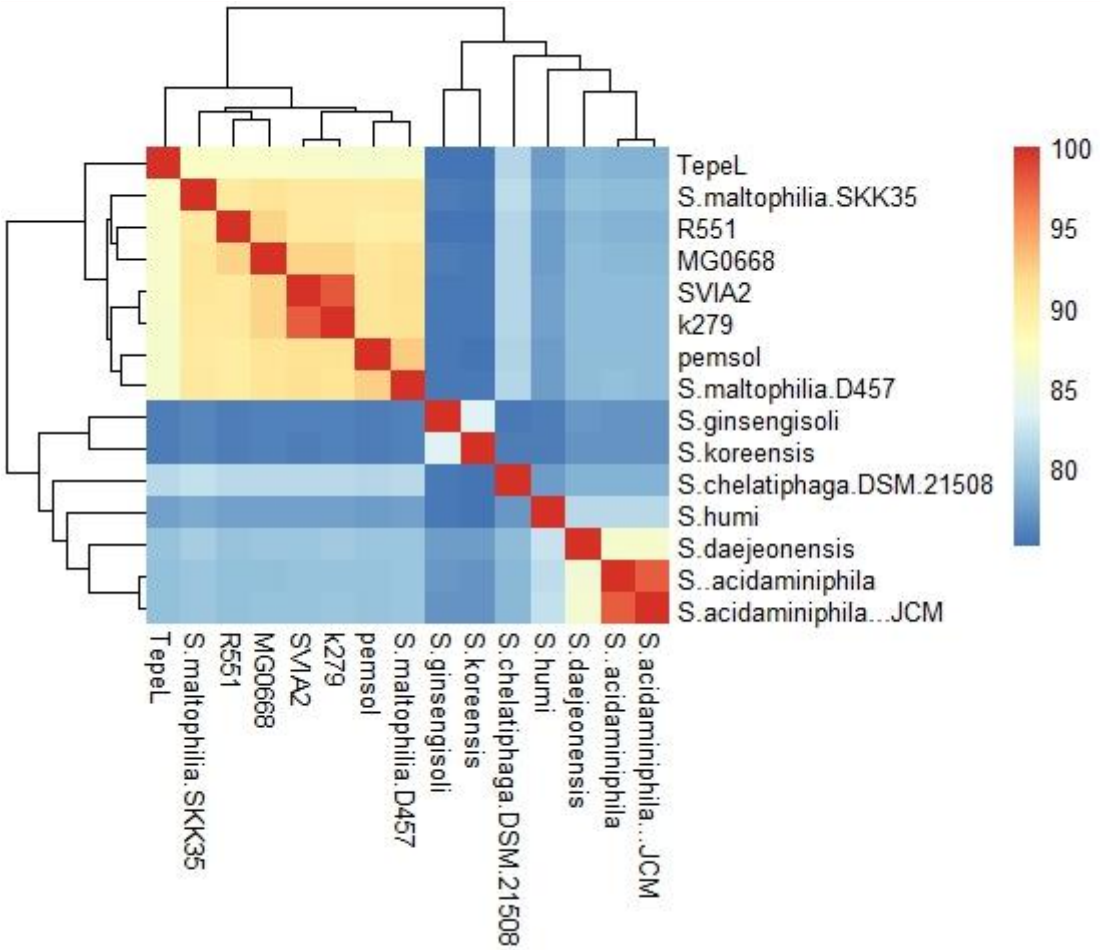


Figure 25. Average Nucleotide Identity (ANI) among the genome sequences of isolated strains and selected members of the genus *Stenotrophomonas*

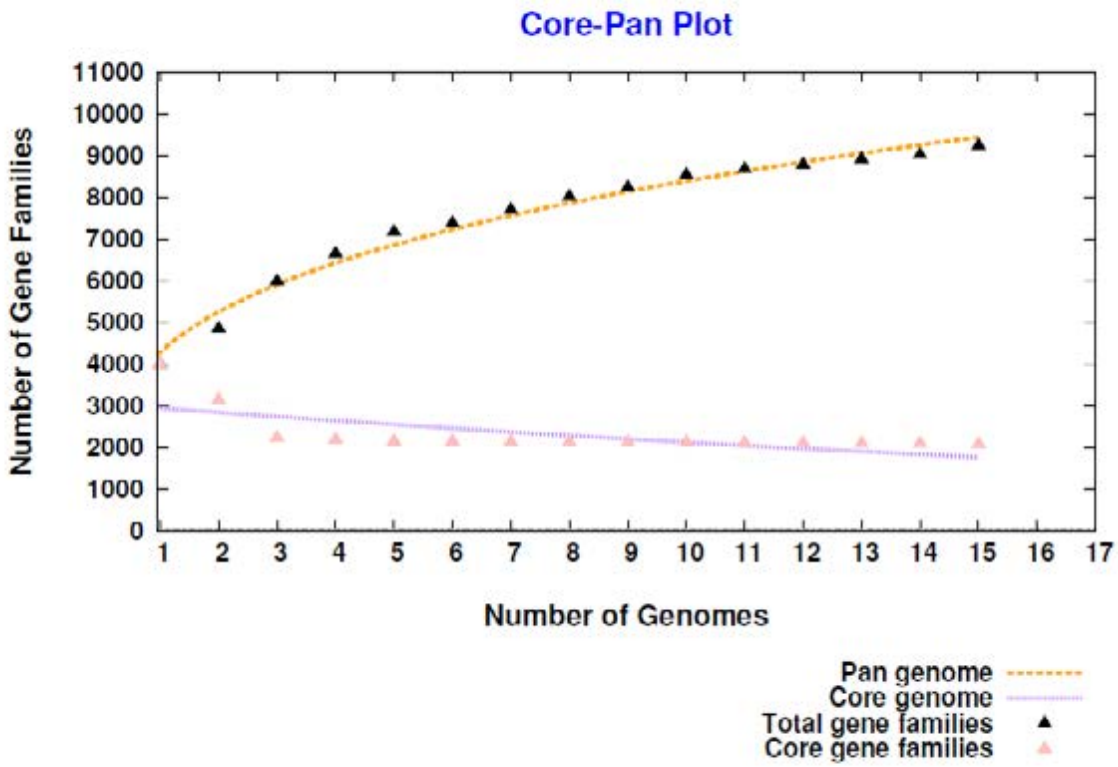


Figure 26. Core-Pan plot of the sequenced strains and other *Stenotrophomonas* Genome retrieved from NCBI database. The Core-Pan Plot was generated with fit law using the Bacterial Pan Genome Analysis (BPGA) tool.

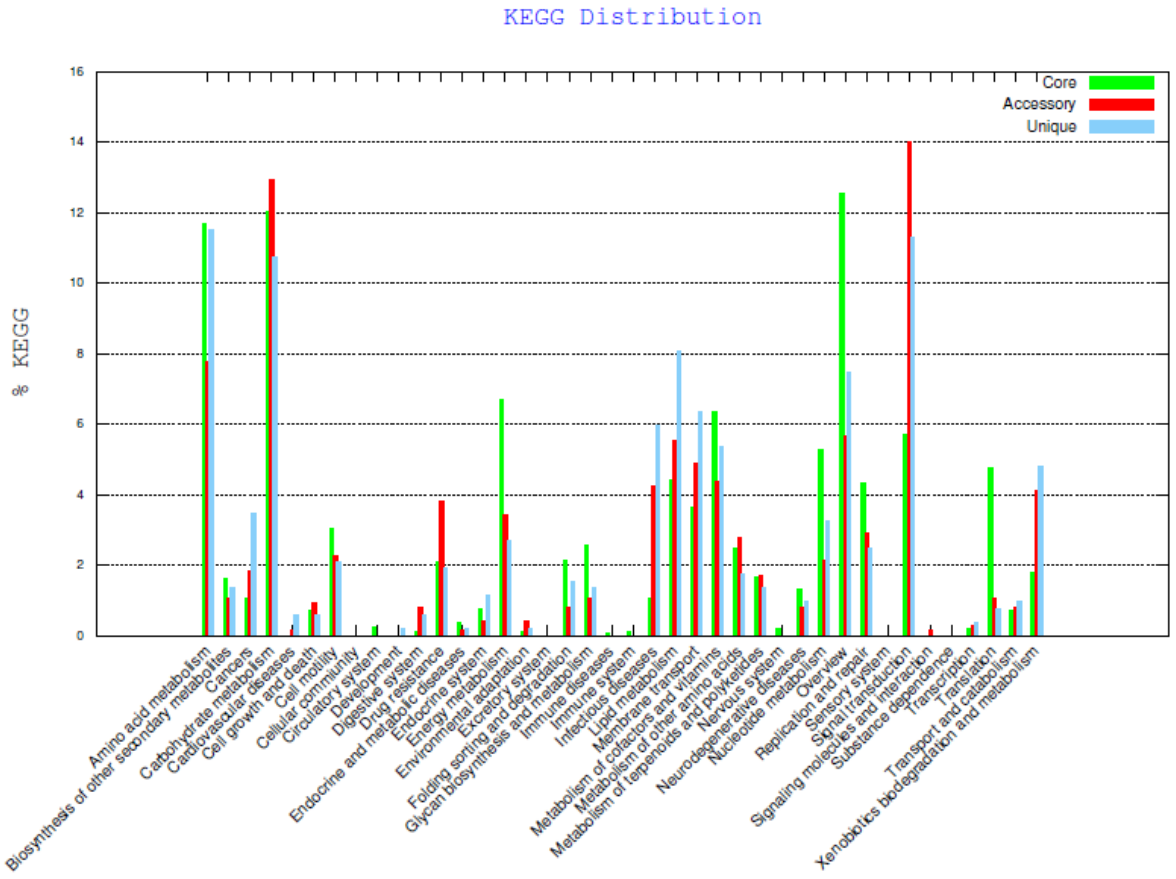


Figure 27. Identified KEGG categories in *Stenotrophomonas* species obtained from the Pan genome analysis of the sequenced isolates using BPGA

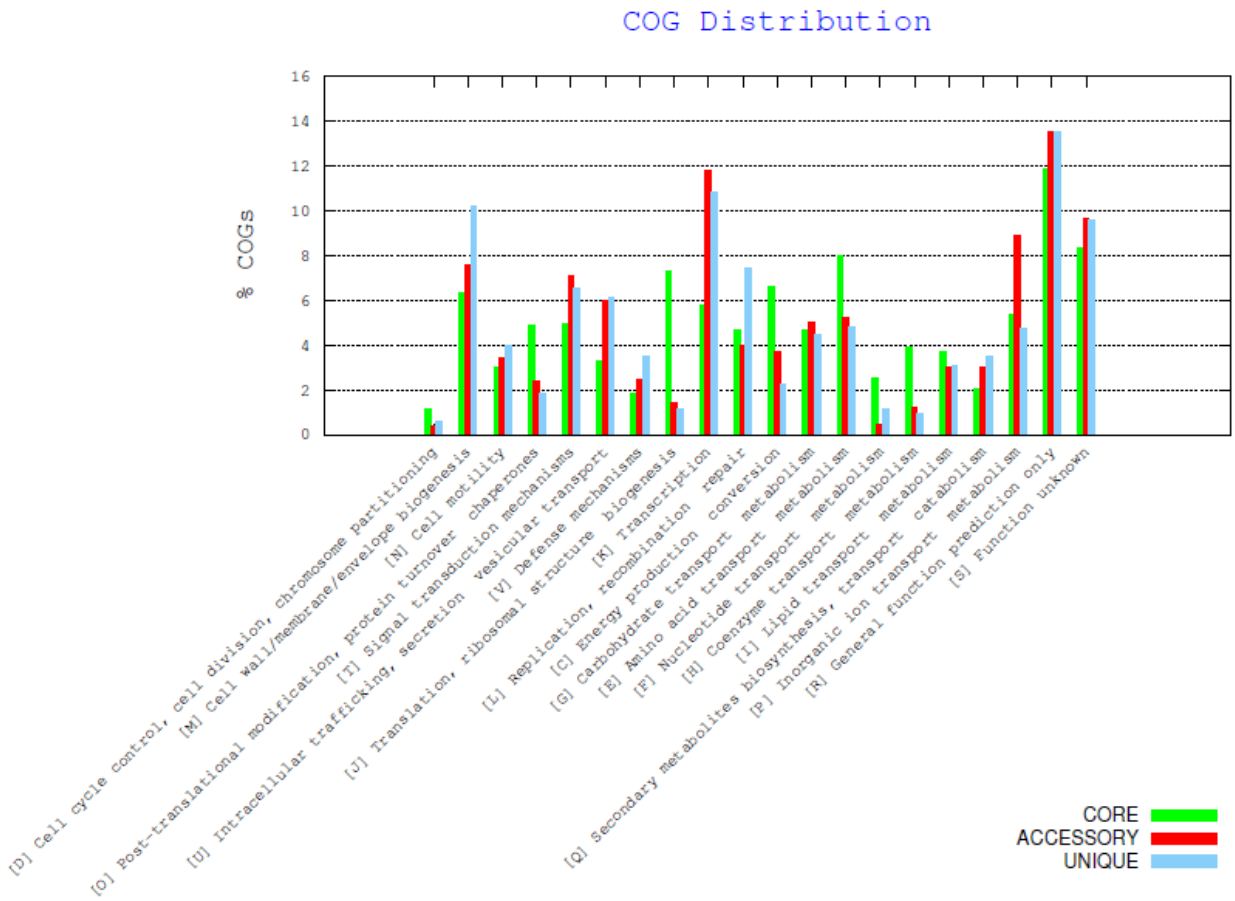


Figure 28. Identified COG categories in *Stenotrophomonas* species obtained from the Pan genome analysis of the sequenced isolates using BPGA

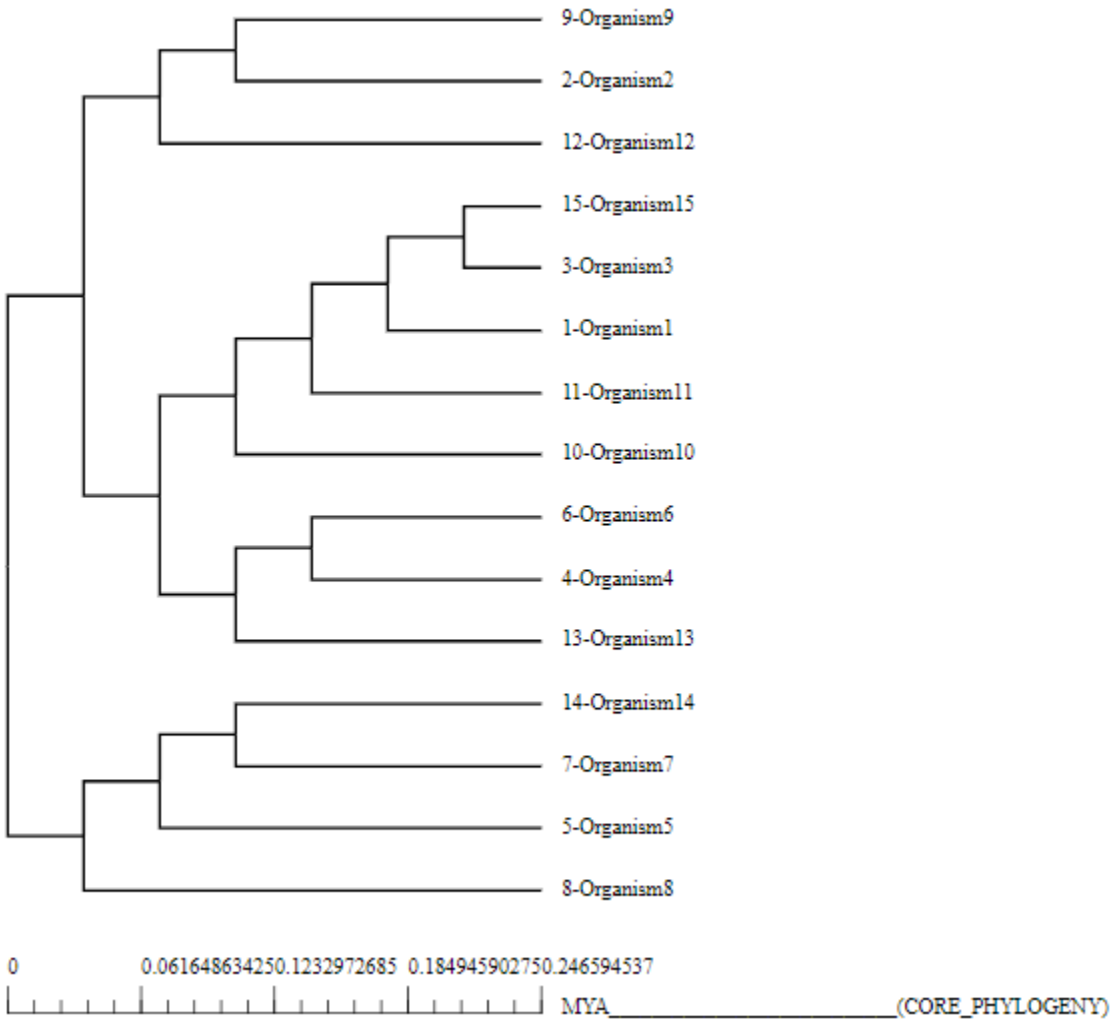


Figure 29. Core Phylogeny genarated based on the Pan genome analysis of isolates and other *Stenotrophomonas* species retrieved from NCBI

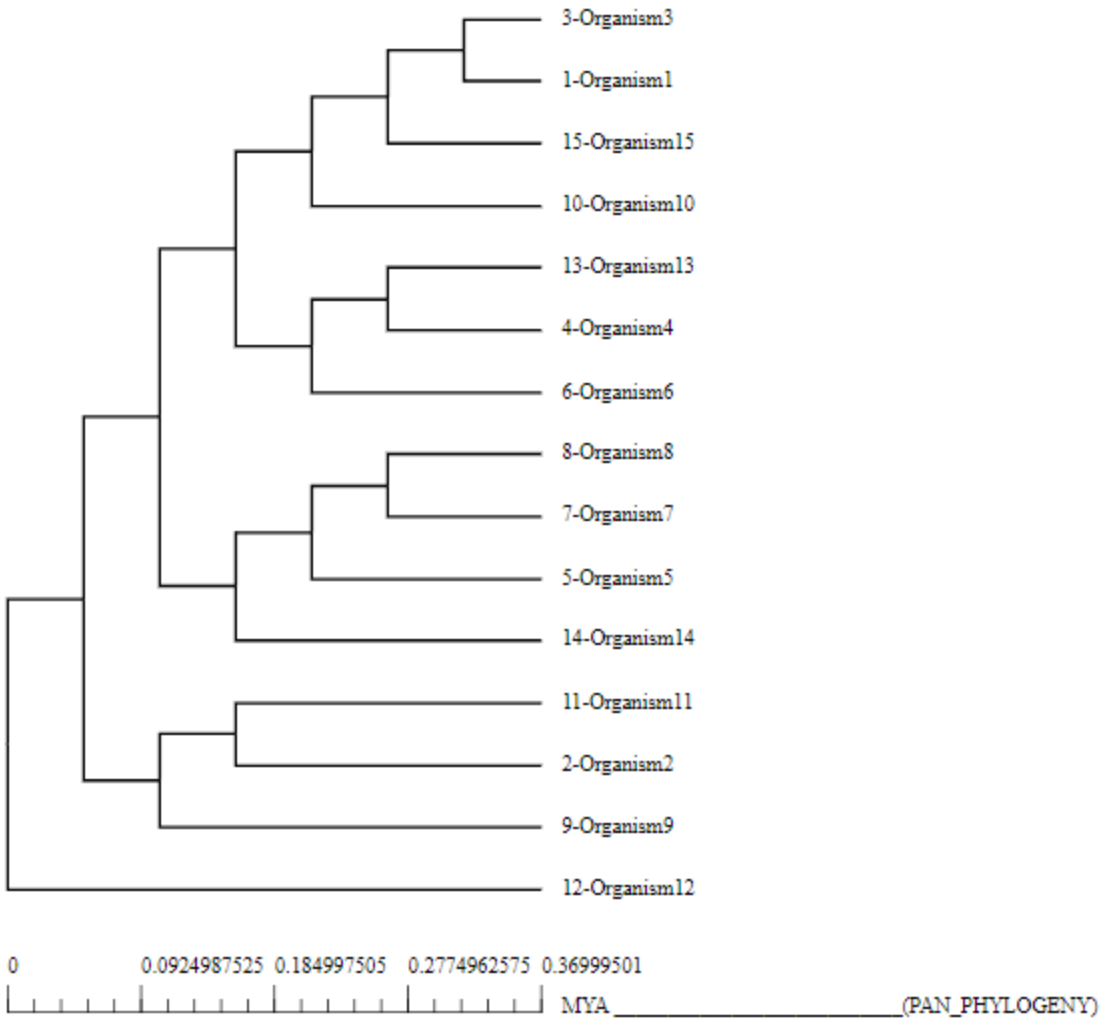


Figure 30. Pan phylogeny generated based on the pan genome analysis of isolates and other *Stenotrophomonas* species retrieved from NCBI

