

Complete Genome Report of *Sphingobium Yanoikuyae S72*: A Bacterium Capable of Degrading Hydrocarbons

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Research Article

Keywords: Naphthalene, Phenanthrene, GC-MS, Biphenyl, Sphingobium, Toluene, genome, sequencing

Posted Date: July 22nd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-687133/v1>

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Abstract

Sphingobium yanoikuyae S72 was isolated from the rhizosphere of sorghum plant, Mexico. Its role on the degradation of polycyclic aromatic hydrocarbon (PAH) was evaluated in a minimal medium containing one of biphenyl, naphthalene, phenanthrene, xylene and Toluene as the only carbon source. It grew effectively in each minimal medium (Bushnell Haas, BH). Gas column chromatography–mass spectrometry (GC-MS) analysis on the metabolite recovered after S72's growth in BH showed that it successfully degraded the PAHs tested resulting in the degradation of biphenyl (85%), phenanthrene (93%), naphthalene (81%), xylene (19%) and toluene (30%). The complete genome of the S72 was sequenced to evaluate the genetic bases of S72's degradation of PAH. The genome was assembled de novo and reduced to 1 contig and consists of 5,532,623 bp, 5005 protein-coding genes. The analysis of the COG categories in S72 in comparison with other *Sphingobium yanoikuyae* and other PAH degrading gene, showed that it has in higher abundance in some categories that have been associated with the degradation of PAH (energy production and conversion (COG C, 6.35), carbohydrate transport and metabolism (COG G, 6.20%), lipid transport and metabolism (COG I, 6.99), secondary metabolites biosynthesis (COG Q, 4.52%), general function prediction only in transport and catabolism (COG R, 9.93).) Pan-core genome analysis revealed that about 126 unique genes in S72 are associated with the degradation of xenobiotics and PAHs. Most gene found in S72 are associated with the peripheral degradation pathway for PAH. Based on our observation we proposed a possible benzoate degradation pathway.

Introduction

Polycyclic Aromatic Hydrocarbon (PAH) are important pollutants which always find their way into the environment through various human activities such as the burning of fossil fuel, oil spillages and sometimes during vehicle repairs (Elufisan, 2020). The release of PAH to surrounding air is worrisome because of the associated hazards on people, animal and plants (United States National Academy of 1983). Exposures to fossil fuels and other PAH contaminants is known to disrupt agricultural lands, contaminate the surrounding air, and may induce cancer and other mutations in aquatic organisms, (White and Claxton 2004). The deleterious effects of the release of PAH to the environments make remediation processes inevitable. Several remediation processes have been employed for the cleaning of PAHs contaminated environment. Among such approaches are the use of chemicals and biological methods. Biological approaches have been identified as effective measures for the cleaning of sites contaminated by low molecular PAH (Gan et al. 2009). The degradation of high molecular weight PAH is said to be difficult because of their molecular structures. However certain bacteria have been reported to possess the capacity to degrade both high and low molecular weight PAHs. These bacteria have shown good potentials for the degradation of PAHs and could possibly be tools for modern bioremediation of PAH contaminated sites. Among such bacteria are member of the genus *Stenotrophomonas*, *Pseudomonas*, *Franconibacter*, *Bacillus*, *Enterobacter* among others (Juhasz and Naidu 2000; Kertesz* and Kawasaki 2010; Elufisan et al. 2020). Although there were arrays of bacteria that have been identified

for the capacity to grow in and degrade PAH, there are still paucity of information on the mechanisms being employed by many of them for the degradation of PAHs. Among such bacteria whose mechanism is yet to be fully elucidated are member of the genus *Sphingomonads* (Kertesz* and Kawasaki 2010).

The *Sphingomonads* belongs to the family *Sphingomonadaceae*. Yabuuchi et al. proposed the family *Sphingomonadaceae* in 1990 (Yabuuchi et al. 1990; 1998; Kosako et al. 2000). The family was later subdivided by Takeuchi et al. 2001 (Takeuchi et al. 2001; Euzéby 2004) into four genera: *Sphingomonas*, *Sphingobium*, *Novosphingobium*, and *Sphingopyxis*. These genera are called the “sphingomonads.” Members of the genera sphingomonads are commonly found in nature. There is an increased attention on these genera in recent times because they play important roles in the remediation of pollutants. They do so by using these pollutants as substrates for growth (Kim and Zylstra 1999; Cho et al. 2005; Cunliffe and Kertesz 2006; Ferraro et al. 2007; Gai et al. 2011). Among the sphingomonads with promising potential for both biodegradation and bioremediation of pollutants are the *Sphingobium* and *Sphingomonas* (Cunliffe and Kertesz 2006; Liu et al. 2012). *Sphingobium* species have been isolated and studied for their ability to degrade different pollutants including PAHs. However, most analysis on *Sphingobium*'s capacity to degrade PAH were limited to the isolation of *Sphingobium* and evaluation of the metabolic products formed from PAH biodegradation.