Legend: 1: *Stenotrophomonas maltophilia* AA1, 2: *Stenotrophomonas maltophilia* D457, 3: *Stenotromaltophilia* FDA-ARGOS, 4: *Stenotrophomonas maltophilia* JV3, 5: *Stenotrophomonas* K279a, 6: *Stenotrophomonas* sp. ASS1, 7: *Stenotrophomonas maltophilia* NCTC_3000, 8: *Stenotrophomonas maltophilia* OUC_Est10, 9: *Stentrophomonas* sp. Pemsol, 10: *Stenotrophomonas maltophilia* R551 11: *Stenotrophomonas* sp TepeL 12: *Stenotrophomonas maltophilia* SVIA2

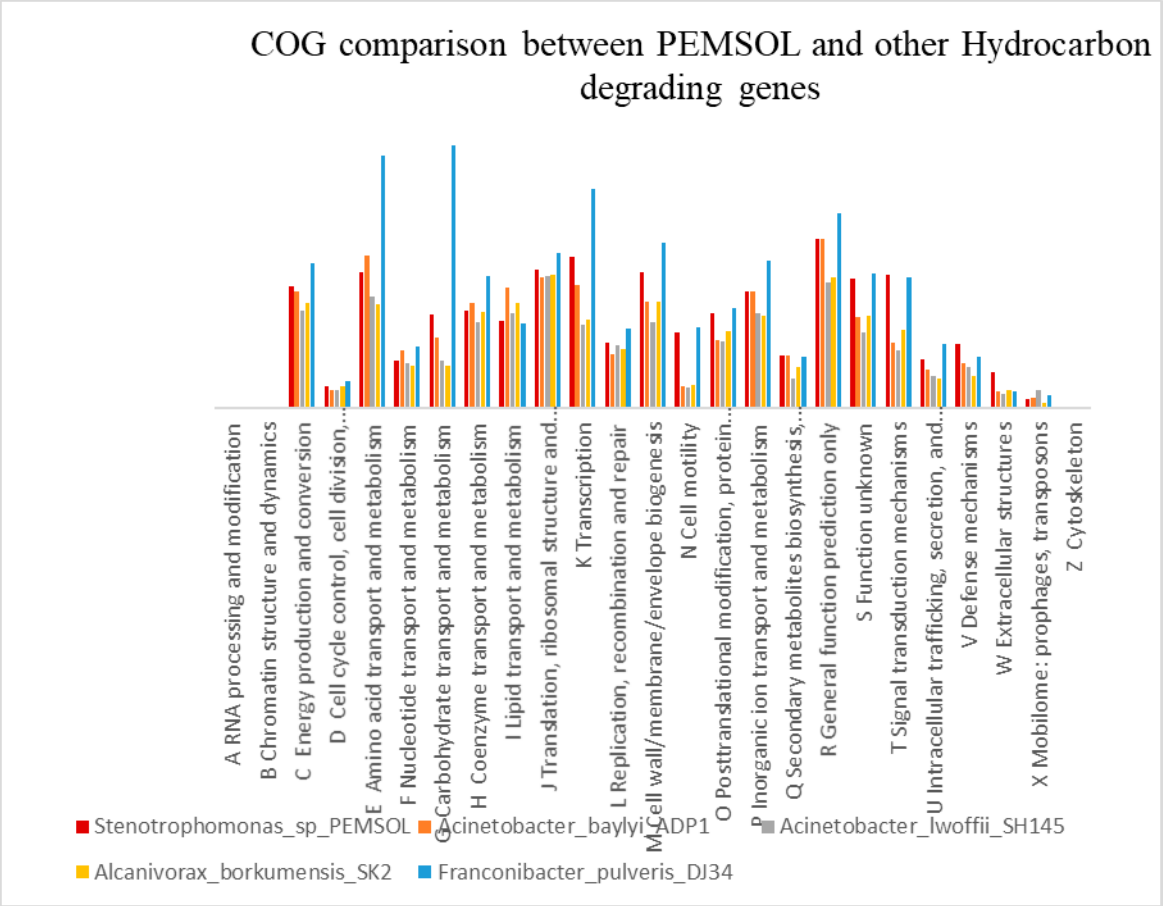


Figure 31 Comparison of COG category between *Stenotrophomonas* sp. Pemsol and other PAH degrading bacteria. The COG categories were analyzed on the JGI-IMG analysis platform

Analysis of Unique gene in Pemsol with Syntax

Pan and core genome analysis of Pemsol revealed the genes that were unique to pemsol. We conducted further analysis on these genes to determine their function and orientation in Pemsol's genome using SYNTAX online software. Further analysis of the genes uniques to Pemsol revealed some clusters that could be associated with the degradation of PAHs. These clusters contain genes that are essential for the transport, gene regulation and the genes that are involve in the degradation of PAHs. Figure 30 contain an example of such cluster. This cluster contain short chain dehydrogenase gene (SDR), lysR gene and ABC transporter.

Figure 32. Unique genes clusters in Pemsol that are associated with the degradation of PAH

3.11. Complete genomic analysis of the genes involved in the bacterial resistance to antibiotics.

The blast search analysis of the complete genome against CARD database revealed several efflux pump and genes, which confer resistance on *Stenotrophomonas* to different antibiotics. The most common gene associated with the efflux system found in them include SmeD, SmeE, SmeR AdeE and AAC (Figure 33), which confer resistance on them to fluoroquinolonones, chloramphenicol and tetracycline and other antimicrobial agents.

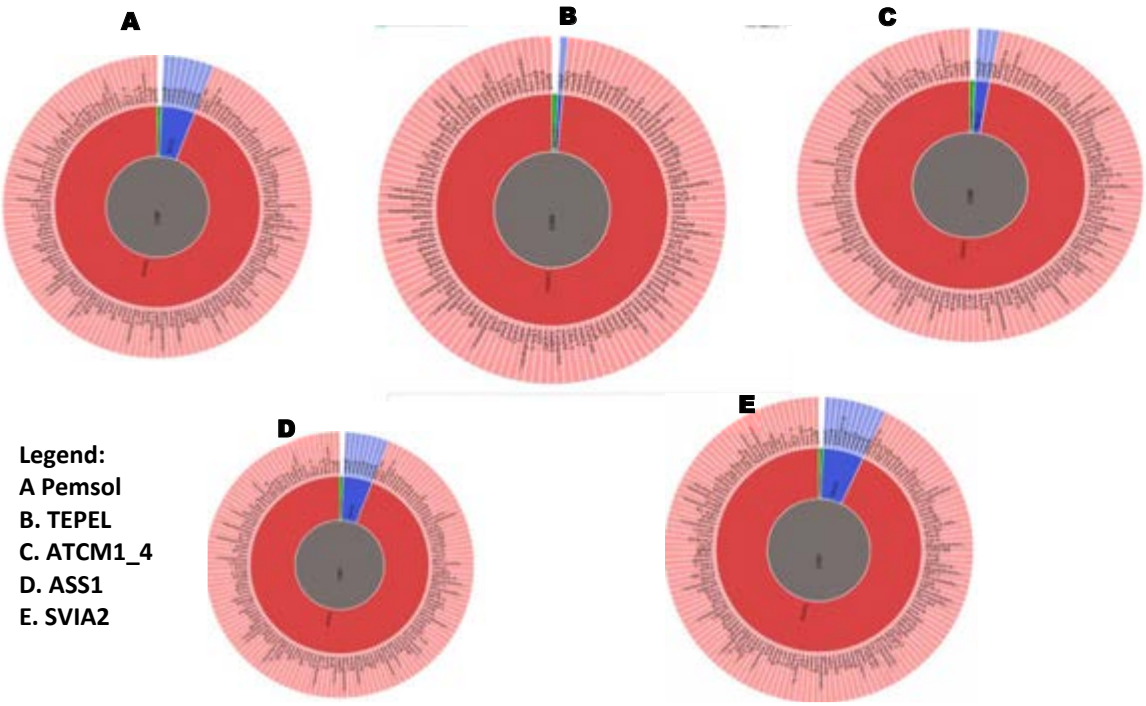


Figure 33. Predicted resistomes in isolated genomes

The genetic basis of PAH degradation in Pemsol, SVIA2 and ASS1 was determined by the functional annotation of these genome on WebMGA online server. The genome functional annotation server successfully predicted the metabolic functions of the genes present in their genomes. The predicted functions were then employed for the identification of the gene associated with the degradation of PAH in Pemsol, SVIA2 and ASS1 (Table 13 and 14). Rast annotation server was employed for the quick overview of the genes associated with the metabolism of aromatic compounds (Figure 33 and 34)

(Please make the following tables much pretty)

Table 13: Identified genes in Pemsol that are associated with the degradation of naphthalene and polycyclic aromatic hydrocarbon

Query_ID	Hit_ID	KEGG_no	KEGG_description	ko_no	EC_no	COG_no	Associated degradation pathways
PEM_00037	aci:ACIAD3598	K00120	glucose-fructose oxidoreductase	ko00625	N/A	COG_no	Chloroalkane and chloroalkene degradation
PEM_00037	nph:NP0254A	K00100	1-deoxy-D-xylulose-5-phosphate reductoisomerase	ko00625	N/A	COG1979;COG2141	Chloroalkane and chloroalkene degradation
PEM_00043	sjp:SJA_C1-21130	K01860	chloromuconate cycloisomerase	ko00623	EC:5.5.1.7	COG0111; COG0677; COG0743; COG1063	Toluene degradation
PEM_00043	pdx:Psed_6064	K01856	muconate cycloisomerase	ko00623	EC:5.5.1.1	N/A	Toluene degradation
PEM_00054	bpm:BURPS1710b_A2071	K01041	glutaconate CoA-transferase, subunit B	ko00626	N/A	N/A	Naphthalene degradation
PEM_00118	bbt:BBta_6889	K00799	glutathione S-transferase	ko00980	EC:2.5.1.18	N/A	Metabolism of xenobiotics by cytochrome P450
PEM_00121	bba:Bd3653	K01077	alkaline phosphatase	ko00627	EC:3.1.3.1	COG0625	Aminobenzoate degradation
PEM_00137	bra:BRADO5603	K05915	2,4'-dihydroxyacetophenone dioxygenase	ko00363	N/A	COG1785	Bisphenol degradation
PEM_00164	gbe:GbCGDNIH1_1792	K00599	trans-aconitate 2-methyltransferase	ko00624	N/A	N/A	Polycyclic aromatic hydrocarbon degradation
PEM_00185	sfu:Sfum_0938	K01826	5-carboxymethyl-2-hydroxymuconate isomerase	ko00362	EC:5.3.3.10	COG0500	Benzoate degradation
PEM_00186	sml:Smlt0609	K01800	maleylacetoacetate isomerase	ko00643	EC:5.2.1.2	COG3232	Styrene degradation
PEM_00297	mbb:BCG_0144	K00680	phospholipid:diacylglycerol acyltransferase	ko00626	N/A	COG0625	Naphthalene degradation
PEM_00342	xac:XAC0815	K00599	trans-aconitate 2-methyltransferase	ko00624	N/A	N/A	Polycyclic aromatic hydrocarbon degradation
PEM_00487	smt:Smal_0786	K01426	amidase	ko00627	EC:3.5.1.4	COG0500	Aminobenzoate degradation
PEM_00496	sml:Smlt0950	K00799	glutathione S-transferase	ko00980	EC:2.5.1.18	COG0154	Metabolism of xenobiotics by cytochrome P450
PEM_00500	reh:H16_B1699	K00001	alcohol dehydrogenase	ko00625	EC:1.1.1.1	COG0625	Chloroalkane and chloroalkene degradation
PEM_00527	mth:MTH234	K01607	4-carboxymuconolactone decarboxylase	ko00362	EC:4.1.1.44	COG0604;COG1062;COG1064;COG1454	Benzoate degradation
PEM_00595	bte:BTH_II0588	K01821	4-oxalocrotonate tautomerase	ko00362	EC:5.3.2.-	COG0599	Benzoate degradation
PEM_00605	rlg:Rleg_4614	K00799	glutathione S-transferase	ko00980	EC:2.5.1.18	COG1942	Metabolism of xenobiotics by cytochrome P450
PEM_00622	bbt:BBta_4719	K00100	1-deoxy-D-xylulose-5-phosphate reductoisomerase	ko00625	N/A	COG0625	Chloroalkane and chloroalkene degradation
PEM_00632	bpd:BURPS668_A2562	K01066	Biphenyl-2,3-diol 1,2-dioxygenase	ko00363	EC:3.1.1.-	COG0111;COG0677;COG0743;COG1063	Bisphenol degradation

PEM_00680	bme:BMEI1067	K00680	phospholipid:diacylglycerol acyltransferase	ko00626	N/A	COG0657	Naphthalene degradation
PEM_00701	reh:H16_B1475	K01066	esterase / lipase	ko00363	EC:3.1.1.-	N/A	Bisphenol degradation
PEM_00701	aba:Acid345_2787	K01061	carboxymethylenebutenolidase	ko00623	EC:3.1.1.45	COG0657	Toluene degradation
PEM_00722	bme:BMEI1388	K00100	1-deoxy-D-xylulose-5-phosphate reductoisomerase	ko00625	N/A	COG0412	Chloroalkane and chloroalkene degradation
PEM_00760	sds:SDEG_0903	K00680	phospholipid:diacylglycerol acyltransferase	ko00626	N/A	COG0111;COG0677;COG0743;COG1063	Naphthalene degradation
PEM_00906	ajs:Ajs_0052	K00492	nitric-oxide synthase, bacterial	ko00363	N/A	N/A	Bisphenol degradation
PEM_01004	sus:Acid_6224	K01692	enoyl-CoA hydratase	ko00362	EC:4.2.1.17	COG0654	Benzoate degradation
PEM_01018	pfl:PFL_2342	K00128	aldehyde dehydrogenase (NAD+)	ko00625	EC:1.2.1.3	COG1024	Chloroalkane and chloroalkene degradation
PEM_01045	buj:BurJV3_1348	K00799	glutathione S-transferase	ko00980	EC:2.5.1.18	COG1012	Metabolism of xenobiotics by cytochrome P450
PEM_01059	lsa:LSA1776	K01607	4-carboxymuconolactone decarboxylase	ko00362	EC:4.1.1.44	COG0625	Benzoate degradation
PEM_01062	bbt:BBta_3786	K00100	1-deoxy-D-xylulose-5-phosphate reductoisomerase	ko00625	N/A	COG0599	Chloroalkane and chloroalkene degradation
PEM_01125	smt:Smal_1438	K00494	alkanal monooxygenase (FMN-linked)	N/A	EC:1.14.14.3	COG0111;COG0677;COG0743;COG1063	N/A
PEM_01174	sml:Smlt1754	K01113	alkaline phosphatase D	ko00627	EC:3.1.3.1	N/A	Aminobenzoate degradation
PEM_01219	buj:BurJV3_1582	K00241	succinate dehydrogenase cytochrome b-556 subunit	ko00623	EC:1.3.99.1	COG3540	Toluene degradation
PEM_01220	buj:BurJV3_1583	K00242	succinate dehydrogenase hydrophobic membrane anchor protein	ko00623	EC:1.3.99.1	COG2009	Toluene degradation
PEM_01221	buj:BurJV3_1584	K00239	succinate dehydrogenase flavoprotein subunit	ko00623	EC:1.3.99.1	COG2142	Toluene degradation
PEM_01222	buj:BurJV3_1585	K00240	succinate dehydrogenase iron-sulfur protein	ko00623	EC:1.3.99.1	COG1053	Toluene degradation
PEM_01261	fra:Francci3_2664	K00517	beta-carotene 15,15'-monooxygenase	ko00363	N/A	COG0479	Bisphenol degradation
PEM_01283	btk:BT9727_2565	K00680	phospholipid:diacylglycerol acyltransferase	ko00626	N/A	COG2124	Naphthalene degradation
PEM_01320	sml:Smlt2023	K00799	glutathione S-transferase	ko00980	EC:2.5.1.18	N/A	Metabolism of xenobiotics by cytochrome P450
PEM_01345	lsl:LSL_0995	K00100	1-deoxy-D-xylulose-5-phosphate reductoisomerase	ko00625	N/A	COG0625	Chloroalkane and chloroalkene degradation
PEM_01355	pna:Pnap_0726	K00114	alcohol dehydrogenase (cytochrome c)	ko00625	EC:1.1.2.8	COG0111;COG0677;COG0743;COG1063	Chloroalkane and chloroalkene degradation
PEM_01385	bcz:BCZK1828	K00517	beta-carotene 15,15'-monooxygenase	ko00363	N/A	N/A	Bisphenol degradation
PEM_01385	rxy:Rxyl_3089	K00494	alkanal monooxygenase (FMN-linked)	N/A	EC:1.14.14.3	COG2124	N/A
PEM_01386	bur:Bcep18194_B1532	K04091	alkanesulfonate monooxygenase	N/A	EC:1.14.14.5	N/A	N/A
PEM_01386	bps:BPSS1672	K00492	nitric-oxide synthase, bacterial	ko00363	N/A	COG2141	Bisphenol degradation
PEM_01386	bur:Bcep18194_B15	K04091	alkanesulfonate monooxygenase	N/A	EC:1.14.14.5	COG0654	N/A

	32						
PEM_01404	pen:PSEEN3116	K00599	trans-aconitate 2-methyltransferase	ko00624	N/A	COG2141	Polycyclic aromatic hydrocarbon degradation
PEM_01407	sml:Smlt2132	K00128	aldehyde dehydrogenase (NAD+)	ko00625	EC:1.2.1.3	COG0500	Chloroalkane and chloroalkene degradation
PEM_01439	rec:RHECIAT_CH0002186	K01113	alkaline phosphatase D	ko00627	EC:3.1.3.1	COG1012	Aminobenzoate degradation
PEM_01462	abo:ABO_0067	K00100	1-deoxy-D-xylulose-5-phosphate reductoisomerase	ko00625	N/A	COG3540	Chloroalkane and chloroalkene degradation
PEM_01474	bme:BMEI0164	K01759	lactoylglutathione lyase	ko00620	EC:4.4.1.5	COG0111;COG0677;COG0743;COG1063	Pyruvate metabolism
PEM_01632	gox:GOX2267	K00680	phospholipid:diacylglycerol acyltransferase	ko00626	N/A	COG0346	Naphthalene degradation
PEM_01654	msm:MSMEG_6370	K01607	4-carboxymuconolactone decarboxylase	ko00362	EC:4.1.1.44	N/A	Benzoate degradation
PEM_01677	dru:Desru_2528	K00121	S-(hydroxymethyl)glutathione dehydrogenase / alcohol dehydrogenase	ko00625	EC:1.1.1.284;1.1.1.1	COG0599	Chloroalkane and chloroalkene degradation
PEM_01677	sen:SACE_5586	K00100	1-deoxy-D-xylulose-5-phosphate reductoisomerase	ko00625	N/A	COG1062	Chloroalkane and chloroalkene degradation
PEM_01677	chu:CHU_1246	K00001	alcohol dehydrogenase	ko00625	EC:1.1.1.1	COG0111;COG0677;COG0743;COG1063	Chloroalkane and chloroalkene degradation
PEM_01733	vfi:VF_A0640	K01759	lactoylglutathione lyase	ko00620	EC:4.4.1.5	COG0604;COG1062;COG1064;COG1454	Pyruvate metabolism
PEM_01834	bat:BAS3185	K00517	beta-carotene 15,15'-monooxygenase	ko00363	N/A	COG0346	Bisphenol degradation
PEM_01834	reu:Reut_C5909	K00494	alkanal monooxygenase (FMN-linked)	N/A	EC:1.14.14.3	COG2124	N/A
PEM_01910	sro:Sros_7956	K01563	haloalkane dehalogenase	ko00361	EC:3.8.1.5	N/A	Chlorocyclohexane and chlorobenzene degradation
PEM_01910	pac:PPA1620	K01066	esterase / lipase	ko00363	EC:3.1.1.-	COG0596	Bisphenol degradation
PEM_01939	fal:FRAAL3541	K04091	alkanesulfonate monooxygenase	N/A	EC:1.14.14.5	COG0657	N/A
PEM_01939	bce:BC3378	K00517	beta-carotene 15,15'-monooxygenase	ko00363	N/A	COG2141	Bisphenol degradation
PEM_01939	cgl:NCgl2137	K00494	alkanal monooxygenase (FMN-linked)	N/A	EC:1.14.14.3	COG2124	N/A
PEM_01939	fal:FRAAL3541	K04091	alkanesulfonate monooxygenase	N/A	EC:1.14.14.5	N/A	N/A
PEM_01943	azo:azo1939	K00492	nitric-oxide synthase, bacterial	ko00363	N/A	COG2141	Bisphenol degradation
PEM_01959	zmo:ZMO1721	K01759	lactoylglutathione lyase	ko00620	EC:4.4.1.5	COG0654	Pyruvate metabolism
PEM_01968	azo:azo2619	K00599	trans-aconitate 2-methyltransferase	ko00624	N/A	COG0346	Polycyclic aromatic hydrocarbon degradation
PEM_02001	gfo:GFO_0971	K00258	isoquinoline 1-oxidoreductase	ko00363	N/A	COG0500	Bisphenol degradation
PEM_02049	buj:BurJV3_2486	K10680	N-ethylmaleimide reductase	ko00633	EC:1.-.-.-	COG3000	Nitrotoluene degradation
PEM_02082	sen:SACE_3584	K00492	nitric-oxide synthase, bacterial/pentachlorophenol monooxygenase	ko00363	N/A	COG1902	Bisphenol degradation
PEM_02090	azo:azo3317	K00001	alcohol dehydrogenase	ko00625	EC:1.1.1.1	COG0654	Chloroalkane and chloroalkene degradation
PEM_02112	ret:RHE_PE00074	K01564	haloalkane dehalogenase	ko00625	N/A	COG0604;COG1062;COG1064;COG1454	Chloroalkane and chloroalkene degradation
PEM_02161	gox:GOX1462	K00100	1-deoxy-D-xylulose-5-phosphate reductoisomerase	ko00625	N/A	N/A	Chloroalkane and chloroalkene degradation

PEM_02171	smt:Smal_2609	K00257	isoquinoline 1-oxidoreductase	ko00626	N/A	COG0111;COG0677;COG0743;COG1063	Naphthalene degradation
PEM_02176	fra:Francci3_1054	K00680	phospholipid:diacylglycerol acyltransferase	ko00626	N/A	COG1960	Naphthalene degradation
PEM_02193	reh:H16_A1564	K00680	phospholipid:diacylglycerol acyltransferase	ko00626	N/A	N/A	Naphthalene degradation
PEM_02199	lsl:LSL_1531	K00100	1-deoxy-D-xylulose-5-phosphate reductoisomerase	ko00625	N/A	N/A	Chloroalkane and chloroalkene degradation
PEM_02199	lps:LPST_C2861	K00244	fumarate reductase flavoprotein subunit	ko00623	EC:1.3.99.1	COG0111;COG0677;COG0743;COG1063	Toluene degradation
PEM_02202	sml:Smlt3208	K00799	glutathione S-transferase	ko00980	EC:2.5.1.18	COG1053	Metabolism of xenobiotics by cytochrome P450
PEM_02214	smt:Smal_2653	K00799	glutathione S-transferase	ko00980	EC:2.5.1.18	COG0625	Metabolism of xenobiotics by cytochrome P450
PEM_02218	msm:MSMEG_6370	K01607	4-carboxymuconolactone decarboxylase	ko00362	EC:4.1.1.44	COG0625	Benzoate degradation
PEM_02305	buj:BurJV3_2901	K01061	carboxymethylenebutenolidase	ko00623	EC:3.1.1.45	COG0599	Toluene degradation
PEM_02315	afm:AFUA_5G10090	K00599	trans-aconitate 2-methyltransferase	ko00624	N/A	COG0412	Polycyclic aromatic hydrocarbon degradation
PEM_02316	chu:CHU_0562	K00258	isoquinoline 1-oxidoreductase	ko00363	N/A	COG0500	Bisphenol degradation
PEM_02399	buj:BurJV3_2988	K09456	putative acyl-CoA dehydrogenase	N/A	N/A	COG3000	N/A
PEM_02405	rba:RB13026	K00517	beta-carotene 15,15'-monooxygenase	ko00363	N/A	COG1960	Bisphenol degradation
PEM_02405	sro:Sros_5419	K00480	salicylate hydroxylase	ko00626	EC:1.14.13.1	COG2124	Naphthalene degradation
PEM_02450	syg:sync_2405	K00257	isoquinoline 1-oxidoreductase	ko00626	N/A	COG0654	Naphthalene degradation
PEM_02458	abo:ABO_1231	K00001	alcohol dehydrogenase	ko00625	EC:1.1.1.1	COG1960	Chloroalkane and chloroalkene degradation
PEM_02489	ava:Ava_2685	K01561	haloacetate dehalogenase	ko00625	EC:3.8.1.3	COG0604;COG1062;COG1064;COG1454	Chloroalkane and chloroalkene degradation
PEM_02489	tpr:Tpau_3844	K05714	2-hydroxy-6-ketonona-2,4-dienedioic acid hydrolase	ko00360	EC:3.7.1.-	N/A	Phenylalanine metabolism
PEM_02532	pdx:Psed_0071	K08195	MFS transporter, AAHS family, 4-hydroxybenzoate transporter	N/A	N/A	COG0596	N/A
PEM_02573	gfo:GFO_2080	K00680	phospholipid:diacylglycerol acyltransferase	ko00626	N/A	COG0477	Naphthalene degradation
PEM_02603	gfo:GFO_2080	K00680	phospholipid:diacylglycerol acyltransferase	ko00626	N/A	N/A	Naphthalene degradation
PEM_02603	gfo:GFO_2080	K00680	phospholipid:diacylglycerol acyltransferase	ko00626	N/A	N/A	Naphthalene degradation
PEM_02626	sml:Smlt3734	K00799	glutathione S-transferase	ko00980	EC:2.5.1.18	N/A	Metabolism of xenobiotics by cytochrome P450
PEM_02671	lch:Lcho_1335	K03862	vanillate monooxygenase	ko00627	EC:1.14.13.82	COG0625	Aminobenzoate degradation
PEM_02673	reu:Reut_A0332	K01826	5-carboxymethyl-2-hydroxymuconate isomerase	ko00362	EC:5.3.3.10	COG2146	Benzoate degradation
PEM_02695	msm:MSMEG_6310	K01041	glutaconate CoA-transferase, subunit B	ko00626	N/A	COG3232	Naphthalene degradation
PEM_02738	vfi:VF_A0640	K01759	lactoylglutathione lyase	ko00620	EC:4.4.1.5	N/A	Pyruvate metabolism
PEM_02753	rha:RHA1_ro02535	K00496	alkane 1-monooxygenase	ko00071	EC:1.14.15.3	COG0346	Fatty acid metabolism
PEM_02799	hma:rrnAC3101	K00599	trans-aconitate 2-methyltransferase	ko00624	N/A	N/A	Polycyclic aromatic hydrocarbon degradation
PEM_02803	bpm:BURPS1710b_1565	K00100	1-deoxy-D-xylulose-5-phosphate reductoisomerase	ko00625	N/A	COG0500	Chloroalkane and chloroalkene degradation
PEM_02845	xtr:496688	K13299	glutathione S-transferase kappa 1	ko00980	EC:2.5.1.18	COG0111;COG0	Metabolism of xenobiotics

						677;COG0743;COG1063	by cytochrome P450
PEM_02851	sml:Smlt3978	K00121	S-(hydroxymethyl)glutathione dehydrogenase / alcohol dehydrogenase	ko00625	EC:1.1.1.284;1.1.1.1	N/A	Chloroalkane and chloroalkene degradation
PEM_02854	agr:AGROH133_07653	K10680	N-ethylmaleimide reductase	ko00633	EC:1.-.-.-	COG1062	Nitrotoluene degradation
PEM_02855	azo:azo2616	K01759	lactoylglutathione lyase	ko00620	EC:4.4.1.5	COG1902	Pyruvate metabolism
PEM_02905	llm:llmg_0271	K00100	1-deoxy-D-xylulose-5-phosphate reductoisomerase	ko00625	N/A	COG0346	Chloroalkane and chloroalkene degradation
PEM_02905	dme:Dmel_CG3609	K00078	dihydrodiol dehydrogenase / D-xylose 1-dehydrogenase (NADP)	ko00980	EC:1.3.1.20;1.1.1.179	COG0111;COG0677;COG0743;COG1063	Metabolism of xenobiotics by cytochrome P450
PEM_02938	mdo:100030403	K00078	dihydrodiol dehydrogenase / D-xylose 1-dehydrogenase (NADP)	ko00980	EC:1.3.1.20;1.1.1.179	N/A	Metabolism of xenobiotics by cytochrome P450
PEM_02960	sml:Smlt4096	K01759	lactoylglutathione lyase	ko00620	EC:4.4.1.5	N/A	Pyruvate metabolism
PEM_02961	bba:Bd0456	K01759	lactoylglutathione lyase	ko00620	EC:4.4.1.5	COG0346	Pyruvate metabolism
PEM_03003	lic:LIC13133	K00100	1-deoxy-D-xylulose-5-phosphate reductoisomerase	ko00625	N/A	COG0346	Chloroalkane and chloroalkene degradation
PEM_03007	reh:H16_A0274	K01759	lactoylglutathione lyase	ko00620	EC:4.4.1.5	COG0111;COG0677;COG0743;COG1063	Pyruvate metabolism
PEM_03013	buj:BurJV3_3604	K00799	glutathione S-transferase	ko00980	EC:2.5.1.18	COG0346	Metabolism of xenobiotics by cytochrome P450
PEM_03054	xac:XAC3819	K00799	glutathione S-transferase	ko00980	EC:2.5.1.18	COG0625	Metabolism of xenobiotics by cytochrome P450
PEM_03079	rha:RHA1_ro02470	K00622	arylamine N-acetyltransferase	ko00633	EC:2.3.1.5	COG0625	Nitrotoluene degradation
PEM_03100	pen:PSEEN0706	K00680	phospholipid:diacylglycerol acyltransferase	ko00626	N/A	COG2162	Naphthalene degradation
PEM_03103	ckl:CKL_0532	K01066	esterase / lipase	ko00363	EC:3.1.1.-	N/A	Bisphenol degradation
PEM_03132	gox:GOX0646	K00100	1-deoxy-D-xylulose-5-phosphate reductoisomerase	ko00625	N/A	COG0657	Chloroalkane and chloroalkene degradation
PEM_03133	azo:azo0148	K00680	phospholipid:diacylglycerol acyltransferase	ko00626	N/A	COG0111;COG0677;COG0743;COG1063	Naphthalene degradation
PEM_03152	btl:BALH_2771	K00680	phospholipid:diacylglycerol acyltransferase	ko00626	N/A	N/A	Naphthalene degradation
PEM_03167	pfl:PFL_4316	K01564	haloalkane dehalogenase	ko00625	N/A	N/A	Chloroalkane and chloroalkene degradation
PEM_03194	hne:HNE_1435	K00100	1-deoxy-D-xylulose-5-phosphate reductoisomerase	ko00625	N/A	N/A	Chloroalkane and chloroalkene degradation
PEM_03223	msm:MSMEG_6370	K01607	4-carboxymuconolactone decarboxylase	ko00362	EC:4.1.1.44	COG0111;COG0677;COG0743;COG1063	Benzoate degradation
PEM_03239	ret:RHE_CH00346	K00462	biphenyl-2,3-diol 1,2-dioxygenase	ko00361	EC:1.13.11.39	COG0599	Chlorocyclohexane and chlorobenzene degradation
PEM_03239	zmo:ZMO0030	K01759	lactoylglutathione lyase	ko00620	EC:4.4.1.5	N/A	Pyruvate metabolism
PEM_03295	reh:H16_A3071	K00680	phospholipid:diacylglycerol acyltransferase	ko00626	N/A	COG0346	Naphthalene degradation
PEM_03309	sml:Smlt4329	K00451	homogentisate 1,2-dioxygenase	ko00643	EC:1.13.11.5	N/A	Styrene degradation
PEM_03332	bba:Bd2246	K00100	1-deoxy-D-xylulose-5-phosphate reductoisomerase	ko00625	N/A	COG3508	Chloroalkane and chloroalkene degradation
PEM_03361	bra:BRADO3440	K00100	1-deoxy-D-xylulose-5-phosphate reductoisomerase	ko00625	N/A	COG0111;COG0677;COG0743;COG1063	Chloroalkane and chloroalkene degradation
PEM_03387	sml:Smlt4422	K03179	4-hydroxybenzoate	ko00130	EC:2.5.1.-	COG0111;COG0	Ubiquinone and other

			octaprenyltransferase			677;COG0743;COG1063	terpenoid-quinone biosynthesis
PEM_03421	cgb:cg0155	K00680	phospholipid:diacylglycerol acyltransferase	ko00626	N/A	COG0382	Naphthalene degradation
PEM_03444	sml:Smlt4504	K00799	glutathione S-transferase	ko00980	EC:2.5.1.18	N/A	Metabolism of xenobiotics by cytochrome P450
PEM_03457	smt:Smal_3879	K03380	phenol 2-monooxygenase	ko00623	EC:1.14.13.7	COG0625	Toluene degradation
PEM_03467	bra:BRADO0841	K00100	1-deoxy-D-xylulose-5-phosphate reductoisomerase	ko00625	N/A	N/A	Chloroalkane and chloroalkene degradation
PEM_03475	tth:TTC1376	K01055	3-oxoadipate enol-lactonase	ko00362	EC:3.1.1.24	COG0111;COG0677;COG0743;COG1063	Benzoate degradation
PEM_03488	gox:GOX1400	K00100	1-deoxy-D-xylulose-5-phosphate reductoisomerase	ko00625	N/A	COG0596	Chloroalkane and chloroalkene degradation
PEM_03493	azo:azo0258	K00680	phospholipid:diacylglycerol acyltransferase	ko00626	N/A	COG0111;COG0677;COG0743;COG1063	Naphthalene degradation
PEM_03569	mil:ML5_5535	K01561	haloacetate dehalogenase	ko00625	EC:3.8.1.3	N/A	Chloroalkane and chloroalkene degradation
PEM_03571	buj:BurJV3_4039	K05782	benzoate membrane transport protein	N/A	N/A	N/A	N/A
PEM_03597	buj:BurJV3_4065	K07323	putative toluene tolerance protein	N/A	N/A	COG3135	N/A
PEM_03603	buj:BurJV3_4071	K00799	glutathione S-transferase	ko00980	EC:2.5.1.18	COG2854	Metabolism of xenobiotics by cytochrome P450
PEM_03604	gox:GOX0476	K00100	1-deoxy-D-xylulose-5-phosphate reductoisomerase	ko00625	N/A	COG0625	Chloroalkane and chloroalkene degradation
PEM_03604	reh:H16_B1699	K00001	alcohol dehydrogenase	ko00625	EC:1.1.1.1	COG0111;COG0677;COG0743;COG1063	Chloroalkane and chloroalkene degradation
PEM_03645	buj:BurJV3_0027	K13953	alcohol dehydrogenase, propanol-preferring	ko00625	EC:1.1.1.1	COG0604;COG1062;COG1064;COG1454	Chloroalkane and chloroalkene degradation
PEM_03645	buj:BurJV3_0027	K13953	alcohol dehydrogenase, propanol-preferring	ko00626	EC:1.1.1.1	COG1064	Naphthalene degradation
PEM_03654	sml:Smlt0084	K09474	acid phosphatase (class A)	ko00627	EC:3.1.3.2	COG1064	Aminobenzoate degradation
PEM_03667	rba:RB2355	K00680	phospholipid:diacylglycerol acyltransferase	ko00626	N/A	COG0671	Naphthalene degradation
PEM_03754	bra:BRADO0018	K00100	1-deoxy-D-xylulose-5-phosphate reductoisomerase	ko00625	N/A	N/A	Chloroalkane and chloroalkene degradation
PEM_03755	rlg:Rleg_0809	K00799	glutathione S-transferase	ko00980	EC:2.5.1.18	COG0111;COG0677;COG0743;COG1063	Metabolism of xenobiotics by cytochrome P450
PEM_03772	buj:BurJV3_0117	K00626	acetyl-CoA C-acetyltransferase	ko00362	EC:2.3.1.9	COG0625	Benzoate degradation
PEM_03776	vfi:VF_0102	K00680	phospholipid:diacylglycerol acyltransferase	ko00626	N/A	COG0183	Naphthalene degradation
PEM_03803	sml:Smlt0199	K00252	glutaryl-CoA dehydrogenase	ko00362	EC:1.3.99.7	N/A	Benzoate degradation
PEM_03811	sml:Smlt0206	K01563	haloalkane dehalogenase	ko00361	EC:3.8.1.5	COG1960	Chlorocyclohexane and chlorobenzene degradation
PEM_03814	lic:LIC13133	K00100	1-deoxy-D-xylulose-5-phosphate reductoisomerase	ko00625	N/A	COG0596	Chloroalkane and chloroalkene degradation
PEM_03823	xac:XAC2121	K00599	trans-aconitate 2-methyltransferase	ko00624	N/A	COG0111;COG0677;COG0743;COG1063	Polycyclic aromatic hydrocarbon degradation
PEM_03827	aci:ACIAD2050	K00680	phospholipid:diacylglycerol acyltransferase	ko00626	N/A	COG0500	Naphthalene degradation

PEM_03855	amd:AMED_8366	K09461	anthraniloyl-CoA monooxygenase	ko00627	EC:1.14.13.40	N/A	Aminobenzoate degradation
PEM_03865	smt:Smal_0220	K00257	isoquinoline 1-oxidoreductase	ko00626	N/A	N/A	Naphthalene degradation
PEM_03866	buj:BurJV3_0222	K01692	enoyl-CoA hydratase	ko00362	EC:4.2.1.17	COG1960	Benzoate degradation
PEM_03939	bba:Bd3653	K01077	alkaline phosphatase	ko00627	EC:3.1.3.1	COG1024	Aminobenzoate degradation

Table 14: Annotation of Predicted genes in the Genomic Island of *Stenotrophomonas* sp. Pemsol