Genome sequence analysis can unravel many underlining factors responsible for bacteria behavior. The needs to understand more about the biology of *Sphigobium* and its detail architecture with respect to PAH degradation necessitates a complete genome sequence analysis of a PAH degrading *Sphingobium*. In this study, we isolated *Sphingobium yanokuyae* S72 from the rhizosphere of sorghum sample collected from the northeastern part of Mexico (25.983333 latitude, 198.1 longitude, and altitude 120M a.s.l.). This strain grew effectively in PAH supplemented medium. Its potential to degrade PAH was further evaluated in a PAH degradation assay. The complete genome was sequenced and analyzed to understand the molecular mechanisms that are associated with its degradation of PAH.

Materials And Methods

1. Bacterial isolation and characterization

S72 was isolated from the rhizosphere of sorghum plant by a colleague in the lab in a previous study. One gram of soil sample recovered from sorghum plant rhizosphere was added to 10ml 0.85% saline solution in a 50ml falcon tube. The mixture was strongly agitated on a vortex and then serially diluted in sterile distilled water to the factor of 10^{-4} . 0.1ml of each diluent was inoculated on an already prepared Tryptic soy agar (TY) and incubated at 30° c for 24-48 hours. The isolates which appeared on the plate were purified by subculturing individual colony in a freshly prepared TY agar and incubated in the same condition previously mentioned. The purified colonies which appeared on plates were identified morphologically with the electron microscope, biochemically using the API®20NE bacterial identification kit (bioMerieux, 200), and molecularly by amplifying the 16S rRNA fragment of the bacterial genome. The biochemical analysis was carried out using the API 20NE gallery system, which consists of a gallery

made up of a series of microtubes with dehydrated medium and substrates. This system is used to identify non-enteric Gram-negative bacilli and some members of the *Sphingomonas* genus (bioMerieux sa, 2006). This test was carried out under the conditions specified by the manufacturer, (bioMerieuxsa). The galleries were incubated at 30° C, and the results were observed at 24 and 48 h. The interpretation of the positive and negative tests was made based on the API 20 gallery color chart (apiweb, bioMerieux, 2010). Subsequently, the results were compared with the API and ID 32 identification database (bioMeerieux) and with biochemical data assigned for *Sphingobium yanoikuyae* (Yabuuchi and Yoshimasa, 2002).

The genomic DNA for the bacterial identification was extracted from an overnight broth culture of the suspected colonies with the wizard Promega genomic DNA extraction kit (USA) according to the manufacturer's instruction. The extracted genomic DNA was amplified with the bacterial universal primer 27f (5' -GAGAGTTTGATCCTGGCTCAG -3') and 1495r (5' - CTACGGCTACCTTGTTACGA-3') (Grifoni et al. 1995). The amplified region was sequenced and blast check analysis on the NCBI database was carried out to determine the identity of the bacteria.

2. Bacterial growth in, and degradation of PAHs

S. yanoikuyae S72 was inoculated in different Bushnell Haas (BH) agar media, which were already supplemented with PAH at a final concentration of 0, 20, 40, 80 100 and 150 µg/mL, respectively. The final bacterial concentration inoculate in the medium was approximately 3.0×10^7 CFU/ml. Each culture was incubated at 30 ° C for 72 hours. The highest concentration of PAH tolerated by the isolates were selected for the degradation studies.

The PAHs-degrading test was carried out in 5 BH liquid media, with each containing one out of the 5 PAHs (naphthalene, phenanthrene, biphenyl, toluene, or xylene) as the sole carbon source. A 1 ml of *S. yanoikuyae* S72 was added to 40 ml of 5 Bushnell Haas Broth (BH) already supplemented with each of the PAHs, in a 250 ml flask, respectively. The culture was then incubated in a rotatory incubator with revolutions of 180 rpm at 30°C for 25 days. The bacterial growth was checked every 48 hours and growth was determined using the spectrophotometer. The cultures were withdrawn on the 12th and 25th day to determine the extent of PAHs degradation by the isolate. The metabolites formed were determined by a Gas Column chromatography mass spectrophotometry (GC-MS) analysis.