Query	Seed Ortholog	evalue	Predicted name	Possible origin for gene transfer	KEGG KO	COG category	Protein Family
PEM_00073	322710.Avin_36080	0		*Azotobacter vinelactii*		T	Histidine kinase
PEM_00075	533247.CRD_00627	5.5E-41	Y0750	*Raphidopsis*		S	Protein of unknown function (DUF1524)
PEM_00076	596151.DesfrDRAFT_0170	6.1E-166	DCM2	*Desulfovibrio fructosivoran*	K00558	L	cytosine-specific methyltransferase
PEM_00077	596151.DesfrDRAFT_0169	2E-60		*Desulfovibrio fructosivoran*			
PEM_00081	190486.XAC2246	1.3E-64		*Xanthomonas axonopodis citris*		S	Protein of unknown function (DUF1629)
PEM_00082	190486.XAC3322	4E-66		*Xanthomonas axonopodis citris*		S	Protein of unknown function (DUF1629)
PEM_00083	190486.XAC2246	1.6E-36		*Xanthomonas axonopodis citris*		S	Protein of unknown function (DUF1629)
PEM_00084	316273.XCV2688	3.3E-63		*Xanthomoas campestris vesicatoria*		S	Protein of unknown function (DUF1629)
PEM_00085	190486.XAC2862	4.1E-63		*Xanthomonas axonopodis citris*		S	Protein of unknown function (DUF1629)
PEM_00086	316273.XCV2685	3.5E-86		*Xanthomoas campestris vesicatoria*		S	Protein of unknown function (DUF1629)
PEM_00087	316273.XCV2684	8.8E-213		*Xanthomoas campestris vesicatoria*			
PEM_00202	522373.Smlt0617	7.4E-215	METB	*Stenotrophomonas maltophilia K279a*	K01739,K01758, K01760,K17217	E	Cystathionine gamma-synthase
PEM_00203	1045855.DSC_02425	2.2E-80	WZM	*Pseudoxanthomonas spadix*	K01992,K09690	V	transporter
PEM_00204	228410.NE0483	7.7E-128	TAGH	*Nitrosomonas europaea*	K09689,K09691,K09693	P	ABC, transporter
PEM_00205	223283.PSPTO_1074	9E-118		*Pseudomonas syringae pv. tomato*	K07011	M	Glycosyl transferase, family 2
PEM_00206	640510.BC1001_0680	6.8E-89		*Burkholderia sp. CCGE1001*	K07011	M	Glycosyl transferase, family 2
PEM_00207	452637.Oter_3973	1.8E-177		*Opitutus terrae (strain DSM 11246 / JCM 15787 / PB90-1)*		M	Glycosyl transferase family 2
PEM_00208	522373.Smlt0784	3.7E-127		*Stenotrophomonas maltophilia (strain K279a)*		M	Glycosyl Transferase
PEM_00209	391008.Smal_0635	9.5E-176		*Stenotrophomonas maltophilia (strain R551-3)*			
PEM_00288	426355.Mrad2831_3035	2.4E-22		*Methylobacterium radiotolerans (strain ATCC 27329 / DSM 1819 / JCM 2831)*			
PEM_00290	266835.mlr4370	0.00000039		*Rhizobium loti (strain MAFF303099)*			

				(Mesorhizobium loti)			
PEM_00581	522373.Smlt1010	5.4E-70		*Stenotrophomonas maltophilia (strain K279a)*		S	Acetyltransferase (GNAT) family
PEM_00582	216591.BCAM1200	3.9E-14		*Burkholderia cenocepacia*		S	Inner membrane protein YmfA
PEM_00583	522373.Smlt0064	2.4E-141	ELI_1277	*Stenotrophomonas maltophilia (strain K279a)*	K06919	L	prophage primase
PEM_00585	190485.XCC3461	2.4E-147		*Xanthomonas campestris pv. campestris*	K16214	S	zeta toxin
PEM_00868	522373.Smlt1390	0	FHAB	*Stenotrophomonas maltophilia (strain K279a)*	K15125	U	filamentous hemagglutinin
PEM_00869	522373.Smlt1396A	2.4E-12		*Stenotrophomonas maltophilia (strain K279a)*		S	Protein of unknown function, DUF596
PEM_00870	391008.Smal_3995	1.3E-11		*Stenotrophomonas maltophilia (strain R551-3)*		S	Protein of unknown function (DUF2628)
PEM_01042	397945.Aave_0535	8.1E-44		*Acidovorax avenae subsp. citrulli*		T	MASE1
PEM_01043	397945.Aave_0536	1.7E-19		*Acidovorax avenae subsp. citrulli*			
PEM_01044	397945.Aave_0535	2.4E-43		*Acidovorax avenae subsp. citrulli*		T	MASE1
PEM_01288	441620.Mpop_2731	1E-60	DCM	*Methylobacterium populi*	K00558	L	C-5 cytosine-specific DNA methylase
PEM_01295	522373.Smlt1978	1.1E-100		*Stenotrophomonas maltophilia (strain K279a)*		S	conserved protein
PEM_01296	522373.Smlt1978	1.7E-48		*Stenotrophomonas maltophilia (strain K279a)*		S	conserved protein
PEM_01297	1127134.NOCYR_5259	3.8E-09		*Nocardia cyriacigeorgica (strain GUH-2)*		K	Helix-turn-helix
PEM_01300	56780.SYN_01916	4.5E-135		*Syntrophus aciditrophicus (strain SB)*		L	integrase family
PEM_01654	522373.Smlt2386	1.9E-76	BMUL_5091	*Stenotrophomonas maltophilia (strain K279a)*		K	Gcn5-related n-acetyltransferase
PEM_01658	216591.BCAM1881	4.8E-81		*Burkholderia cenocepacia*			
PEM_01659	216591.BCAM1882	2.9E-48		*Burkholderia cenocepacia*			
PEM_01661	522373.Smlt2497	6.1E-130		*Stenotrophomonas maltophilia (strain K279a)*			
PEM_01666	522373.Smlt2501	3.4E-102		*Stenotrophomonas maltophilia (strain K279a)*	K07141	S	2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase
PEM_01667	391008.Smal_1990	8.1E-181		*Stenotrophomonas maltophilia (strain R551-3)*	K07402	O	Xanthine dehydrogenase accessory factor
PEM_01690	522373.Smlt2534	2.8E-122		*Stenotrophomonas maltophilia (strain K279a)*		S	Protein of unknown function DUF72
PEM_01691	522373.Smlt2535	2.3E-71		*Stenotrophomonas maltophilia (strain K279a)*		S	BLUF
PEM_01693	522373.Smlt2711	8.2E-11	OCAR_6262	*Stenotrophomonas maltophilia (strain K279a)*		T	response regulator
PEM_01694	391008.Smal_1741	1.8E-30		*Stenotrophomonas maltophilia (strain R551-3)*		S	domain protein
PEM_01695	522373.Smlt2543	3.6E-83		*Stenotrophomonas maltophilia (strain K279a)*		M	Glycosyl transferase
PEM_01696	522373.Smlt2544	2.1E-106		*Stenotrophomonas maltophilia (strain K279a)*		S	Nodulation protein S (NodS)
PEM_01697	522373.Smlt2545	4.2E-131		*Stenotrophomonas*		S	GlcNAc-PI de-N-

				maltophilia (strain K279a)			acetylase
PEM_01698	1045855.DSC_05565	2.2E-68		*Pseudoxanthomonas spadix (strain BD-a59)*		S	acyl-CoA dehydrogenase
PEM_01699	522373.Smlt2547	3.5E-70	BMUL_5515	*Stenotrophomonas maltophilia (strain K279a)*		S	Inherit from bactNOG: Diguanylate cyclase phosphodiesterase
PEM_01700	522373.Smlt2548	5.7E-220	YBDR	*Stenotrophomonas maltophilia (strain K279a)*	K00098	C	Dehydrogenase
PEM_01702	314565.XC_3765	2.5E-76		*Xanthomonas campestris pv. campestris (strain 8004)*			
PEM_01703	522373.Smlt2552	5E-81		*Stenotrophomonas maltophilia (strain K279a)*		Q	Isochorismatase family
PEM_01704	522373.Smlt2553	2E-39		*Stenotrophomonas maltophilia (strain K279a)*			
PEM_01705	522373.Smlt2554	4.8E-58		*Stenotrophomonas maltophilia (strain K279a)*			
PEM_01706	742159.HMPREF0004_4690	8.4E-70		*Achromobacter piechaudii ATCC 43553*			
PEM_01707	391008.Smal_2029	1.3E-164	PTXS	*Stenotrophomonas maltophilia (strain R551-3)*	K02525, K02529, K03435	K	Transcriptional regulator
PEM_01806	743721.Psesu_2031	3.1E-30	ECPD	*Pseudoxanthomonas suwonensis (strain 11-1)*	K07346, K07357, K15540	N, U	Pfam:Pili_assembly_C
PEM_01808	1097668.BYI23_B003340	2.2E-155	HTRE	*Burkholderia sp. YI23*	K07347	M	outer membrane usher protein
PEM_01809	340100.Bpet4466	1.9E-32	ECPD	*Bordetella petrii (strain ATCC BAA-461 / DSM 12804 / CCUG 43448)*	K07346, K07357 K15540	O	Pili assembly chaperone
PEM_01810	626418.bglu_1g22890	0.000000023	FIMA	*Burkholderia glumae (strain BGR1)*	K07345, K12517	N, U	Inherit from proNOG: Fimbrial protein
PEM_01811	522373.Smlt0732	1.6E-10	YEHC	*Stenotrophomonas maltophilia (strain K279a)*	K07346, K15540	N, U	Chaperone
PEM_01814	350701.BDAG_03369	5.5E-33		*Burkholderia dolosa AU0158*	K15540	O	Pili assembly chaperone
PEM_01816	667129.HMPREF0758_4622	1.1E-157	YEHB	*Serratia odorifera DSM 4582*	K07347	M	outer membrane usher protein
PEM_01817	340100.Bpet4466	6.6E-44	ECPD	*Bordetella petrii (strain ATCC BAA-461 / DSM 12804 / CCUG 43448)*	K07346, K07357, K15540	O	Pili assembly chaperone
PEM_01818	266265.Bxe_C1153	3.9E-09	FIMA	*Bordetella petrii (strain ATCC BAA-461 / DSM 12804 / CCUG 43448)*	K07345	N, U	Fimbrial
PEM_01819	716541.ECL_04444	4.1E-61		*Enterobacter cloacae subsp. cloacae (strain ATCC 13047 / DSM 30054 / NBRC 13535 / NCDC 279-56)*		M	Inherit from proNOG: opacity protein and
PEM_02561	522373.Smlt2892	2.2E-195	BMUL_3317	*Stenotrophomonas maltophilia (strain K279a)*	K08224	G	Major Facilitator
PEM_02562	522373.Smlt2893	2.2E-166		*Stenotrophomonas maltophilia (strain K279a)*		K	LysR family transcriptional regulator
PEM_02563	391008.Smal_2344	1E-266		*Stenotrophomonas maltophilia* (R551-3)		S	amidohydrolase
PEM_02564	522373.Smlt2895	8.4E-118	OCAR_5703	*Stenotrophomonas maltophilia (strain K279a)*	K06867	F, J	Ankyrin repeat-containing protein

PEM_02565	522373.Smlt2896	1.3E-160		*Stenotrophomonas maltophilia (strain K279a)*	K19242	K	lysr family transcriptional regulator
PEM_02566	391008.Smal_2347	9.3E-48		*Stenotrophomonas maltophilia* (R551-3)			
PEM_02567	391008.Smal_2347	3.3E-72		*Stenotrophomonas maltophilia* (R551-3)			
PEM_02568	522373.Smlt2898	1E-213	YAHJ	*Stenotrophomonas maltophilia (strain K279a)*	K01485	F	deaminase
PEM_02569	522373.Smlt2899	5.9E-110		*Stenotrophomonas maltophilia (strain K279a)*			
PEM_02680	391008.Smal_2251	0		*Stenotrophomonas maltophilia* (R551-3)		M	rhs family
PEM_02689	391008.Smal_1583	1.4E-200	MCHE	*Stenotrophomonas maltophilia* (R551-3)	K02022,K13408	U	secretion protein
PEM_02690	391008.Smal_1584	0	CVAB	*Stenotrophomonas maltophilia* (R551-3)	K06147,K13409	V	AtP-binding protein
PEM_02692	190486.XAC3298	4.5E-105		*Xanthomonas axonopodis pv. citri (strain 306)*		L	Integrase
PEM_02741	391008.Smal_3245	0		*Stenotrophomonas maltophilia* (R551-3)	K19232	U, W	Pfam:HIM
PEM_03048	522373.Smlt4157	2.6E-66	OCAR_5815	*Stenotrophomonas maltophilia (strain K279a)*	K03746	S	Histone family protein nucleoid-structuring protein H-NS
PEM_03049	391008.Smal_3563	1.3E-62	YQJF	*Stenotrophomonas maltophilia (strain K279a)*	K15977	S	Inner membrane protein YqjF
PEM_03050	522373.Smlt4159	7.4e-309	PBP-2	*Stenotrophomonas maltophilia (strain K279a)*		V	Beta-lactamase
PEM_03054	522373.Smlt0332	1.5E-177	SCLAV_0044	*Stenotrophomonas maltophilia (strain K279a)*	K11904	S	Rhs element vgr protein
PEM_03055	522373.Smlt0324	1E-161	Q	*Stenotrophomonas maltophilia (strain K279a)*		S	portal protein
PEM_03244	391008.Smal_2784	5.4E-39		*Stenotrophomonas maltophilia* (R551-3)			
PEM_03245	391008.Smal_2785	1.3E-35	BMUL_3731	*Stenotrophomonas maltophilia* (R551-3)		S	Protein of unknown function (DUF1653)
PEM_03246	391008.Smal_2787	6.7E-49	EMRE	*Stenotrophomonas maltophilia* (R551-3)	K03297	P	multidrug resistance protein
PEM_03248	391008.Smal_2789	2E-105	RHTB	*Stenotrophomonas maltophilia* (R551-3)		E	Homoserine homoserine lactone efflux protein
PEM_03249	391008.Smal_2790	6.8E-108	CLPP	*Stenotrophomonas maltophilia* (R551-3)	K01358	O	Cleaves peptides in various proteins in a process that requires ATP hydrolysis. Has a chymotrypsin-like activity. Plays a major role in the degradation of misfolded proteins (By similarity)
PEM_03250	391008.Smal_2791	1.2E-53		*Stenotrophomonas maltophilia* (R551-3)			
PEM_03251	391008.Smal_2792	9E-48		*Stenotrophomonas maltophilia* (R551-3)		K	Transcriptional regulator
PEM_03252	522373.Smlt3366	3.2E-109	YGEA	*Stenotrophomonas maltophilia* (strain K279a)	K01779	E	aspartate racemase

PEM_03254	391008.Smal_2795	4.5E-84		*Stenotrophomonas maltophilia* (R551-3)		S	Protein of unknown function (DUF3011)
PEM_03255	391008.Smal_2797	0.00000012		*Stenotrophomonas maltophilia* (R551-3)		S	acetyltransferase, GNAT family
PEM_03256	1001585.MDS_1719	3.7E-42		*Pseudomonas mendocina* (strain NK-01)	K01104	T	LMWPc
PEM_03257	384676.PSEEN3596	2.5E-11		*Pseudomonas entomophila* (strain L48)			
PEM_03258	390235.PputW619_3104	5.4E-57	OCAR_5492	*Pseudomonas putida* (strain W619)		S	Antioxidant protein with alkyl hydroperoxidase activity. Required for the reduction of the AhpC active site cysteine residues and for the regeneration of the AhpC enzyme activity (By similarity)
PEM_03259	395492.Rleg2_1661	6.7E-44		*Rhizobium leguminosarum bv. trifolii* (strain WSM2304)		J	endoribonuclease L-PSP
PEM_03261	743721.Psesu_1124	1.6E-56	INTE	*Pseudoxanthomonas suwonensis* (strain 11-1)		L	Integrase
PEM_03263	391008.Smal_2798	4.8E-61	MERR			K	Transcriptional Regulator, MerR family
PEM_03264	391008.Smal_2799	3.9E-48	IHFA	*Stenotrophomonas maltophilia* (strain R551-3)	K04764,K05788	K	This protein is one of the two subunits of integration host factor, a specific DNA-binding protein that functions in genetic recombination as well as in transcriptional and translational control (By similarity)
PEM_03265	391008.Smal_2800	0	PHET	*Stenotrophomonas maltophilia* (strain R551-3)	K01890	J	phenylalanyl-tRNA synthetase beta subunit
PEM_03266	522373.Smlt3374	3.4E-189	PHES	*Stenotrophomonas maltophilia* (strain K279a)	K01889	J	Phenylalanyl-tRNA synthetase alpha subunit
PEM_03381	522373.Smlt4372	1.8E-78	ARR	*Stenotrophomonas maltophilia* (strain K279a)	K19062	M	rifampin ADP-ribosyl transferase
PEM_03382	522373.Smlt4373	2.4E-73		*Stenotrophomonas maltophilia* (strain K279a)			
PEM_03385	76114.ebA2464	7.1E-54		*Aromatoleum aromaticum* (strain EbN1) (*Azoarcus* sp. (strain EbN1))			
PEM_03390	1045855.DSC_14975	3.6E-48		*Pseudoxanthomonas spadix* (strain BD-a59)		M	Inherit from COG: YD repeat protein
PEM_03427	522373.Smlt4430	0		*Stenotrophomonas maltophilia* (strain K279a)		M	rhs family

PEM_03658	522373.Smlt4693	0	YIDC	*Stenotrophomonas maltophilia* (strain K279a)	K03217	U	Required for the insertion and or proper folding and or complex formation of integral membrane proteins into the membrane. Involved in integration of membrane proteins that insert both dependently and independently of the Sec translocase complex, as well as at least some lipoproteins. Aids folding of multispanning membrane proteins (By similarity)
PEM_03659	522373.Smlt4694	5.6E-84	RNPA	*Stenotrophomonas maltophilia* (strain K279a)	K03536	J	RNaseP catalyzes the removal of the 5'-leader sequence from pre-tRNA to produce the mature 5'-terminus. It can also cleave other RNA substrates such as 4.5S RNA. The protein component plays an auxiliary but essential role in vivo by binding to the 5'-leader sequence and broadening the substrate specificity of the ribozyme (By similarity)
PEM_03660	522373.Smlt4695	5.3E-16	RPMH	*Stenotrophomonas maltophilia* (strain K279a)	K02914	J	50S ribosomal protein L34
PEM_03661	522373.Smlt0001	8.7E-249	DNAA	*Stenotrophomonas maltophilia* (strain K279a)	K02313	L	it binds specifically double-stranded DNA at a 9 bp consensus (dnaA box) 5'-TTATC CA A CA A-3'. DnaA binds to ATP and to acidic phospholipids (By similarity)

PEM_03662	522373.Smlt0002	2.4E-196	DNAN	*Stenotrophomonas maltophilia* (strain K279a)	K02338	L	DNA polymerase III is a complex, multichain enzyme responsible for most of the replicative synthesis in bacteria. This DNA polymerase also exhibits 3' to 5' exonuclease activity. The beta chain is required for initiation of replication once it is clamped onto DNA, it slides freely (bidirectional and ATP- independent) along duplex DNA (By similarity)
PEM_03663	522373.Smlt0004	6.6E-202	RECF	*Stenotrophomonas maltophilia* (strain K279a)	K03629	L	it is required for DNA replication and normal SOS inducibility. RecF binds preferentially to single-stranded, linear DNA. It also seems to bind ATP (By similarity)
PEM_03664	522373.Smlt0005	0	GYRB	*Stenotrophomonas maltophilia* (strain K279a)	K02470	L	DNA gyrase negatively supercoils closed circular double-stranded DNA in an ATP-dependent manner and also catalyzes the interconversion of other topological isomers of double-stranded DNA rings, including catenanes and knotted rings (By similarity)
PEM_03665	391008.Smal_0005	5.7E-129		*Stenotrophomonas maltophilia* (strain R551-3)	K07052	S	CAAX amino terminal protease family
PEM_03666	391008.Smal_0006	7.6E-130		*Stenotrophomonas maltophilia* (strain R551-3)		O	peptidase M48, Ste24p
PEM_03667	522373.Smlt0008	3E-200	ORF398	*Stenotrophomonas maltophilia* (strain K279a)		S	domain protein
PEM_03668	391008.Smal_0008	3.4E-108		*Stenotrophomonas maltophilia* (strain R551-3)	K03832	M	Pfam:TonB
PEM_03770	522373.Smlt0157	0		*Stenotrophomonas maltophilia* (strain K279a)	K02014	P	TonB-dependent receptor
PEM_03771	500635.MITSMUL_03861	1.4E-31		*Mitsuokella multacida* DSM 20544		S	phosphoglycerate mutase family protein
PEM_03773	391008.Smal_3568	2.7E-14	AVA_1330	*Stenotrophomonas maltophilia* (strain R551-3)	K07497	L	Integrase core domain
PEM_03776	391612.CY0110_22834	0.000000046	BMUL_4716	*Cyanothece sp.* CCY0110		S	aaa ATPase
PEM_03777	402626.Rpic_4575	0.00000089		*Ralstonia pickettii* (strain 12J)			
PEM_03778	1045855.DSC_02605	3.5E-22		*Pseudoxanthomonas spadix*	K03746	S	nucleoid-structuring

				(strain BD-a59)			protein
PEM_03779	318167.Sfri_2854	3.8E-80		*Shewanella frigidimarina* (strain NCIMB 400)			
PEM_03780	589865.DaAHT2_1056	1.4E-242		*Desulfurivibrio alkaliphilus (strain DSM 19089 / UNIQEM U267 / AHT2)*	K02168	P	Choline carnitine betaine transporter
PEM_03781	709797.CSIRO_3742	4.7E-92		*Bradyrhizobiaceae bacterium* SG-6C		S	Predicted membrane protein (DUF2254)
PEM_03782	522373.Smlt0115	5.2E-41		*Stenotrophomonas maltophilia* (strain K279a)		V	Beta-lactamase
PEM_03783	543728.Vapar_2066	1.1E-27		*Variovorax paradoxus* (strain S110)	K02014	P	receptor
PEM_03784	1045855.DSC_06700	1.7E-79		*Pseudoxanthomonas spadix* (strain BD-a59)	K07090	S	Sulfite exporter TauE/SafE
PEM_03785	316273.XCV2442	7.7E-37		*Xanthomonas campestris pv. vesicatoria* (strain 85-10)		S	Putative phosphatase (DUF442)
PEM_03786	316273.XCV2443	8.4E-52		*Xanthomonas campestris pv. vesicatoria* (strain 85-10)	K07112	S	YeeE YedE family protein
PEM_03787	316273.XCV2445	2.7E-135		*Xanthomonas campestris pv. vesicatoria* (strain 85-10)	K01069	P	beta-lactamase domain protein
PEM_03789	690566.Sphch_3377	7.1E-223		*Sphingobium chlorophenolicum* L-1	K02014,K16089	P	TonB-dependent Receptor Plug
PEM_03790	272630.MexAM1_META1p2602	1.6E-36	HMRR	*Methylobacterium extorquens* (strain ATCC 14718 / DSM 1338 / JCM 2805 / NCIMB 9133 / AM1)		K	MerR family transcriptional regulator
PEM_03791	314266.SKA58_04115	1.2E-51		*Sphingomonas* sp. (strain SKA58)		P	Cation efflux protein
PEM_03792	395492.Rleg2_1661	3.3E-43		*Rhizobium leguminosarum bv. trifolii* (strain WSM2304)		J	endoribonuclease L-PSP
PEM_03793	398578.Daci_5638	7.9E-121		*Delftia acidovorans* (strain DSM 14801 / SPH-1)		K	transcriptional Regulator LysR family
PEM_03794	339670.Bamb_3531	2.2E-111		*Burkholderia ambifaria* (strain ATCC BAA-244 / AMMD) (*Burkholderia cepacia* (strain AMMD))		S	Short-chain dehydrogenase reductase sdr
PEM_03795	267608.RSc2594	3.2E-74	GSTCH1	*Ralstonia solanacearum* (strain GMI1000) (*Pseudomonas solanacearum*)	K00799, K03599	O	Glutathione S-transferase, C-terminal domain
PEM_03796	640512.BC1003_0658	2.2E-74	LDB1079	*Burkholderia* sp. (strain CCGE1003)		L	integrase family
PEM_03800	517433.PanABDRAFT_0490	3.9E-99	LYSR18	*Pantoea* sp. aB	K18900	K	lysr family transcriptional regulator
PEM_03801	682634.SOD_a07460	8.7E-191	SCLAV_5667	*Serratia plymuthica* 4Rx13		F	amidohydrolase
PEM_03802	1001585.MDS_3582	3.9E-42		*Pseudomonas mendocina* (strain NK-01)	K09936	S	Protein of unknown function, DUF606
PEM_03803	78245.Xaut_0790	2.4E-32		*Xanthobacter autotrophicus* (strain ATCC BAA-1158 / Py2)			

PEM_03804	572477.Alvin_2808	2.8E-80		*Allochromatium vinosum* (strain ATCC 17899 / DSM 180 / NBRC 103801 / NCIMB 10441 / D) (*Chromatium vinosum*)			
PEM_03806	384676.PSEEN3569	1.2E-243		*Pseudomonas entomophila* (strain L48)		S	Inherit from proNOG: ATP-dependent OLD family endonuclease
PEM_03807	391008.Smal_0122	4.4E-195	GLNL	*Stenotrophomonas maltophilia* (strain R551-3)	K02482, K07708, K07710, K13532, K13533	T	Signal transduction histidine kinase, nitrogen specific
PEM_03808	522373.Smlt0159	2.5E-257	NTRC	*Stenotrophomonas maltophilia* (strain K279a)	K07712, K07714	T	two components, sigma54 specific, transcriptional regulator, Fis family
PEM_03809	522373.Smlt0160	2.3E-97	SODC	*Stenotrophomonas maltophilia* (strain K279a)	K04565	P	Destroys radicals which are normally produced within the cells and which are toxic to biological systems (By similarity)
PEM_03810	522373.Smlt0161	7.8E-107	SODC	*Stenotrophomonas maltophilia* (strain K279a)	K04565	P	Destroys radicals which are normally produced within the cells and which are toxic to biological systems (By similarity)
PEM_03811	522373.Smlt0163	4.9E-163		*Stenotrophomonas maltophilia* (strain K279a)			
PEM_03812	522373.Smlt0164	1E-241	FADA2		K00626, K00632	I	Catalyzes the final step of fatty acid oxidation in which acetyl-CoA is released and the CoA ester of a fatty acid two carbons shorter is formed (By similarity)
PEM_03813	391008.Smal_0129	1.8E-227	HEMY	*Stenotrophomonas maltophilia* (strain R551-3)	K02498	H	HemY protein
PEM_03814	383407.Xoryp_010100001530	2.9E-68	HEMX	*Xanthomonas oryzae pv. oryzicola* (strain BLS256)	K02496, K13543	H	c-methyltransferase
PEM_03815	522373.Smlt0167	1.4E-119	HEMD	*Stenotrophomonas maltophilia (strain K279a)*	K01719, K13542,K13543	H	synthase
PEM_03816	522373.Smlt0168	6.1E-80		*Stenotrophomonas maltophilia (strain K279a)*		S	Putative thioesterase (yiiD_Cterm)
PEM_03817	314565.XC_0211	1.3E-28		Xanthomonas campestris pv. campestris (strain 8004)			
PEM_03818	522373.Smlt0170	3.9E-64	YIBN	*Stenotrophomonas maltophilia* (strain K279a)	K01011	P	Rhodanese domain protein

PEM_03819	522373.Smlt0171	2.2E-91	SECB	*Stenotrophomonas maltophilia* (strain K279a)	K03071	U	One of the proteins required for the normal export of preproteins out of the cell cytoplasm. It is a molecular chaperone that binds to a subset of precursor proteins, maintaining them in a translocation-competent state. It also specifically binds to its receptor SecA (By similarity)

Number of Predicted genomic island in Pemsol = 35

Total number of Associated gene = 227

Table 15: Identified PAH degrading gene in SVIA2

	Hit_ID	e-value	KEGG_no	KEGG_description	ko_no	ko_description	EC_no	GO_no	COG_no
LBG_00003	rrs:RoseRS_3822	2.00E-08	K01561	haloacetate dehalogenase	ko00625	Chloroalkane and chloroalkene degradation	EC:3.8.1.3	18785	N/A
LBG_00082	azo:azo0258	3.00E-28	K00680	phospholipid:diacylglycerol acyltransferase	ko00626	Naphthalene degradation	N/A	N/A	N/A
LBG_00087	gox:GOX1400	1.00E-42	K00100	1-deoxy-D-xylulose-5-phosphate reductoisomerase	ko00625	Chloroalkane and chloroalkene degradation	N/A	N/A	COG0111;COG0677; COG0743;COG1063
LBG_00090	yen:YE0909	8.00E-08	K01726	cyanate lyase	ko00626	Naphthalene degradation	N/A	N/A	N/A
LBG_00116	bra:BRADO0841	5.00E-97	K00100	1-deoxy-D-xylulose-5-phosphate reductoisomerase	ko00625	Chloroalkane and chloroalkene degradation	N/A	N/A	COG0111;COG0677; COG0743;COG1063
LBG_00128	smt:Smal_3879	1.00E-135	K03380	phenol 2-monooxygenase	ko00623	Toluene degradation	EC:1.14.13.7	18662	N/A
LBG_00142	sml:Smlt4504	1.00E-134	K00799	glutathione S-transferase	ko00480	Glutathione metabolism	EC:2.5.1.18	4364	COG0625
LBG_00165	cgb:cg0155	8.00E-29	K00680	phospholipid:diacylglycerol acyltransferase	ko00626	Naphthalene degradation	N/A	N/A	N/A
LBG_00235	bra:BRADO3440	1.00E-130	K00100	1-deoxy-D-xylulose-5-phosphate reductoisomerase	ko00625	Chloroalkane and chloroalkene degradation	N/A	N/A	COG0111;COG0677; COG0743;COG1063
LBG_00262	bba:Bd2246	1.00E-20	K00100	1-deoxy-D-xylulose-5-phosphate reductoisomerase	ko00625	Chloroalkane and chloroalkene degradation	N/A	N/A	COG0111;COG0677; COG0743;COG1063
LBG_00284	sml:Smlt4329	0	K00451	homogentisate 1,2-dioxygenase	ko00350	Tyrosine metabolism	EC:1.13.11.5	4411	COG3508
LBG_00298	reh:H16_A3071	1.00E-36	K00680	phospholipid:diacylglycerol acyltransferase	ko00626	Naphthalene degradation	N/A	N/A	N/A
LBG_00329	azo:azo0148	1.00E-144	K00680	phospholipid:diacylglycerol acyltransferase	ko00626	Naphthalene degradation	N/A	N/A	N/A
LBG_00330	gox:GOX0646	1.00E-73	K00100	1-deoxy-D-xylulose-5-phosphate reductoisomerase	ko00625	Chloroalkane and chloroalkene degradation	N/A	N/A	COG0111;COG0677; COG0743;COG1063
LBG_00360	fnu:FN1306	3.00E-99	K00599	trans-aconitate 2-methyltransferase	ko00624	Polycyclic aromatic	N/A	N/A	COG0500

						hydrocarbon degradation			
LBG_00363	pen:PSEEN0706	2.00E-68	K00680	phospholipid:diacylglycerol acyltransferase	ko00626	Naphthalene degradation	N/A	N/A	N/A
LBG_00389	reu:Reut_B4044	4.00E-38	K00622	arylamine N-acetyltransferase	ko00633	Nitrotoluene degradation	EC:2.3.1.5	4060	COG2162
LBG_00414	xac:XAC3819	3.00E-77	K00799	glutathione S-transferase	ko00980	Metabolism of xenobiotics by cytochrome P450	EC:2.5.1.18	4364	COG0625
LBG_00474	sml:Smlt4155	1.00E-107	K00799	glutathione S-transferase	ko00980	Metabolism of xenobiotics by cytochrome P450	EC:2.5.1.18	4364	COG0625
LBG_00556	mdo:100030403	6.00E-17	K00078	dihydrodiol dehydrogenase / D-xylose 1-dehydrogenase (NADP)	ko00980	Metabolism of xenobiotics by cytochrome P450	EC:1.3.1.20;1.1.1.179	0047115;0047837	N/A
LBG_00587	llm:llmg_0271	6.00E-39	K00100	1-deoxy-D-xylulose-5-phosphate reductoisomerase	ko00625	Chloroalkane and chloroalkene degradation	N/A	N/A	COG0111;COG0677;COG0743;COG1063
LBG_00636	agr:AGROH133_07653	7.00E-98	K10680	N-ethylmaleimide reductase	ko00633	Nitrotoluene degradation	EC:1.-.-.-	N/A	COG1902
LBG_00639	sml:Smlt3978	0	K00121	S-(hydroxymethyl)glutathione dehydrogenase / alcohol dehydrogenase	ko00626	Naphthalene degradation	EC:1.1.1.284;1.1.1.1	0004327;0004022	COG1062
LBG_00651	dre:436833	7.00E-10	K13299	glutathione S-transferase kappa 1	ko00980	Metabolism of xenobiotics by cytochrome P450	EC:2.5.1.18	4364	N/A
LBG_00690	bpm:BURPS1710b_1565	1.00E-106	K00100	1-deoxy-D-xylulose-5-phosphate reductoisomerase	ko00625	Chloroalkane and chloroalkene degradation	N/A	N/A	COG0111;COG0677;COG0743;COG1063
LBG_00694	hma:rrnAC3101	5.00E-36	K00599	trans-aconitate 2-methyltransferase	ko00624	Polycyclic aromatic hydrocarbon degradation	N/A	N/A	COG0500
LBG_00741	rha:RHA1_ro02535	9.00E-10	K00496	alkane 1-monooxygenase	ko00071	Fatty acid metabolism	EC:1.14.15.3	18685	N/A
LBG_00798	msm:MSMEG_6310	1.00E-12	K01041	glutaconate CoA-transferase, subunit B	ko00626	Naphthalene degradation	N/A	N/A	N/A
LBG_00885	ava:Ava_2685	2.00E-12	K01561	haloacetate dehalogenase	ko00625	Chloroalkane and chloroalkene degradation	EC:3.8.1.3	18785	N/A
LBG_00916	azo:azo3181	6.00E-58	K00001	alcohol dehydrogenase	ko00625	Chloroalkane and chloroalkene degradation	EC:1.1.1.1	0004022;0004023;0004024;0004025	COG0604;COG1062;COG1064;COG1454
LBG_00969	sco:SCO3245	5.00E-16	K00480	salicylate hydroxylase	ko00626	Naphthalene degradation	EC:1.14.13.1	18658	COG0654
LBG_01063	sml:Smlt3439	1.00E-110	K01061	carboxymethylenebutenolidase	ko00623	Toluene degradation	EC:3.1.1.45	8806	COG0412
LBG_01121	bme:BMEII0295	5.00E-39	K00462	biphenyl-2,3-diol 1,2-dioxygenase	ko00361	Chlorocyclohexane and chlorobenzene degradation	EC:1.13.11.39	18583	N/A
LBG_01151	smt:Smal_2777	0	K00257	isoquinoline 1-oxidoreductase	ko00626	Naphthalene degradation	N/A	N/A	COG1960
LBG_01158	hne:HNE_1435	7.00E-44	K00100	1-deoxy-D-xylulose-5-phosphate reductoisomerase	ko00625	Chloroalkane and chloroalkene degradation	N/A	N/A	COG0111;COG0677;COG0743;COG1063
LBG_01162	bra:BRADO1897	5.00E-50	K01253	microsomal epoxide hydrolase	ko00980	Metabolism of xenobiotics by cytochrome P450	EC:3.3.2.9	4301	COG0596
LBG_01189	pfl:PFL_4316	1.00E-114	K01564	haloalkane dehalogenase	ko00625	Chloroalkane and chloroalkene degradation	N/A	N/A	N/A

LBG_01204	btl:BALH_2771	2.00E-27	K00680	phospholipid:diacylglycerol acyltransferase	ko00626	Naphthalene degradation	N/A	N/A	N/A
LBG_01278	sml:Smlt3220	1.00E-122	K00799	glutathione S-transferase	ko00980	Metabolism of xenobiotics by cytochrome P450	EC:2.5.1.18	4364	COG0625
LBG_01289	rba:RB2355	7.00E-07	K00680	phospholipid:diacylglycerol acyltransferase	ko00626	Naphthalene degradation	N/A	N/A	N/A
LBG_01290	sml:Smlt3208	1.00E-125	K00799	glutathione S-transferase	ko00980	Metabolism of xenobiotics by cytochrome P450	EC:2.5.1.18	4364	COG0625
LBG_01293	lsl:LSL_1531	2.00E-17	K00100	1-deoxy-D-xylulose-5-phosphate reductoisomerase	ko00625	Chloroalkane and chloroalkene degradation	N/A	N/A	COG0111;COG0677; COG0743;COG1063
LBG_01322	smt:Smal_2609	0	K00257	isoquinoline 1-oxidoreductase	ko00626	Naphthalene degradation	N/A	N/A	COG1960
LBG_01381	ret:RHE_PE00074	1.00E-119	K01564	haloalkane dehalogenase	ko00625	Chloroalkane and chloroalkene degradation	N/A	N/A	N/A
LBG_01401	azo:azo3317	1.00E-104	K00001	alcohol dehydrogenase	ko00626	Naphthalene degradation	EC:1.1.1.1	0004022;0004023;0004024;0004025	COG0604;COG1062; COG1064;COG1454
LBG_01415	sub:SUB1360	8.00E-39	K00244	fumarate reductase flavoprotein subunit	ko00623	Toluene degradation	EC:1.3.99.1	19739	COG1053
LBG_01449	sml:Smlt2978	0	K10680	N-ethylmaleimide reductase	ko00633	Nitrotoluene degradation	EC:1.-.-.-	N/A	COG1902
LBG_01470	reh:H16_B2397	1.00E-36	K00680	phospholipid:diacylglycerol acyltransferase	ko00626	Naphthalene degradation	N/A	N/A	N/A
LBG_01567	azo:azo2619	6.00E-65	K00599	trans-aconitate 2-methyltransferase	ko00624	Polycyclic aromatic hydrocarbon degradation	N/A	N/A	COG0500
LBG_01596	azo:azo1939	1.00E-28	K00492	nitric-oxide synthase, bacterial	ko00626	Naphthalene degradation	N/A	N/A	COG0654
LBG_01599	fnu:FN1295	1.00E-05	K00680	phospholipid:diacylglycerol acyltransferase	ko00626	Naphthalene degradation	N/A	N/A	N/A
LBG_01600	bce:BC3378	3.00E-44	K00517	beta-carotene 15,15'-monooxygenase	ko00624	Polycyclic aromatic hydrocarbon degradation	N/A	N/A	COG2124
LBG_01795	lps:LPST_C2861	3.00E-08	K00244	fumarate reductase flavoprotein subunit	ko00623	Toluene degradation	EC:1.3.99.1	19739	COG1053
LBG_01799	reh:H16_A1070	1.00E-16	K01726	cyanate lyase	ko00626	Naphthalene degradation	N/A	N/A	N/A
LBG_01828	dru:Desru_2528	1.00E-94	K00121	S-(hydroxymethyl)glutathione dehydrogenase / alcohol dehydrogenase	ko00626	Naphthalene degradation	EC:1.1.1.284;1.1.1.1	0004327;0004022	COG1062
LBG_01848	mma:MM_1656	9.00E-54	K00100	1-deoxy-D-xylulose-5-phosphate reductoisomerase	ko00625	Chloroalkane and chloroalkene degradation	N/A	N/A	COG0111;COG0677; COG0743;COG1063
LBG_01880	aci:ACIAD2104	4.00E-30	K00100	1-deoxy-D-xylulose-5-phosphate reductoisomerase	ko00625	Chloroalkane and chloroalkene degradation	N/A	N/A	COG0111;COG0677; COG0743;COG1063
LBG_02004	xac:XAC0815	1.00E-114	K00599	trans-aconitate 2-methyltransferase	ko00624	Polycyclic aromatic hydrocarbon degradation	N/A	N/A	COG0500
LBG_02021	kfl:Kfla_1983	6.00E-06	K01563	haloalkane dehalogenase	ko00361	Chlorocyclohexane and chlorobenzene degradation	EC:3.8.1.5	18786	COG0596
LBG_02050	mbb:BCG_014	3.00E-12	K00680	phospholipid:diacylglycerol	ko00626	Naphthalene degradation	N/A	N/A	N/A