GC-MS chromatography analysis

For the GC-MS analysis, an Agilent Technologies brand gas chromatograph, model 6890N (Net Work GC system), equipped with a DB-5 column, 5% -phenyl-methyl-polysiloxane (Agilent Technologies), 60 meters long, 0.25 mm in internal diameter and 0.25 µm thick film was used. The starting temperature was 50 ° C, which was maintained for 5 min, subsequently the temperature was raised to 280 ° C using a heating ramp of 20 ° C / min, for 20 min. Helium was used as carrier gas at a flow of 1 mL / min, the injector temperature was 250 ° C, split injection, with a split ratio of 50: 1. Once the chromatogram was obtained, the identification of each of the peaks was carried out by mass spectrometry using an Agilent

Technologies model 5975 inert XL mass spectrometer. Mass spectra were obtained by electron impact ionization at 70 eV, for identification the mass spectra obtained for each compound were compared with a database (HP Chemstation-NIST 05 Mass Spectral search program, version 2.0d), in addition to the comparison with a standard, analyzed under the same conditions, which was used as an external standard for quantification.

The GC-MS analysis started with the separation of the organic phase and the aqueous phase of the culture medium in a rotatory funnel. The culture medium (40 ml) was poured in a separating funnel in which 20 mL of HPLC grade hexane (J.T. Baker®) were added. The funnels were manually shaken for 10 min. Funnels were kept at rest until the two phases were clearly separated into two. From the upper phase containing the PAH, 1 mL aliquot was transferred to 1.5 mL dark vials. The was used for the analysis of the metabolite formed in a gas chromatograph (Agilent Technologies® Model 6890 N). Once the chromatograms were obtained, each of the chromatographic peaks was identified by mass spectrometry, using a mass detector (Agilent Technologies® Mod. 5975 inert XL). The mass spectra observed in the metabolites were confirmed with the HP Chemstation-NIST 05 Mass Spectral Search Program, version 2.0d software. The spectra were compared with the standard for each PAH and the spectra from the control in which no bacterium was inoculated under the same condition. Experiment was set, in which treatments consisted of flasks inoculated with or without *S. yanoikuyae*, and with or without PAH. Each type of PAH was separately analyzed, and each treatment had five replicates in the experiment. The experiments were repeated three times. Data were analyzed using the SAS statistical program (Dunne 2005).

Genome sequencing and analysis of *S. yanoikuyae* S72.

We sequenced the genome of S72 to fully understand its genetic basis for the degradation of PAH.

The genomic DNA was extracted with the Promega® Wizard Genomic DNA purification kit (Promega Corp., Madison, WI, USA) according to manufacturer's recommended protocol. The whole genome sequencing was performed using the Illumina MiSeq™ platform (Illumina, Inc., San Diego, CA, USA). The reads obtained from the genome assembly were trimmed with trim-galore, which also check the quality of the read with its embedded fastqc. The genome assembly was carried out in 2 stages: i) de novo assembly with the Spades Genome Assembler (Bankevich et al. 2012) and Velvet (Zerbino and Birney 2008) and ii). reference-based assembly.

The de novo assembly used two different bacterial genome assembly pipelines, which are the Spades based de novo assembly (3.9.1) and the velvet assembly pipeline. The assembled genome from the two software were compared using the Metassembler software. A consensus assembly produced from the two assembling techniques was then used for the reference-based assembly (Wences and Schatz 2015). The reference-based assembly was carried out to reduce the genome to 1 contigs. This approach employs the alignment of the consensus assembly with *Sphingomonas* genome (ATCC512030) recovered from NCBI database using the Nucmer software and the Consed suite (Gordon and Green 2013; Marçais et al. 2018). The assembled genome was annotated with Prokka (version 1.12) and NCBI

prokaryotic genome annotation pipeline were used for automatic gene prediction and annotation (Seemann 2014; Tatusova et al. 2016). RNAmmer (Lagesen et al. 2007) and tRNAscan (Lowe and Eddy 1996) were used for the identification of rRNA and tRNA. The KEGG and Orthologous gene cluster analysis was carried out on the web based PATRIC server (Wattam et al. 2014). Alternatively, COGs for unique genes were assigned with BlastP against the COGs database downloaded from NCBI. The bacterial pan genome analysis (BPGA) tool was used for the analysis of the isolate's pan genome (Chaudhari et al. 2016). The Average Nucleotide Identity based on Mummer (ANIm) was determined with PYANI (Pritchard et al. 2016). The genomic island in S72 was identified with an online web-based Island viewer4 (Bertelli et al. 2017) and pairwise analysis on Nucmer (Marçais et al. 2018). The deep gene sequence analysis for the identification of the genes that were associated with the degradation of PAH and comparative genomics such as COG abundance was carried out on the JGI-IMG database (Mavromatis et al. 2009).

Results

S72 is a gram-negative rod and moves by means of a monotrichous flagellum. It is between 1.0-3.5 μm in length and 0.6-1 μm in width (Fig. S1). S72 effectively use D glucose, L arabinose, Mannose, N acetyl glucosamine, maltose, potassium gluconate, Malic acid, citrate, galactose, citrate and esculin for its growth. It however could not use mannitol, adipic acid, capric acid, phenyl acetic acid as a substrate for its growth (Table 1).

The preliminaries analysis showed that S72 can grow in the minimal media (BH) supplemented with polycyclic aromatic hydrocarbons (PAH) (biphenyl, phenanthrene, xylene, toluene, and naphthalene). The number of colonies forming units appearing on the plates decreased as the concentration (0, 20, 40, 80 100 and 150 $\mu\text{g}/\text{mL}$) of the PAH in the BH medium increased. The preliminary analysis also revealed that the S72 could better tolerate the hydrocarbons at a concentration that is $\leq 100\mu\text{g}/\text{ml}$, but their growth in PAH began to decline at any concentration greater than $100\mu\text{g}/\text{ml}$. S72 did not show any growth when inoculated in BH medium containing $150\mu\text{g}/\text{ml}$ toluene or xylene and showed limited growth in plate with naphthalene, phenanthrene or biphenyl at this concentration. Also, the rate at which S72 grew in medium containing toluene and xylene is lower than other PAHs tested at $100\mu\text{g}/\text{ml}$. (Fig. 1). Based on the reported observations $100\mu\text{g}/\text{ml}$ was selected as the study concentration for the experiment.

S72 degraded all the PAHs tested at $100\mu\text{g}/\text{ml}$ with varying efficiencies. S72 showed the best activity in phenanthrene, resulting in the 93 % degradation. The degradation of biphenyl, and naphthalene lead to 85 and 81% reduction, respectively (Fig. 2). However, a low degradation efficiency was observed in the medium containing xylene (19% and 31%) respectively. The degradation was confirmed by the reduction in the peak for the PAHs as compared to the control from the GC-MS analysis (Fig 3, S2).

Genomic analysis has been used severally to unravel several details about bacterial behavior. As a result of this, the genome of S72 was sequenced and analyzed to relate its function with its genetic constituent. The sequenced genome of *S. yanoikuyae* S72 was reduced to 1 contig, consisting of 5,532,633 bp, 5005