	4			acyltransferase					
LBG_02204	abo:ABO_0067	1.00E-122	K00100	1-deoxy-D-xylulose-5-phosphate reductoisomerase	ko00625	Chloroalkane and chloroalkene degradation	N/A	N/A	COG0111;COG0677;COG0743;COG1063
LBG_02263	gfo:GFO_2080	4.00E-14	K00680	phospholipid:diacylglycerol acyltransferase	ko00626	Naphthalene degradation	N/A	N/A	N/A
LBG_02287	gfo:GFO_2080	3.00E-14	K00680	phospholipid:diacylglycerol acyltransferase	ko00626	Naphthalene degradation	N/A	N/A	N/A
LBG_02304	sml:Smlt3734	1.00E-121	K00799	glutathione S-transferase	ko00980	Metabolism of xenobiotics by cytochrome P450	EC:2.5.1.18	4364	COG0625
LBG_02349	bcz:BCZK3126	6.00E-12	K00680	phospholipid:diacylglycerol acyltransferase	ko00626	Naphthalene degradation	N/A	N/A	N/A
LBG_02382	sml:Smlt2132	0	K00128	aldehyde dehydrogenase (NAD+)	ko00625	Chloroalkane and chloroalkene degradation	EC:1.2.1.3	4029	COG1012
LBG_02385	pen:PSEEN3116	5.00E-16	K00599	trans-aconitate 2-methyltransferase	ko00624	Polycyclic aromatic hydrocarbon degradation	N/A	N/A	COG0500
LBG_02410	bur:Bcep18194_B1532	0	K04091	alkanesulfonate monooxygenase	N/A	N/A	EC:1.14.14.5	8726	COG2141
LBG_02411	bcz:BCZK1828	1.00E-46	K00517	beta-carotene 15,15'-monooxygenase	ko00624	Polycyclic aromatic hydrocarbon degradation	N/A	N/A	COG2124
LBG_02451	pna:Pnap_0726	8.00E-15	K00114	alcohol dehydrogenase (cytochrome c)	ko00625	Chloroalkane and chloroalkene degradation	EC:1.1.2.8	N/A	N/A
LBG_02461	lsl:LSL_0995	2.00E-93	K00100	1-deoxy-D-xylulose-5-phosphate reductoisomerase	ko00625	Chloroalkane and chloroalkene degradation	N/A	N/A	COG0111;COG0677;COG0743;COG1063
LBG_02486	sml:Smlt2023	1.00E-130	K00799	glutathione S-transferase	ko00980	Metabolism of xenobiotics by cytochrome P450	EC:2.5.1.18	4364	COG0625
LBG_02571	buj:BurJV3_1585	1.00E-157	K00240	succinate dehydrogenase iron-sulfur protein	ko00623	Toluene degradation	EC:1.3.99.1	104	COG0479
LBG_02572	sml:Smlt1798	0	K00239	succinate dehydrogenase flavoprotein subunit	ko00623	Toluene degradation	EC:1.3.99.1	104	COG1053
LBG_02573	buj:BurJV3_1583	2.00E-60	K00242	succinate dehydrogenase hydrophobic membrane anchor protein	ko00623	Toluene degradation	EC:1.3.99.1	104	COG2142
LBG_02698	btl:BALH_2627	2.00E-19	K00680	phospholipid:diacylglycerol acyltransferase	ko00626	Naphthalene degradation	N/A	N/A	N/A
LBG_02749	bbt:BBta_3786	9.00E-09	K00100	1-deoxy-D-xylulose-5-phosphate reductoisomerase	ko00625	Chloroalkane and chloroalkene degradation	N/A	N/A	COG0111;COG0677;COG0743;COG1063
LBG_02766	buj:BurJV3_1348	1.00E-112	K00799	glutathione S-transferase	ko00980	Metabolism of xenobiotics by cytochrome P450	EC:2.5.1.18	4364	COG0625
LBG_02794	pfl:PFL_2342	1.00E-156	K00128	aldehyde dehydrogenase (NAD+)	ko00625	Chloroalkane and chloroalkene degradation	EC:1.2.1.3	4029	COG1012
LBG_02904	ajs:Ajs_0052	1.00E-83	K00492	nitric-oxide synthase, bacterial	ko00626	Naphthalene degradation	N/A	N/A	COG0654
LBG_03061	sds:SDEG_0903	6.00E-04	K00680	phospholipid:diacylglycerol acyltransferase	ko00626	Naphthalene degradation	N/A	N/A	N/A
LBG_03102	bme:BMEI1388	4.00E-34	K00100	1-deoxy-D-xylulose-5-phosphate reductoisomerase	ko00591	Linoleic acid metabolism	N/A	N/A	COG0111;COG0677;COG0743;COG1063
LBG_03124	aba:Acid345_2787	1.00E-20	K01061	carboxymethylenebutenolidase	ko00623	Toluene degradation	EC:3.1.1.45	8806	COG0412
LBG_03145	bme:BMEI1067	2.00E-05	K00680	phospholipid:diacylglycerol acyltransferase	ko00626	Naphthalene degradation	N/A	N/A	N/A
LBG_03204	bbt:BBta_4719	1.00E-117	K00100	1-deoxy-D-xylulose-5-phosphate reductoisomerase	ko00625	Chloroalkane and chloroalkene degradation	N/A	N/A	COG0111;COG0677;COG0743;COG1063
LBG_03219	mav:MAV_5078	2.00E-17	K00100	1-deoxy-D-xylulose-5-phosphate reductoisomerase	ko00625	Chloroalkane and chloroalkene degradation	N/A	N/A	COG0111;COG0677;COG0743;COG1063
LBG_03223	rle:RL0349	2.00E-72	K00799	glutathione S-transferase	ko00980	Metabolism of xenobiotics by cytochrome P450	EC:2.5.1.18	4364	COG0625

LBG_03354	reh:H16_B1699	2.00E-73	K00001	alcohol dehydrogenase	ko00626	Naphthalene degradation	EC:1.1.1.1	0004022;0004023;0004024;0004025	COG0604;COG1062;COG1064;COG1454
LBG_03359	sml:Smlt0950	1.00E-113	K00799	glutathione S-transferase	ko00980	Metabolism of xenobiotics by cytochrome P450	EC:2.5.1.18	4364	COG0625
LBG_03543	reh:H16_B0018	2.00E-27	K00680	phospholipid:diacylglycerol acyltransferase	ko00626	Naphthalene degradation	N/A	N/A	N/A
LBG_03592	gbe:GbCGDNIH1_1792	1.00E-90	K00599	trans-aconitate 2-methyltransferase	ko00624	Polycyclic aromatic hydrocarbon degradation	N/A	N/A	COG0500
LBG_03619	bra:BRADO5603	3.00E-59	K05915	2,4'-dihydroxyacetophenone dioxygenase	ko00626	Naphthalene degradation	N/A	N/A	N/A
LBG_03641	bbt:BBta_6889	4.00E-30	K00799	glutathione S-transferase	ko00980	Metabolism of xenobiotics by cytochrome P450	EC:2.5.1.18	4364	COG0625
LBG_03699	bpm:BURPS1710b_A2071	0	K01041	glutaconate CoA-transferase, subunit B	ko00626	Naphthalene degradation	N/A	N/A	N/A
LBG_03710	pdx:Psed_6064	8.00E-40	K01856	muconate cycloisomerase	ko00623	Toluene degradation	EC:5.5.1.1	18849	N/A
LBG_03716	aci:ACIAD3598	1.00E-116	K00120	glucose-fructose oxidoreductase	ko00626	Naphthalene degradation	N/A	N/A	COG1979;COG2141
LBG_03846	smt:Smal_0220	0	K00257	isoquinoline 1-oxidoreductase	ko00626	Naphthalene degradation	N/A	N/A	COG1960
LBG_03886	aci:ACIAD2050	2.00E-14	K00680	phospholipid:diacylglycerol acyltransferase	ko00626	Naphthalene degradation	N/A	N/A	N/A
LBG_03890	xac:XAC2121	6.00E-53	K00599	trans-aconitate 2-methyltransferase	ko00624	Polycyclic aromatic hydrocarbon degradation	N/A	N/A	COG0500
LBG_03903	sml:Smlt0206	1.00E-168	K01563	haloalkane dehalogenase	ko00361	Chlorocyclohexane and chlorobenzene degradation	EC:3.8.1.5	18786	COG0596
LBG_03940	vfi:VF_0102	2.00E-06	K00680	phospholipid:diacylglycerol acyltransferase	ko00626	Naphthalene degradation	N/A	N/A	N/A
LBG_04012	rba:RB2355	2.00E-06	K00680	phospholipid:diacylglycerol acyltransferase	ko00626	Naphthalene degradation	N/A	N/A	N/A
LBG_03531	gga:423169	4.00E-26	38	120	300	10	192	137	326
LBG_04033	buj:BurJV3_0027	0	K13953	alcohol dehydrogenase, propanol-preferring	ko00626	Naphthalene degradation	EC:1.1.1.1	4022	COG1064
LBG_04076	gox:GOX0476	6.00E-85	K00100	1-deoxy-D-xylulose-5-phosphate reductoisomerase	ko00625	Chloroalkane and chloroalkene degradation	N/A	N/A	COG0111;COG0677;COG0743;COG1063
LBG_04077	sml:Smlt4679	1.00E-103	K00799	glutathione S-transferase	ko00980	Metabolism of xenobiotics by cytochrome P450	EC:2.5.1.18	4364	COG0625
LBG_04083	sml:Smlt4673	1.00E-99	K07323	putative toluene tolerance protein	N/A	N/A	N/A	N/A	COG2854
LBG_03326	mth: MTH234	7.00E-04	K01607	4-carboxymuconolactone decarboxylase	ko00362	Benzoate degradation	EC:4.1.1.44	N/A	COG0599
LBG_02752	lsa:LSA1776	1.00E-10	K01607	4-carboxymuconolactone decarboxylase	ko00362	Benzoate degradation	EC:4.1.1.44	N/A	COG0599

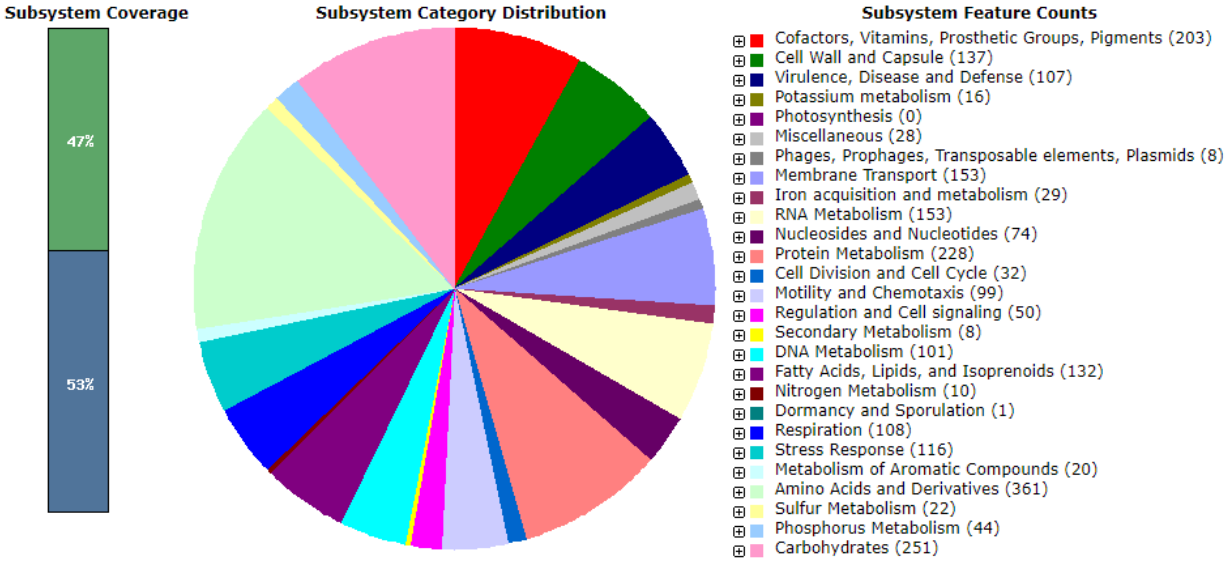


Figure 33. RAST Annotation of *Stenotrophomonas* sp. ATCM1_4

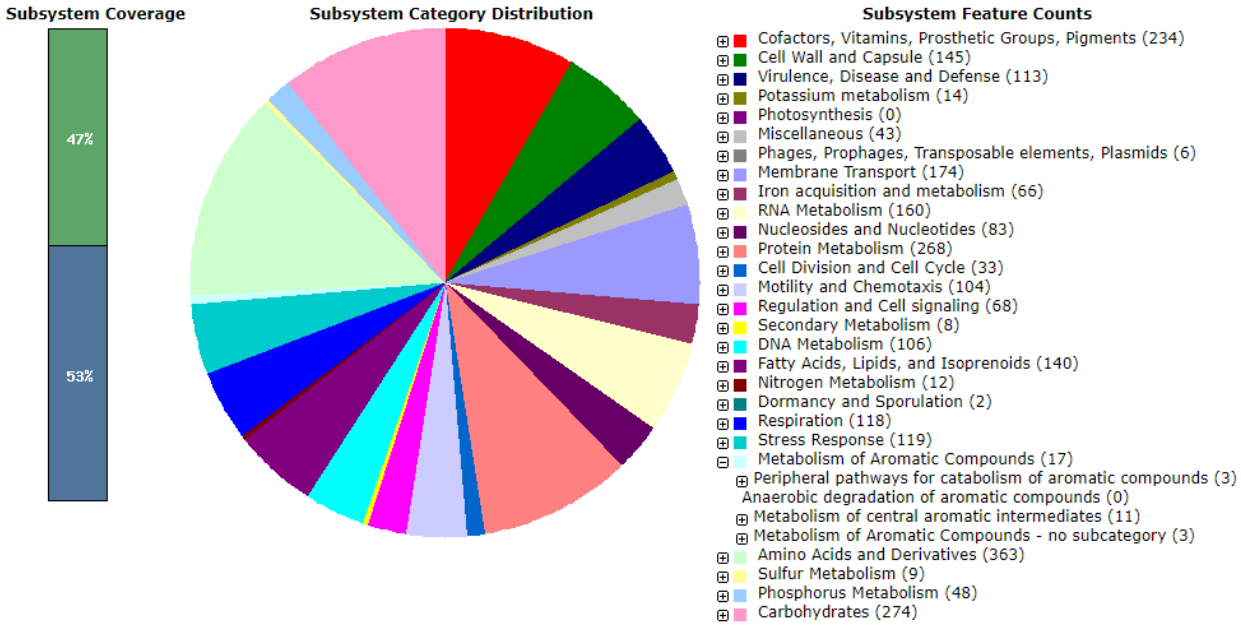


Figure 34 Rast Annotation of Stenotrophomonas maltophilia SVIA2

CHAPTER FOUR

Discussion

4.1 Isolation and identification of *Stenotrophomonas* species

Stenotrophomonas species are ubiquitous, they can be found in a wide range of environment such as soil, Plant rhizosphere, human and extreme environments. *Stenotrophomonas* species can survive in many environments because of their versatile metabolic ability (Ryan et al., 2009). In this study, we isolated 48 strains of *Stenotrophomonas* species from different habitats in Mexico using two isolation methods as stated earlier in the methodology. The habitats include sewage, contaminated soils, agricultural soil, crude oil contaminated soil, Plant rhizosphere, sewage and textile effluents and hospital environment. Several researches have reported isolation of Stenotrophomonas species from related sources (Brooke 2012; Fülöp et al. 2001; M. Lee et al. 2011a; Ryan et al. 2009).

Although some media are now available for the isolation of *Stenotrophomonas* species, none of them has a conventional acceptance for the isolation of *Stenotrophomonas* species. We noted 70% *Stenotrophomonas* recovery rate from environmental samples, with *Stenotrophomonas* SVIA agar (Himedia, India). However, the medium which contains tryptic soy agar supplemented with 20mg/ml Methionine and 16mg/L Imipenem 5mg/L Amphotericin B and 2.5 mg/L Vancomycin yielded 100% *Stenotrophomonas* recovery rate from environmental samples. It can be deduced that SVIA agar (Himedia) is appropriate for the isolation of *Stenotrophomonas* species but a freshly formulated medium such as contain tryptic soy agar supplemented with 20mg/ml Methionine and 16mg/L Imipenem 5 mg/L Amphotericin B and 2.5 mg/L Vancomycin may be more appropriate for quick isolation of *Stenotrophomonas* species.

We identified isolates using three different approaches (Phenotype, MALDI-TOF and 16S rRNA sequencing) to ascertain their appropriate identity of each isolate. The identity of isolates based on biochemical characteristics confirmed 54 environmental isolates as members of the genus *Stenotrophomonas*. However, the use of MALDI-TOF technique which based on bacterial cell membrane protein profiling analysis (Sogawa et al. 2011) revealed that only 36 out of the environmental isolates were member of the genus *Stenotrophomonas*. To reach a consensus, we amplified and sequenced the 16S rRNA gene fragments of the isolates. 48 environmental isolates and 9 recovered from the hospital environment were confirmed to be the members of the genus *Stenotrophomonas* via the sequencing of the 16S rRNA gene fragments, the blast search and phylogenetic analysis of these fragments. The isolates comprise two *Stenotrophomonas* spp., one

Stenotrophomonas nitritireducens, one *Stenotrophomonas* sp. sharing its identity with *S. acidaminiphila* and *S. nitritireducens*, and other clusters as *S. maltophilia*. The higher rate of recovery of *S. maltophilia* in comparison with the other members of the genus confirmed the abundance of the *S. maltophilia* more than the other members of the genus in the environment. (Alavi et al., 2014a; Brooke, 2012; Martínez et al., 2015).MALDI-TOF worked successfully in identifying the isolates, even though it could not correctly identify some strains as *Stenotrophomonas* that were confirmed to be not *Stenotrophomonas* after sequencing their 16S rRNA regions. The inability of MALDI-TOF to identify some *Stenotrophomonas* strains in this study may be due to the limited information on MALDI-TOF since most of the isolates were retrieved from the environment.