CDS, and 4 identical copies of the rRNA gene operon (23S, 16S and 5S). The overall G + C content of the assembled genome is 64.23 %. The genome has 5231 putative genes and 75 RNAs (67tRNA and 12 rRNA). 1140 genes are in operons, 2515 are parts of the clusters of orthologous gene (COGs) Table 2. S72 has some COG categories in abundance than some other *Sphingobium* species which has been previously reported. These COG categories include energy production and conversion (COG C, 6.35%), carbohydrate transport and metabolism (COG G, 6.20%), lipid transport and metabolism (COG I, 6.99%), secondary metabolites biosynthesis (COG Q, 4.52%), general function prediction only in transport and catabolism (COG R, 9.93) (Table 3). In a related analysis S72 was found to have higher COG abundance than 17 other hydrocarbon degrading bacteria retrieved from IMG database Table 4). It showed abundance in the COG categories Mobilome: prophages, transposons (COG X, 2.03%), Secondary metabolites biosynthesis, transport and catabolism (COG Q, 4.52%), Carbohydrate transport and metabolism (COG G, 6.20%), Lipid transport and metabolism (COG I, 6.99%), Cell wall/membrane/envelope biogenesis (COG M, 6.58), Replication, recombination and repair (COG L, 3.33%), Inorganic ion transport and metabolism (COG P, 6.22%), Transcription (COG K, 7.8), Posttranslational modification, protein turnover, chaperones (COG O, 4.27%), and General function prediction only (COG R, 9.93%) Some of these COG categories have been previously reported to be associated with the degradation and mineralization of PAHs (Pal et al. 2017; Elufisan et al. 2019). The deep sequence analysis of S72 showed that it possesses 37 genes in different COG categories which are associated with the degradation of xenobiotics and polycyclic aromatic hydrocarbons. These genes include 2 pyruvate decarboxylase (A6768_17510, COG R), 4 carboxy – 2 – hydroxy muconic semialdehyde dehydrogenase (A6768_17470, COG R). These two genes have been linked to the degradation of Benzoate and Fluorobenzoate by bacteria. Bacteria have been reported to use the enzyme encoded by these genes for the degradation of benzoate via hydroxylation (Oltmanns et al. 1989). Others include S-(hydroxymethyl) glutathione dehydrogenase/alcohol dehydrogenase (A6768_05785, COG R) that is associated with the degradation of naphthalene and chloroalkane (Yang et al. 2017). S-(hydroxymethyl) glutathione dehydrogenase/alcohol dehydrogenase has been previously reported to be associated with the degradation of naphthalene (Das et al. 2015; Pal et al. 2017). Thus, the presence of S-(hydroxymethyl) glutathione dehydrogenase/alcohol dehydrogenase in S72 could be associated with its need to degrade naphthalene as a substrate for growth. Alcohol dehydrogenase (cytochrome c) (A6768_00755, COG G), aldehyde dehydrogenase (NAD⁺) (A6768_10925, COG E), aldo/keto oxidoreductase (A6768_11370, COG R), Phenylacetaldehyde dehydrogenase (A6768_11850, COG C) known to participate in the degradation of fluorobenzoate toluene and naphthalene are also present in S72 (Pal et al. 2017; Elufisan et al. 2020). Other PAH degrading genes identified in S72 include propanol-preferring alcohol dehydrogenase (A6768_17370, EC:1.1.1.1), carboxymethylenebutenolidase (A6768_23915), catechol 1,2-dioxygenase (A6768_16795), muconate cycloisomerase (A6768_16805), aryl-alcohol dehydrogenase (A6768_11850), 2-keto-4-pentenoate hydratase (A6768_14055), oxalocrotonate tautomerase (A6768_04785) benzoate 1,2-dioxygenase alpha subunit (A6768_16790), benzoate/toluene 1,2-dioxygenase beta subunit (Ben B), dihydroxy cyclohexadiene carboxylate dehydrogenase (Ben D). Similarly, a gene encoding the lactoylglutathione lyase was found in S72. The

lactoylglutathione lyase enzyme has been described to be actively involved in the cleavage of aromatic bond in many aromatic hydrocarbons (Mesarch et al. 2000). Among the observed genes in S72 are genes which have been reported to be specifically associated with the degradation of toluene. The genes include 4 genes encoding 3-hydroxyacyl-CoA dehydrogenase (EC:1.1.1.35) one gene for aryl-alcohol dehydrogenase (EC:1.1.1.90), and a gene for Catechol 1,2-dioxygenase (EC:1.13.11.1). Others include 2 genes encoding the enzyme oxidoreductases (EC:1.14.13.-) which often act on paired donors, with incorporation or reduction of molecular oxygen. 3 copies of the genes encoding Ferredoxin–NAD (+) reductase (EC:1.18.1.3), 7 copies of Acyltransferases (EC:2.3.1), 3 copies of carboxymethylenebutenolidase and 1 copy of muconate cycloisomerase.

S72 relatedness to other *Sphingobium* strain (105) was evaluated on PYANI (Pritchard et al. 2016) using the average nucleotide identity mummer (ANIm) comparison measure. The ANIm result showed that S72 is closely related to *S. yanoikuyae* strain UBA2097 sharing 97% average identity with it (Fig. 4). The analysis of S72 pan genome showed that it shared 1734 core genome with other *Sphingobium* species and possess 403 unique genes. We noted that 126 of the unique genes are associated with the catabolism of xenobiotics. Twenty out of the unique genes have been previously reported to be involved in the degradation of toluene, xylene, ethylbenzene, biphenyl, benzoate, naphthalene, anthracene, tetrachloroethene, 1, 4-dichlorobenzene, bisphenol, trinitrotoluene in bacteria. These genes are involved in both the catabolism of central aromatic intermediate and the peripheral catabolic pathway for aromatic hydrocarbon. (Table S1).