The phenotypic characteristics of isolates in this study revealed that the environment could influence the behavior of bacteria. Some *S. maltophilia* strains retrieved from sewage possess the phenotype, which differs from those traits typical for *S. maltophilia* (Table 7). Such phenotypes include the use of arabinose, mannitol as sole carbon source and varied utilization of citrate and trehalose. Bacteria have potential to evolve special phenotype if they find themselves in fluctuating ecosystem as a means of survival (Cooper & Lenski, 2000). For example, complex intracellular *S. maltophilia* strains have been isolated from Amoeba, and micro *S. maltophilia* colonies with special adaptation for the internal environments of the lungs in cystic fibrosis patients, were previously reported (Corsaro et al., 2013; Vidigal et al., 2014)

4.2 Antimicrobial resistance and Biofilm formation

Stenotrophomonas species are intrinsically resistance to a wide range of antimicrobial agents(Hu et al. 2012; Ryan et al. 2009; Sanchez, Hernandez, and Martinez 2009). Antimicrobial susceptibility testing is thus an important phenotypic characteristic for the genus. The result of antimicrobial susceptibility testing for *Stenotrophomonas* species in the study confirmed their multi-resistance property. The susceptibility pattern showed that most isolates were multi-resistant with each exhibiting more than 50% resistance to at least three antibiotics tested with an average multiple antibiotic resistance indexes (MARI= $\frac{\textbf{Number of Antibiotics to which bacteria is resistant}}{\textbf{Number of Antibiotics tested}}$) being around 50%. The pattern of resistance to antimicrobial agents tested in this study varied according to antibiotic families and generations.

Another important observation in this study is that the isolated *Stenotrophomonas* species showed high rate of resistance to sulfamethoxazole-trimethoprim (SXT) (83.3%) (Table 10 and

11). SXT is the primary drug recommended for the treatment of infections associated with *Stenotrophomonas* species (Brooke, 2011; Denton and Kerr, 1998). The rate of resistant of *S. maltophilia* to SXT has been said to vary with geographical distribution (Chung et al., 2012; Neela et al., 2012; Samonis et al., 2012; Wu et al., 2012; Farrell et al., 2010; Valdezate et al., 2001). A SENTRY surveillance study reported 3.8% resistance to SXT by *S. maltophilia* (Fedler, Biedenbach, and Jones 2006). In the SENTRY surveillance program in Europe between 1997 and 1999, the rate at which *S. maltophilia* resisted SXT in Europe was recorded to be around 10% (Gales, 2001). The rate of resistance in Latin America was 4.5% (Gales et al. 2001). A recent study from Mexico reported *S. maltophilia* resistance to SXT as 32.8%. (Flores-Treviño *et al.*, 2014). The observation from the study of Trevino indicated that there is a high rate of *S. maltophilia* resistance to SXT in Mexico. We isolated *S. maltophilia* from the environment (Soil, Sewage, and Fish tumor), evaluated their susceptibility to SXT using the CLSI guideline, and noted a high rate of resistance of around 80%. This high incidence of resistance revealed that some factors from the environment could contribute to resistance development by *S. maltophilia* (Andersson & Hughes, 2011). It also implies that SXT may no longer be the best drug of choice for the treatment of *S. maltophilia* infections. Other antimicrobial agents such as ofloxacin and levofloxacin, which has about 100% rate of susceptibility by the tested isolates, may be considered for clinical trials. Another important observation in this study is a low level of resistance to ceftazidime, (12) 26% in contrast to previous reports. Tanimoto reported 84.8% resistance to ceftazidime in a study on the susceptibility and genomic variability of clinically isolated *S. maltophilia* species in Japan (ref, 2013). In addition, Çıkman, *et al*., in a study in turkey reported 72% resistance to ceftazidime in clinically isolated *S. maltophilia* strains (Çıkman et al. 2016). Similarly, a multi-center surveillance study in Taiwan, reported 70% *Stenotrophomona*s' resistance to ceftazidime (Wu *et al*., 2012). Ceftazidime resistance reported in other studies by *Stenotrophomonas* species may be associated with previous exposure to ceftazidime because these studies focused on hospital isolates. The isolates in this study were of environmental origin and may be the reason for the low resistant profile noted against treatment with ceftazidime. (i.e., resistance to ceftazidime may be inducible). This fact, however, emphasizes the importance of the controlled use of antibiotics either in hospital setting or in the neighborhood.

Several mechanisms are involved in the resistance of *S. maltophilia* species to antibiotics. These mechanisms include the intrinsic expression of beta-lactamases (Nicodemaz and Paez, 2007)

which confer on them resistant to beta-lactam antibiotics. Two beta-lactamase enzymes in the genomes of many *Stenotrophomonas* species (L1, which is a metallo-β-lactamase with a broad substrate spectrum including carbapenems, and L2, a molecular class A, functional class 2e β-lactamase, Avison et al., 2001) are responsible for their intrinsic resistance to beta-lactam antibiotics. In addition to the possession of L1 and L2 beta-lactamase in their genomes, *Stenotrophomonas* also possess other mechanisms through which they resist beta-lactamases. Other genes such as the possession $bla_{NDM-1,}$ $bla_{PER,}$ bla_{TEM} and bla_{shv}, which are associated with extended beta-lactamase resistance in bacteria, have been reported responsible for *Stenotrophomonas'* resistance to beta-lactam antibiotics particularly the carbapenems (Liu et al., 2012; Conesa et al., 2005; Pedro, Furlan, Pitondo-silva, & Stehling, 2018) . We investigated factors that may be responsible for the observed resistance in the isolated strains by amplifying some of the genes known to be responsible for *Stenotrophomonas*' resistant to beta-lactam antibiotics ($bla_{TEM,}$ bla_{SHV} bla_{NDM-1}). Only 55% of the isolated strains possess any of the beta lactamase genes screened. The result from this assay showed that resistance to beta-lactam antibiotics might not only be due to the presence of the amplified gene. It may involve some other beta-lactamases such as the L1 and L2 or other mechanisms such as the activities of different efflux pumps present in their genomes (Al-Hamad, Burnie, and Upton 2011; Alonso and Martinez 2000; Crowder et al. 1998). *Stenotrophomonas* are not only resistant to beta-lactam antibiotics, they also exhibit resistance to other antibiotics such as tetracycline, chloramphenicol, fluoroquinolones and Trimethoprim-Sulfamethoxazole (Anvarinejad et al. 2016; Chang et al. 2015; Flores-Treviño et al. 2014; Jia et al. 2015).

Isolates showed a high rate of resistance to the two types of tetracycline ($tetracycline^R$ and Doxycline) tested in this study. Due to limited resources availableon the molecular basis for the resistance to all the tetracycline, tetA and tetB genes was tested by amplification, which have been reported to confer resistance on bacteria against tetracycline (Roberts 2012). Only one of the isolates (ATCM1_4) displayed the presence of tetB gene in its genome and none of the isolates possess tetA genes. Resistance to tetracycline by the isolates in this study may be due to other mechanisms or other resistant genes such as tetH and tetW (Roberts 2012). Another important of observation in this study is the high rate of resistance in isolates against SXT. SXT is the drug of choice for the treatment of *S. maltophilia* associated infection. It is therefore important to understand the molecular basis of isolates resistant to SXT. It was, however, unfortunate that we could not investigate the molecular mechanisms involved in isolates

resistance to SXT, but it is possible that the isolates employ one of the studied mechanisms of *Stenotrophomonas* resistance to SXT. These mechanisms include the expressions of resistance through integrons such as the Sul3 gene which confers on them resistance to SXT (Grape, 2005), transposons, and Plasmid. Sul 1, Sul2, and Sul3 genes (if you have some genes or transposons, plasmids in your strains, please write here with related information of mechanisms. If not, please do not say too much in this paragraph) have been extensively studied and identified as important genes in *Stenotrophomonas* through which they resist the activities of SXT ((Adesoji, Ogunjobi, and Olatoye 2016; Chung et al. 2015) As described earlier *Stenotrophomonas* possess array of mechanism through they resist different antimicrobial agents, and these may be responsible for the identified resistance to most of the antimicrobial agents. Although there are limited resources for the analysis of the complete genome of all the strains isolated in this study, the analysis of the complete genome of six sequence for the presence of resistant genes revealed that they possess plethora of genes associated with the bacterial resistance to antimicrobial agents. We noted the presence of various efflux pump related genes in the isolates. They include SmeA, SmeB, SmeC, SmeD, SmeE, SmeF, SmeR AAC, AdeF. SmeDEF RND is a membrane fusion protein, resistance nodulation division MDR efflux which was identified by Alonzo and Martínez, 2000 as one of the efflux systems responsible for the resistance in *Stenotrophomonas maltophilia* D457R to chloramphenicol, quinolone, tetracycline, trimethoprim-sulfamethoxazole, and macrolides. SmeDEF has since been reported in other *S. maltophilia* strains. SmeDEF has been reported not to be associated with resistance to aminoglycosides (Huang, et al., 2017). The SmeABC and SmeDEF efflux system were identified to be responsible for the extrusion ciprofloxacin and meropenem by *S. maltophilia* (Chang *et al*., 2004). The overexpression of SmeVWX and SmeDEF efflux system was reported as the reason for observed resistance to quinolone in clinical S. maltophilia isolates (Garcia, et al., 2015). Other RND type MDR efflux system has been linked with resistance to other antibiotics, for example SmeYZ contributes to the resistance in *S. maltophilia* to aminoglycoside and trimethoprim-sulfamethoxazole (Lin et al., 2015) while SmeOP-TOlCsm extruded nalidixic acid, doxycycline, amikacin, gentamicin, erythromycin, leucomycin, carbonyl cyanide 3-chlorophenylhydrazone, crystal violet, sodium dodecyl sulfate, and tetrachlorosalicylanilide from the cell of *S. maltophilia* (Lin, et al., 2014). SmeIJK is responsible for the intrinsic resistance of *S. maltophilia* to gentamicin, amikacin, tetracycline, minocycline, ciprofloxacin, and leucomycin. SmeIJK also associated with acquired resistance to levofloxacin, when overexpressed alone or in coordinate hyperproduction with

SmeYZ (Crossman *et al*., 2008; Gould, Okazaki, &Avison, 2012; Huang, *et al*., 2014; Wang *et al*., 2015).

Biofilm formation is a phenotypical mechanism through which bacterial resist a harmful agent or harsh environment. Biofilm is formed by the production of extracellular slime or glycocalyx with which they attach to a surface(G. Di Bonaventura et al. 2004). *Stenotrophomonas* species have been associated with different infection via their attachment to abiotic surfaces, thus enhances their resistance to antibiotics (G. D. I. Bonaventura et al. 2007). The analysis of the biofilm formed in this study showed that most isolates could form biofilms. Variation in the genetic makeup of bacteria can give insight into the differences in their phenotypes such as pathogenicity, geographical distribution, antibiotic resistance, carbohydrate metabolism, and virulence, even though they are the same species (Andrei and Zervos, 2006).

4.3 Molecular Typing

Bacterial typing could can give insight into the ecology and dynamics of bacterial population Li *et al*., 2009). Genotypic typing technique is more useful than phenotypic typing for bacteria because phenotypic markers are not very stable and genotypic typing does not suffer from the setback of altered convergent evolution (Goncalves, et al., 2014**).** There are many genotypic techniques available for the in-depth analysis of species. We analyzed the genetic diversity of *Stenotrophomonas* species recovered from soils, sewage and different infection sites in hospitalized patients.

Several studies have shown that *S. maltophilia* in CF patients and other environment displayed high genetic diversity (Vidigal *et al*, 2014; Wu *et al*., 2011; Pompilio et al., 2011a; Marzuillo *et al*., 2009; Nazik *et al*., 2007; Valdezate *et al*., 2001b; Denton *et al.*, 1998). (Denton et al) reported to use ERIC-PCR technique for typing analysis of 45 *S. maltophilia* recovered from 41 pediatric cystic fibrosis patients (ref., 1998). ERIC-PCR was adopted to characterize and type strains in our test. Another study from Spain used ribotyping technique and pulse field gel electrophoresis as typing tool on 76 *S. maltophilia* strains recovered from 25 cystic fibrosis patients. DNA restriction enzyme digestion using XbaI was applied to identify 47 PFGE profiles (SID value of 0.97) whereas when digested with HindIII (SID value of 0.899) and BamHI (SID value of 0.915), 21 and 20 ribotypes were detected, respectively (Valdezate et al., 2001b). In addition, the use of random amplified polymorphic DNA (RAPD-PCR) for the analysis of 11 *S. maltophilia* recovered from CF patients in turkey yielded 9 RAPD types (Nazik *et al*., 2007).

Recently, Vidigal *et al* (2014) employed DiversiLab (DL) system and ERIC-PCR typing technique for analyzing the genomic diversity of *S. maltophilia* recovered from the clinic and the environment (ref). They analyzed 102 isolates with semi-automated DL and 9 with ERIC-PCR. Their result revealed 70 distinct types among which are 30 singletons. The ERIC-PCR, however, gave it distinct type with strains recovered from the ICU clustering separately. The observation from their results make ERIC-PCR typing seems like an aberration to previous studie but a singular study will not be enough to disprove previous report (Vidigal et al, 2014). The ERIC-PCR analysis of the *Stenotrophomonas* isolates in this study conform with previous report in which *Stenotrophomonas* species were highly heterogeneous. We noted 37 distinct types with three singletons from the ERIC-PCR. However, no band pattern was produced with REP-PCR from these isolates. Bacterial exposure to an unfavorable environment can induce niche adaptation, which could have effect on their molecular make-up (Vidigal et al., 2014). It is thus possible that the distinct types presented by the isolates were due to various environmental adaptations. The outcome from our finding is in concordance with the report of Denton *et al* where they noted 41 distinct ERIC-PCR types and with the report from Spain in which PFGE yielded 47 distinct PFGE types where XbaI was the restriction enzyme. This result further emphasizes the heterogeneous nature of *Stenotrophomonas* species. In a related observation, the phylogenetic analysis of the isolates using the sequences of 16S rRNA showed that isolates have close phylogeny with specific adaptation as some strains from soil clustered on a branch of the phylogenetic tree.

4.4 Hydrocarbon Tolerance and Degradation

Stenotrophomonas species are versatile bacteria because of their ability to grow and survive in different environments (Ryan et al., 2009). The versatility of *Stenotrophomonas* species has resulted in their recovery from different environments including those that are toxic to bacteria. Such environments include PAH contaminated soil, acidic lake and some other sites in nature (Samanta, *et al*., 2002; Chen, et al., 2010; Brooke, 2012). In this study, five strains (ASS1, ASS2, Pemsol, SVIA1 and SVIA2) of *Stenotrophomonas* were isolated from crude oil contaminated soil from Tabasco, Mexico and three strains (TepeL, Tepegreen and Tepe) from Textile effluents in Tepettila, Tlaxcala, Mexico. Their survival potential in different concentration of PAHs (Naphthalene, Phenanthrene, Phenanthridine, Biphenyl, Xylene, Anthracene, and Anthraquinone) and four Textile dyes (Acid blue, Reactive black, Brilliant blue and Brilliant Yellow dye, (Sigma Aldrich, USA)) were tested respectively. We noted that these

isolates grew effectively on Bushnell Hass medium, (minimal medium) with each PAH as the sole energy source in different concentrations. The isolates recovered from textile dyes grew and decolorize textile dyes added to glucose-peptone-sodium chloride broth. *Stenotrophomonas* species are highly metabolically diverse and have been recovered from diverse environment and employed for bioremediation purposes (Ryan et al., 2009; Mangwani *et al*., 2014). *S. maltophilia* with potentials to decolorize Azo dye was reported by Galai, Limam, & Marzouki, 2009. The versatile nature of *Stenotrophomonas* makes them important tool for biotechnological applications. Many studies have reported the use of *Stenotrophomonas* spp in the degradation of PAHs and other xenobiotics (Boochan, Britz &Stanley, 1998; Guan et al, 2008; Chen et al., 2010 Gao et al; 2013; Mangwani, et al., 2014). Magwani et al., 2014 reported the degradation of PAH by *S. acidaminiphila* NCW- 702 biofilm and planktonic formation. The biofilm from *S. acidaminiphila* NCW- 702 enhanced the degradation of PAH by 71.1% ± 3.1% in contrast to the 38.7% ± 2.5% in 7days degradation study (Mangwani, et al., 2014). Similarly, Gao et al reported *Stenotrophomonas*' degradation of Phenanthrene via multiple metabolic degradation pathways (Gao et al., 2012). They reported that *S. maltophilia* C6 is capable of completely degrading phenanthrene via multiple pathway with the production of protocatechuate as one of the final metabolites formed.

The degradation of PAH by the isolates from this study was evaluated using naphthalene as a PAH compound. The strain *Stenotrophomonas* sp. Pemsol completely degraded naphthalene after 30 days of degradation study. The GC-MS analysis revealed the lost in the peak corresponding to naphthalene while the peak persists in the control. The FTIR spectrometry following 15^{th} day of degradation study revealed that the degradation of naphthalene may involve the formation of 1, 2 dihydroxy naphthalene because of the introduction of an OH peak on the experimental intermediate product, which is absent in the control. The degradation of naphthalene and phenanthrene by *Stenotrophomonas* and other bacteria has been reported to involve the production of 1, 2-dihydroxy naphthalene in some degradation pathway (Eaton & Chapman, 1992; Gao *et al*., 2012). Similarly, the complete degradation of naphthalene by *Stenotrophomonas* sp. Pemsol revealed the formation of catechol as the major metabolite. The GC-MS and UPLC MS/MS analysis of the degradation product after the 30^{th} day of experimental studies showed that naphthalene was completely degraded and a peak corresponding in molecular weight (110.03) to that of catechol was observed. Several studies have reported that the degradation of PAH by bacteria usually involve the formation of salicylic acid or catechol

(Eaton &Chapman, 1992; Chen et al., 2010; Mangwani, et al., 2014). *Stenotrophomonas* sp. Pemsol like previously reported *Stenotrophomonas* species (Mangwani, et al., 2014, Urszula, 2009; Tebyanian, 2013) successfully degraded PAH.

Bacteria employ various mechanisms for the degradation of PAH; one of such mechanisms is the production of bio-surfactants. Biosurfactants are substances produced by bacterial species, which allow them to access to PAH and other hydrocarbons trapped on surfaces (Das & Chandran, 2011). Several methods could be employed to determine bacterial potential of producing bio-surfactants. They include bio-emulsification assay, cell droplet analysis and micelle formation test (Das & Chandran, 2011). We evaluated the possibility of using biosurfactant production for the uptake of hydrocarbon by our isolates using the bio-emulsifying assay. Analysis of the emulsification process in the strains showed that surfactant production may be a strategy for the degradation of PAH in four of the isolated strains (ASS1, ASS2, SVIA1, and SVIA2) while Pemsol may not use bio surfactant production for the uptake of PAH, as it could not emulsify the tested hydrocarbons. Singh *et al*., 2015 reported the degradation of pyrene by bio surfactant producing *S. maltophilia* BR-12. This may imply that *S. maltophilia* uses bio surfactant as a mechanism for degradation. The inability of Pemsol to form surfactant indicated that biosurfactant production is not the precondition but likely one of other mechanisms for PAH degradation.

The Phenotypic characteristics of bacteria and other organism is associated with their genetic makeup. Many studies for example have shown that resistance in bacteria to antimicrobial agents has link with gene possession and expression. In *Stenotrophomonas maltophilia* for example, intrinsic resistance was attributed to the presence of the inducible beta-lactamase L1 and L2 genes in their genome (Flores-Trevino *et al*., 2014). Similarly, microbial degradation of PAH has been linked with the genes in their genomes such as the dioxygenases and dehydrogenase (Das and Chandran, 2012). In a bid to understand the mechanism associated with the reported phenotypes in SVIA2 ASS1, TepeL, ATCM1_4 and Pemsol, we sequenced and analyzed their genomes.

4.5 Whole genome Sequencing and Analysis

Whole genome sequencing has given many insights to the understanding of the underlying mechanisms associated with bacterial behavior and phenotype (Schneiker, *et al*., 2006; Das et al., 2014 Pals *et al*., 2017).