Horizontal gene transfer is a common phenomenon through which bacteria often acquire some genes that are essential for their survival (Elufisan et al. 2020). The acquired genes are commonly found on the genomic islands in bacteria or as mobile genetic elements. The analysis of the S72's genome with the online based Island viewer 4.0, revealed that it has 37 genomic islands (Fig. 5). Five large regions were found in the genomic Island with regions II and III being the largest consisting of 317,308 kb and 579,060 kb in length, respectively. An in-depth look into the genomic island showed the presence of many genes that are associated with the degradation of xenobiotics and PAHs. Among such genes are SDR family NAD(P)-dependent oxidoreductase (A6768_07840), aldo/keto reductase (A6768_07825), cytochrome P450 (A6768_11830), 4-hydroxybenzoate 3-monooxygenase (A6768_12975), and aromatic alcohol reductase (A6768_RS13080). The other PAH degrading genes found on the genomic island can be seen in supplementary file.

Discussion

The biochemical characteristics and the molecular identification of S72 confirmed that it is a *S. yanoikuyae* strain. It grew effectively in the tested PAH concentration until 100microgram/ml implying that S72 can use the tested PAH as substrates for growth. S72's growth rate, however, decreased as the PAH's concentration increased. The reduction in growth as shown in Fig. 1 could be due to increased toxicity that is associated with increased in the PAH concentration. This toxicity may be due to the reduction in rate of solubility that may occur as PAH concentration increases. Previous study has shown

that as concentration of hydrocarbon increases the rate of solubility decreases resulting in the reduction in growth for bacteria (Volkering et al. 1992). Low solubility could make it difficult for bacteria to utilize certain compounds as nutrient for growth (Leahy and Colwell 1990).

S72 successfully degraded the PAHs during the 25-days degradation study. S72 showed the most activities in the medium containing phenanthrene. The activity of S72 resulted in the degradation of Phenanthrene by 93% at a concentration of 100 microgram/mL. This observation is evident by the reduction in the spectra for Phenanthrene following the activity of S72 in contrast to the control which lack bacteria but only PAH (Fig. 3). This observation is like a previous report in which *Sphingobium chlorophenolicum* C3R degraded Phenanthrene by 65% (Liu et al. 2019). Hence, S72 possess the capability to degrade Phenanthrene but has a lower efficacy compared to *Sphingobium chlorophenolicum* C3R because it cannot degrade the PAHs at a conc greater than 100µl/mL. S72's ability to degrade PAH is not limited to Phenanthrene (PHE), it also degraded biphenyl and naphthalene by 85 and 81% respectively. This may imply that S72 had learnt to survive in and use different PAH as substrates for growth. S72 degradation efficiency is slightly weaker than what was reported for *Burkholderia fungorum* FM-2 which degraded PHE by 65% after 3days (Liu et al. 2019). It however has an advantage of surviving in PHE and ensuring its degradation after 25days. On the other hand, S72 did not effectively degrade xylene and toluene. Several reason could be responsible for the inability of S72 to degrade toluene and xylene, one of such reason could be the presence of a CH₃ and two CH₃ groups as an attachment to their benzene rings, respectively. It may be difficult for S72 to degrade the single benzene ring in xylene and toluene unlike in the other PAHs with more than one benzene ring and other moieties. In addition, the tested concentrations are not the molar equivalent but weight concentration. The same weight concentration in the media may contain more molecules of xylene and toluene than in the other PAHs, which may also increase toxicity of these compounds (higher molar concentration) on S72. Previous studies have shown that high concentration can inhibit the ability of bacteria to degrade PAH (Leahy and Colwell 1990). Also, the in-depth analysis of the genome of S72 showed that the genes encoding the enzymes required for the degradation of toluene are not complete in it and such may be another reason for its inability to degrade toluene. It has only four genes that that are directly associated with the degradation of toluene, but these genes are taking part in different reactions in the pathway for the degradation of toluene. The genes are aryl-alcohol dehydrogenase [EC:1.1.1.90], an Oxidoreductases acting on the CH-OH group of donors with NAD⁺ or NADP⁺ as acceptor, muconate cycloisomerase [EC:5.5.1.1] a lyase which isomerizes 3 methyl cis cis muconate to 3 methyl muconolactone. The other two genes encode catechol 1, 2 dioxygenase and carboxymethylenebutenolidase [EC:3.1.1.45]. Catechol 1, 2 dioxygenase catalyzes the decyclizing of the aromatic ring of methyl catechol to generate 3-methyl-cis, cis-hexadienedioate, while carboxymethylenebutenolidase [EC:3.1.1.45] is acting as dienelactone hydrolase converting 3-chloro-2-methyldienelactone to 3-Chloro-2-methylmaleylacetate in the breakdown of toluene. This observation is same for the genes required for the degradation of xylene as reported by the KEGG database.

S72's ability to grow in and degrade PAHs has underlying molecular mechanisms. The molecular mechanisms responsible for the survival of S72 in and degradation of PAHs was evaluated by the

sequencing of its genome. The genomic analysis of the S72 genome show the presence of some genes which are essential for the degradation of PAHs. Genes such as S-(hydroxymethyl) glutathione dehydrogenase/alcohol dehydrogenase (A6768_05785, COG R) which is known to enhance the degradation of PAH by catalyzing the oxidation of long chain primary alcohol (Uniprot). Alcohol dehydrogenase which are essential for the degradation of PAH via their oxido-reductase activities. We also noted several copies of other essential genes for the degradation of PAH. For example, there were 18 genes that are associated with the Cytochrome P450 superfamily. The cytochrome P450 is a monooxygenase which are involved in the degradation of PAH. Recent report showed that cytochrome P450 can play important role in the degradation of naphthalene and pyrene (England et al. 1998). The presence of cytochrome P450 monooxygenase (CP450) in S72 may be due to its role in helping S72 in the degradation of PAHs. CYP450's importance in S72 is evident with the presence of many of its copies in S72. 18 genes which are member of CYP450 superfamily were found in S72. The role of the unique CYP450 gene could not be fully explained here but it is likely that they are involved in some pathways that are associated with the degradation of PAH.

Similarly, several genes which have been linked with the degradation of tetrachloroethane were seen in the genome of S72. These genes include nitrilotriacetate monooxygenase (A6768_04895, EC 1.14.13.), Aldehyde dehydrogenase (A6768_04870, EC 1.2.1.3) and Alcohol dehydrogenase (A6768_22375, EC 1.1.1.1). There are 22 genes in these three categories that have been associated with the degradation of tetrachloroethane. Tetrachloroethane (CCl₄) and trichloroethanes are organic solvents commonly used for laundries and metal cleaning, as a result they are often find as environmental pollutant (Ryoo et al. 2001). No aerobic bacteria have been reported to possess the ability to degrade CCl₄ (Gerritse et al. 1997), The presence of genes needed to degrade CCl₄ may suggest that S72 possess the tendencies to decontaminate an area polluted by CCl₄. it a potential bacterium for the aerobic decontamination of CCl₄ polluted sites. S72's ability to grow in and degrade naphthalene is associated with the presence of some genes in its genome.

The genome has six genes which are involved in the pathway for the degradation of naphthalene (Alcohol dehydrogenase, Aldehyde dehydrogenase, and Nitrilotriacetate monooxygenase). Although these genes are not the only one that are needed in the pathway for the degradation of naphthalene, their presence in S72's genome may be the reason for its naphthalene degradation. More so, there are several hypothetical genes in S72 which are in the same operon with some of the genes that are involved in the degradation of PAHs (Fig. S3) and, as such may be complementing the roles of the identified genes in the degradation of naphthalene (Fig. S3). For example, we noted two genes encoding hypothetical protein upstream to the gene encoding nitronate monooxygenase and one hypothetical protein gene downstream nitronate monooxygenase. Most genes in this region had been previously reported to play essential role in the adaptation to PAH environment, uptake of PAH, utilization and degradation of PAH. They include the lysR family protein gene which is known to play significant role in the regulation of bacterial adaptation to stress and the use of PAH.