The *de novo* assembly of the genome with Spades of the genomes (Pemsol, ASS1, SVIA2 TepeL and ATCM1_4) resulted in 62, 42, 1, 109, 100 2016 contigs respectively. The contigs were reduced to 1, 1, 5, and 8 scaffolds and contigs respectively. The reduction of all except ASS1 the initial genome contigs to one scaffold was achieved using the MeDuSa genome scaffolder (Bosi et al., 2015) with *Stenotrophomonas maltophilia* K279a as the reference genome and *Stenotrophomonas nitritireducens* 2001 as the reference genome in the case of ATCM1_4. The reduction of ASS1 into one contig was achieved manually with Consed v29.0 (Gordon & Green, 2013) by aligning and manually joining its contigs. MeDuSa has been used to obtain improved contigs from different bacterial genome (Aliyu, et al., 2016; Furi et al., 2016). Medusa with the help of a reference genome provide an easier platform for the aligning and joining of short reads. Analysis of the obtained reads via NCBI blasts showed that the reduced contigs were complete.

Annotation of bacterial genome usually provides information about the genes they possess and insight into the relationship between their genetic make-up and their phenotype. Several methods can be used for the annotation of bacterial genome. They include the use of Stand-alone software such as Prokka (Seaman, 2014), RASTik (Aziz et al., 2008) or web-based databases such as Rapid Annotation using Subsystem Technology (RAST) (Aziz et al., 2008), Bacteria automated annotation server (Basys) automated annotation server, (Van Domselaa, 2005) and solicited annotator such as the NCBI Prokaryote Genome Annotation pipeline (PGAP). We used both Prokka and PGAP for the annotation of the sequenced genome. The ease of manipulating the stand-alone annotation pipelines make them useful for the annotation of bacterial genome. This usually makes it faster to have annotation result unlike web-based servers that may be over loaded and, thus, delay the annotation process. PGAP was employed for the annotation of the sequenced strain as quality control measure for the Prokka based annotation. The analysis of the two annotation methods showed that there are no significant differences in their results. The annotation predicted between 3.9Mb-4.5Mb protein coding sequences for the genomes.

The functional annotation of the genomes was carried out to determine the metabolic function of the isolates and to compare their phenotype with their genetic make-up. The functional annotation of the genome was evaluated by determining the COG categories of the isolates and the KEGG functions. The COG and KEGG function of the isolates was evaluated via a web-based functional annotation server called the WEBMGA, which is a customizable web server for fast metagenomic sequence analysis (Wu et al., 2011). The analysis of the bacterial COG

categories revealed the presence of gene in the cluster of orthologous group A, B C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, S, T, U, V, X, and Z. The isolates showed the abundance of COG category with the function unknown (S), Transcription (K), others include amino acid transport and metabolism, secondary metabolism and transport categories. The abundance of transport categories may be associated with their adaptation mechanism. Possession of many metabolic genes by the isolates could be due to the versatile nature of the bacteria. The comparison of the cluster of orthologous genes in isolates showed that all the isolates and the reference strain *S. maltophilia* K279a shared 2435 orthologous gene clusters. The orthologous gene analysis showed that ATCM1_4 has the highest number of unique genes orthologues, which implies that ATCM1_4 may obtain more genes by horizontal transfer for adaptation. Niche adaptation has been reported in many *Stenotrophomonas* species (Ochoa and Vinueza, 2017; Vidigal, et al., 2014). Such adaptation may involve genetic manipulations, which confer on them traits for survival. This type of genetic modification may result in speciation, thus resulting in the evolution of new species. New *Stenotrophomonas* species have been isolated from specialized habitats such as the bentonite, stone chamber interior, indicating the possibility of environmentally stimulated speciation (Handa et al. 2016; Sánchez-Castro et al. 2017; Weber et al. 2018).

Phages often play a role in the survival or adaptation strategy of *Stenotrophomonas* species. Lee et al., 2014 isolated a temperate phage Smp131 from *Stenotrophomonas maltophilia* T13 (C. N. Lee et al. 2014). Similarly, a bacteriophage DLP5 was isolated from *S. maltophilia* D1614. The phage was found to be playing major role in its host survival (Peters and Dennis 2018). Prophages are synonymous with *Stenotrophomonas* as most of them usually possess phage-originated sequences in their genome (C. N. Lee et al. 2014). In our study, different prophage sequences in the genomes of the sequenced strains were identified. The analysis of the prophage sequences revealed that some of them are important for the adaptation of their hosts. In strain *Stenotrophomonas* sp. Pemsol, for example, we identified one intact prophage which characterization revealed to be having an oxidoreductase activity. Oxidoreductase enzymes are one of the important enzymes essential for the degradation of hydrocarbon (Brooijmans, Pastink, and Siezen 2009). We identified four prophages in ATCM1_4, two for SVIA2, one in TepeL, one in Pemsol and one in ASS1.

Adaptation in bacteria can be influenced by the variation in their environment, also known as selective pressure. Bacteria employ various mechanisms to cope with it. One of such mechanisms is the acquisition of genes encoding features that enhance the survival. This may include mobile genetic elements such as transposon, insertion sequence and complete gene island, also known as genomic island, all of which can be obtained by conjugation (Lacour, Gaillard, and Van Der Meer 2006). Many new features that contributes to the survival of bacteria have been recovered from different genomic islands. The genomic island has been described as the bacteria ecological fitness center through which they adapt to various environment (Hacker and Carniel 2001). *Stenotrophomonas* species enjoys interaction with other bacteria and viruses in their environment. In this study, we identified genomic island regions in the sequenced *Stenotrophomonas* species ranging from 19 in strain ASS1, 22 in ATCM1_4, 25 in SVIA2. The analysis of the genes in the genomic island of the isolates revealed that some of the genes were important for adaptation to their environment. AzoR, an azoreductase gene reportedly known to be involved in the decolorization of azo dyes in *Pseudomonas aeruginosa* (Saratale, *et al*., 2011) was found in the genomic island of TepeL. TepeL demonstrated an effective capability for the decolorization and the degradation of Azo dyes. Similarly, in ASS1, Pemsol, and SVIA2 there are several genes which are associated with hydrocarbon degradation such as glycosyl transferase, 2,3-dihydroxybenzoate-dehydrogenase, and 3-oxoacyl-[acyl-carrier-protein] reductase which act on the CH-CH bond in siderophores (Young and Gibson, 1969).

The comparative analysis of the sequenced strains using MAUVE alignment showed that isolate genomes have the same orientation as the reference strains. The average nucleotide identity analysis, however, revealed that four (ASS1, Pemsol, TepeL and ATCM1_4) out of the isolates may likely be novel while SVIA2 is *Stenotrophomonas maltophilia.* This is because the average nucleotide identity of the four strains were below the threshold (>93) (Konstantinidis, & Tiedje, 2005) for bacteria of the same species. It will therefore be important to verify the species status of these strains with further experiment. Similarly, the genome-to-genome distance Calculator which works effectively as the DNA-DNA hybridization (Auch et al., 2005), confirmed that these strains are likely new species in the genus *Stenotrophomonas.* They shared over 2000 core genes with other members of the species. The pan genome analysis using the power fit showed that the pan genome is still open, indicating that the species in the genus are very diversified. The phylogenetic tree generated by the pan genome concatenation showed that there is closer

relationship between isolates with the capacity to degrade hydrocarbon as they originate from the same branch of the phylogenetic tree.

The genetic basis for the degradation of PAH as observed in the experimental assay in Pemsol and the tolerance assay of other strains (ASS1 and SVIA2) was determined by metabolic pathway analysis on WEBMGA. The analysis revealed that there are 147 genes in Pemsol for n the degradation of xenobiotics and PAHs. Some of the genes include alcohol dehydrogenases for cleaving PAH aromatic rings and other genes which are involved in the peripheral pathway for the degradation of PAH like acetyl coA transferase. Nine genes encod lactoylglutathione lyase family enzyme in COG0346, which has been reported to be involved in the degradation of aromatic compound (Mesarch, Nakatsu, & Nies, 2000). One of the lactoylglutathione lyase family was predicted to be catechol 2, 3 dioxygenases. This gene is essential for the conversion of salicylate aldehyde to catechol as reported in the pathway for the degradation of naphthalene by bacteria (Grund, Denecke, and Eichenlaub 1992). The genome also has a gene encoding for salicylate hydroxylase (COG0654) (EC:1.14.13.1) (NahG), first identified as salicylate 1-monooxygenase gene in the bacterium *Pseudomonas putida* in the operon through which environmental naphthalene is converted to pyruvate and acetaldehyde. NahG is responsible for the conversion of salicylic acid, an intermediate in the degradation of naphthalene, to catechol by removal of the carboxyl group at position 1 and the introduction of a hydroxyl group at that position (Bosch et al. 1999; Goyal and Zylstra 1997). Two other genes, homogentisate 1,2-dioxygenase involved in the catabolism of aromatic rings (Borowski, Georgiev, and Siegbahn 2005) and 2, 4 dihydroxyacetophenonedioxygenase (PEM_00137), which helps in the cleavage of carbon-carbon bond in a substituent aromatic ring, were also detected in the genome of *Stenotrophomonas* sp. Pemsol (Keegan et al. 2014). The Branched-chain acyl-CoA dehydrogenase, identified as isoquinoline 1 reductase (PEM_03865, COG1960), may be playing an important role in conversion of gentisate to Acetyl COA, thus ensuring the complete mineralization of PAH.

There are several genes associated with the degradation of toluene, benzoate styrene, bisphenol and other hydrocarbons in the genome of Pemsol. The genes include chloromuconate isomerase (PEM_00043, EC:5.5.1.7), which has been reported as a broad specific protein involved in the transformation of toluene (Dobslaw and Engesser 2015); and carboxymethylenebutenolidase, a hydrolase which converts 4-carboxymethyl-4-methylbut-2-en-4-olide formed from conversion of methyl catechol during the degradation of toluene in *Burkholderia* to 4-oxohex-2-enedioate

(Dobslaw and Engesser 2015). 4-oxalocrotonate tautomerase is known to be associated with the degradation of toluene, o-xylene, 3-ethyltoluene, and 1,2,4-trimethylbenzene by catalyzing their conversion into citric acid intermediates (Chen et al. 1992) in the beta-ketoadipate pathway for the degradation of aromatic compounds. A gene coding for biphenyl-2,3-diol 1,2-dioxygenase (PEM_03239, EC:1.13.11.39) is associated with the degradation of biphenyl and gamma-hexachlorocyclohexane degradation (Yam et al. 2009).

Stenotrophomonas sp. Pemsol also possesses a gene for alkane 1-monooxygenase (EC:1.14.15.3, PEM_02753) and three genes for alkanesulfonate monooxygenases (PEM_01386, and PEM_01939, PEM_1834, EC:1.14.14.5, COG2141). Alkane 1 monooxygenase has been reported to play a significant role in the degradation of alkanes. Pal *et al*., 2017 reported that alkane 1 monooxygenase in *Franconibacter pulveris*, which shared 93% similarity with a flavin dependent enzyme in *Enterobacter*, played important role in *F. pulveris* with respect to the degradation of alkanes. Thus, distinct genes encoding alkane 1 monooxygenase in *Stenotrophomonas* sp. Pemsol might be involved the degradation and complete mineralization of alkane. The complete mineralization of alkane after oxidation has been reported to involve the activities of the enzymes, alcohol dehydrogenase and aldehyde dehydrogenase, in second and third steps of oxidation respectively. *Stenotrophomonas* sp. Pemsol has 6 genes related to alcohol dehydrogenase (PEM_00500, PEM_01355, PEM_01677, PEM_02090, PEM_02458, PEM_03604, EC: 1.1.1.1, COG0604; COG1062; COG1064; COG1454) and two genes encoding aldehyde dehydrogenase (PEM_01439, PEM_01407; EC: 1.2.1.3; COG1012). Alcohol dehydrogenase is not only involved in the degradation of aliphatic hydrocarbon but can also catalyze the degradation of aromatic hydrocarbon and other xenobiotics in a similar pathway described above (Pal et al. 2017). Aldehyde dehydrogenase plays an important role in the detoxification of toxic aldehyde produced from the different metabolic processes. Aldehyde dehydrogenase are therefore significant in the degradation of many hydrocarbon compounds (Sierra-García et al. 2014).

Three genes encode the enzyme alkane sulfonate monooxygenase. The involvement of alkane sulfonate monooxygenase in the degradation of alkane and sulfonated alkanes has been well elaborated by the studies of Abbasian *et al*., 2016. This method of degradation is common among the *Actinomycetales, Clostridiales, Burkholderiales, Psedumonadales and Rhizobiales* (Pat et al., 2017). Alkane monooxygenase and Cytochrome P450 have been reported to be associated with the degradation of small, medium and long chain N-alkanes via its oxidation to either primary

alcohol, monocarboxylic fatty acids, or secondary alcohol and ketones (Ciferri 1999). Fatty acids are common biodegradation products of alkane (Rojo 2009) and the strain *Stenotrophomonas* sp. Pemsol possesses long-chain fatty acid transport protein (PEM_03883 COG2067) with which it can transport the fatty acid produced during the degradation of N alkane.

The presence of a functional maleylacetoacetate isomerase (PEM_00186, COG0625) in the genome of Pemsol in contrast to its non-functional potein *S. maltophilia* K279a may be associated with its versatile capability to degrade different PAH. Maleylacetoacetate isomerase has been reported to be essential for the conversion of maleylacetoacetate produced from the oxidation of homogentisate to Fumarylacetoate, which is broken down by FAcAchydrolase to Fumarate and acetate (EDWARDS and KNOX 1956).

Emulsification has been described as one of the mechanisms in bacteria through which they take up hydrocarbon to PAH and hydrocarbon trapped on surfaces in their environment. Emulsification enables microbes to have contacts with trapped hydrocarbons by solubilizing them and reducing interfacial surface tension and thus they can degrade these hydrocarbons. *Stenotrophomonas* sp. Pemsol only has one (phosphomannomutase, PEM_00211) of the genes that are needed to produce biosurfactant (Pat et al., 2017). The absence of the other genes essential for biosurfactant production might the reason for its inability to emulsify PAH in our emulsification experimental studies. Thus, in the Pemsol, the degradation of PAH occurs without the help from emulsification.

The comparison of the COG categories in *Stenotrophomonas* sp. Pemsol and four other hydrocarbon-degrading gene revealed that it has an abundant of COG in the categories T V and W. The more abundance of the COG category S than the other four hydrocarbon degrading bacteria may be associated with the need for *Stenotrophomonas* sp. Pemsol to sense and respond to the changes in its environment. Similarly, higher percentage of the COG category (W) associated with the mechanisms, responsible for *Stenotrophomonas* sp. Pemsol's ability to protect itself from the harmful nature of PAH. It could also be involved in one of the mechanisms through which *Stenotrophomonas* sp. Pemsol move towards the useful or away from the harmful components of the PAHs. This is because majority of the genes that constitute the extracellular COG category are associated with the organelles for bacterial movement.

Likewise, the abundance of defense COG category could be associated with their capability to resist PAH.

The genomic analysis of Pemsol corroborates its phenotype because it possesses some genes that take part in the degradation of PAHs. Similarly, the complete genome analysis of strain ASS1 and SVIA2 revealed the presence of many genes required for the degradation of PAHs . The capability to emulsify PAHs in biosurfactant production assay is another phenomenon, which support the possibilities in ASS1 and SVIA2 degradation of PAHs, different from the strain Pemsol without emulsification. They could show different mechanisms for PAH degradation. The genomic analysis of ATCM1_4 retrieved from soil samples could be related to *Stenotrophomonas nitritireducens* and may have genes that are involved in nitrate reduction or nitrogene fixation. However, further investigation is essential to confirm these functions. TepeL and TepeS successfully decolorized azo dyes and other dyes tested in a preliminary study. The analysis of TepeL genome revealed that it posseses the genes for the decolorization of Azo dyes such as FAMN dependent Azo reductase, peroxidase and tyrosinase. These results showed that genome analysis is an accessible way to understand the phenotypes of bacterial strains and provide a good basis for its biotechnological application.

Conclusion

In conclusion, the isolation and genomic characterization confirmed that the genus *Stenotrophomonas* is diverse metabolically. *Stenotrophomonas* have intrinsic resistance to many antibiotics and possess the potentials to develop resistance to new or previously effective antimicrobial agents. Environmental *Stenotrophomonas* strains showed high rate of resistance to Trimethoprim-Sulfamethoxazole which indicates that the environment could influence the adaptation of *Stenotrophomonas*. Resistance in *Stenotrophomonas* has been attributed to various mechanism through either phenotypic or genotypic factors. For example, biofilm formation is a phenotypic factor responsible for their resistance, through which majority of the *Stenotrophomonas* species are resistance to many antimicrobial agents. The genetic composition of the isolates also played important role in the resistance to many antimicrobial agents. Bla_{NDM} genes were found in 55% of the isolates, which were resistant to beta-lactam antibiotics. The complete genome analysis revealed many multidrug efflux pumps that confer resistance on isolates. It is, however, important to carry out a further study that can correlate the presence of these pumps and resistant phenotype in isolates. Niche Adaptation is a common phenomenon in *Stenotrophomonas* species. Brooke (2012) reported the isolation of microcolonies of *S.*

maltophilia from water faucet and Goncalves-Vidigal (2014) reported theadaptation in lungs of some *S. maltophilia* strains recovered from cystic fibrosis patients. In this thesis, we identified two strains of *S. maltophila* from sewage channels that grew as plaque forming units, indicating a new biological trait for *Stenotrophomonas*. Similarly, isolates recovered from crude oil contaminated sites showed the potentials to grow and degrade different components of crude oil as revealed by FTIR, GC-MS/MS and UPLC-MS analysis. Furthermore, the strain recovered from textile effluents showed good activity for the decolorization of textile dyes. *Stenotrophomonas* heterogeneity was confirmed by the different fingerprint obtained from the ERIC-PCR typing from most isolates. The new phenotypic and genotypic strains in genus *Stenotrophomonas* are still emerging because their potentials to acquire genes from other strains develop new genotypic and phenotypic properties. New species have emerged recently from extreme environments such as bentolite and tumulicola clay (Handa *et al*., 2015; Sanchez-Castro *et al*., 2017). In this thesis, four potential novel species candidates were identified due to their phenotypic differences, their average nucleotide identity blast (ANIb) and genome-genome distance calculation (GGDC). Fatty acid methyl ester analysis will therefore be a good tool to provide new evidence. Whole genome sequencing and analysis is an important tool for understanding bacterial behaviour, metabolic versatility, adaptation mechanisms and uniqueness.

Finally, continuous studies on *Stenotrophomonas* is important because of their genomic plasticity. *Stenotrophomonas* species can alter their genetic content via the acquisition of genetic materials from other species, or via mutation to enhance their survival. This attribute confers on *Stenotrophomonas* species high potentials to be manipulated for beneficial purposes. It also calls for the continuous surveillance on *Stenotrophomonas*, especially on the opportunistic pathogens of *S. maltophilia*, which help us to effectively manage the menace of resistance to antimicrobial agents.

Further studies

There is a need to carry out the transcriptomic analysis of the genes identified to be associated with the degradation of PAH to corroborate the role of these genes in the degradation pathway. This will make it easy to find new metabolic pathways employed by the strains for the degradation of PAH.

Molecular identification of basis of resistance studies will be of great importance for an in-depth understanding of the resistant behavior in the isolates. This can be achieved by sequencing more

genomes to analyze the genes that could be responsible for bacterial resistance to antimicrobial agents and confirming the functions with genetic research.

Fatty acid methyl ester analysis will therefore be a good tool to provide new evidence for the new species identification.

New drug design study that aims at combating resistance in *Stenotrophomonas* is important.

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