Comparative genomic analysis of S72 showed that it is more closely related to *S. yanoikuyae* strain UBA2097 with which it shares 97% average nucleotide identity confirming it a *Sphingobium yanoikuyae* strain. Further analysis on its genome showed that it has higher abundance in some cog categories (Mobilome: prophages, transposons (COG X), Secondary metabolites biosynthesis, transport and catabolism (COG Q), Carbohydrate transport and metabolism (COG G), Lipid transport and metabolism (COG I), Cell wall/membrane/envelope biogenesis (COG M), Replication, recombination and repair (COG L), Inorganic ion transport and metabolism (COG P), Transcription (COG K), Posttranslational modification, protein turnover, chaperones (COG O), and General function prediction only (COG R)) when compared with previously described PAH degrading bacteria retrieved from JGI_IMG database. Many enzyme that are essential for the degradation of PAH by bacteria have been reported to be encoded by genes in some of these COG categories (Das et al. 2015). For example, it has a higher abundance of the COG category K which is the transcription category. There are 307 genes in this cog category, 49 of which belong to the LTTR family. The LTTR has been described as regulator essential for the survival of bacteria in diverse environment. Pal et al 2017, (Pal et al. 2017) reported that the abundance of the LTTR family in *Franconibacter* could be associated with their utilization of PAH Binnewies et al., had previously reported that the LTTR family plays important role in regulating genes responsible for aromatic compound catabolism, motility of cell and quorum sensing (Binnewies et al. 2006). The abundance of the LTTR gene family in S72 implies that it highly metabolically active and thus could be associated with its capacity to degrade PAHs. Twenty-one proteins of the OmpR family transcriptional regulator of the two components system, 6 Mer family regulator protein, 21 proteins belonged to the multiple antibiotic resistance regulator (MarR) family (COG1846) (Wilkinson and Grove 2006); S72 has 41 TetR regulation protein (COG1309 and COG3226), and 14 GntR family transcriptional regulators protein (COG1167, COG1802, COG2186 and COG2188). These transcription regulators are known to play essential roles in the degradation of aromatic hydrocarbon (Gerischer 2002). Other transcriptional regulating protein in S72 include 23 AraC family (COG, 2207, COG4977, COG1609) known to be involved in the metabolism of different sugars (Martin and Rosner 2001), 8 proteins of the IclR family transcriptional regulator (COG1414), 2 proteins in the AsnC transcriptional regulator and 8 proteins of the ArsR family transcriptional regulator. S72 also has gene in the two-component system which are responding to the limitation of phosphate in the environment. It has a Pho R and Pho B genes which are phosphate regulon regulator in the OmpR family which can play essential role in the phosphate transport system (Pal et al. 2017).

S72 also has in abundance genes in the COG category for Mobilome: prophages, transposons (COG X), This COG category comprise genes that were acquired via horizontal genes transfer, some studies have shown that bacterial adaptation to polycyclic aromatic hydrocarbon could be mediated by an acquired mobile genetic element such as transposons, integron, plasmids or even prophages (Top and Springael 2003). There are about 80 genes in this COG category. The high abundance of gene from the COG X category could be an indication that certain genes which are essential for the degradation of PAH might have be a part of the mobilome, prophage or transposon.

Conclusions

S. yanoikuyae S72 was isolated from northern Mexico. S72 used selected PAHs as unique carbon source for its growth. GC-MS analysis showed that S72 effectively degraded biphenyl, phenanthrene, and naphthalene. Genome analysis revealed the genes responsible for degradation of the tested PAH in *S. yanoikuyae* S72 genome. Most genes found in S72 are associated with the peripheral pathway for the degradation of the tested PAH. Although, some genes that are associated with the degradation of PAH were not found in S72, there were many hypothetical genes in S72 in the same region with the genes that are involved in the degradation of PAHs. These hypothetical genes may have roles in the degradation of the tested PAH as observed in the experimental study. *S. yanoikuyae* S72 strain has a potential to be used as an agent for cleaning up environments contaminated with petroleum and other chemical pollutants. However, the use of microorganisms for bioremediation are highly dependent on other abiotic and biotic soil parameters. Thus, necessitating further “*in situ*” studies to investigate its activity and ability to survive in hydrocarbons contaminated soils would be essential.

Nucleotide sequence accession number

The complete genome sequence of *S. yanoikuyae* S72 was deposited at GenBank with accession number CP023741. This strain is deposited at laboratory interaction environment-microorganism culture collection center.

Declarations

Declaration

Ethical approval: The study does not involve human or animal and so does not require any ethical approval.

Consent for publication: No individual data was used in the study and so does not require any consent before sending out for publication.

Competing interest: There are no financial or Nonfinancial competing interest on the manuscript.

Conflict of interest: Temidayo O Elufisan, Patricia, Bustos, Paola Charles-Mendoza, Alberto Herrera Mendoza and Xianwu declare that they do not have conflict of interest.

Funding: There is no special funding for this study.

Authors' contribution

Author Claudia Paola, Charles-Mendoza and Eric Sanchez Lopez designed and carried out the experimental study.

Author Eric Sanchez-Lopez wrote the manuscript.

Author Xianwu Guo and Author Alberto Herrera Mendoza supervised the study.

Author Patricia Bustos carried out the genome assembly and analysis.

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Author Temidayo O Elufisan holds a PhD in biotechnology with a focus on bacteria genomics. He has previously worked on the Isolation and Genomic characterization of *Stenotrophomonas* from the environment. He also worked on the application of *Stenotrophomonas* species on the degradation of Polycyclic Aromatic Hydrocarbon. He is currently working on the purification of town water supply using moringa seeds.

Acknowledgements

To Lourdes Vital, Ma. Antonia Cruz and Susana Fernandez for technical assistance and suggestion to improve the genome analysis.

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Figures

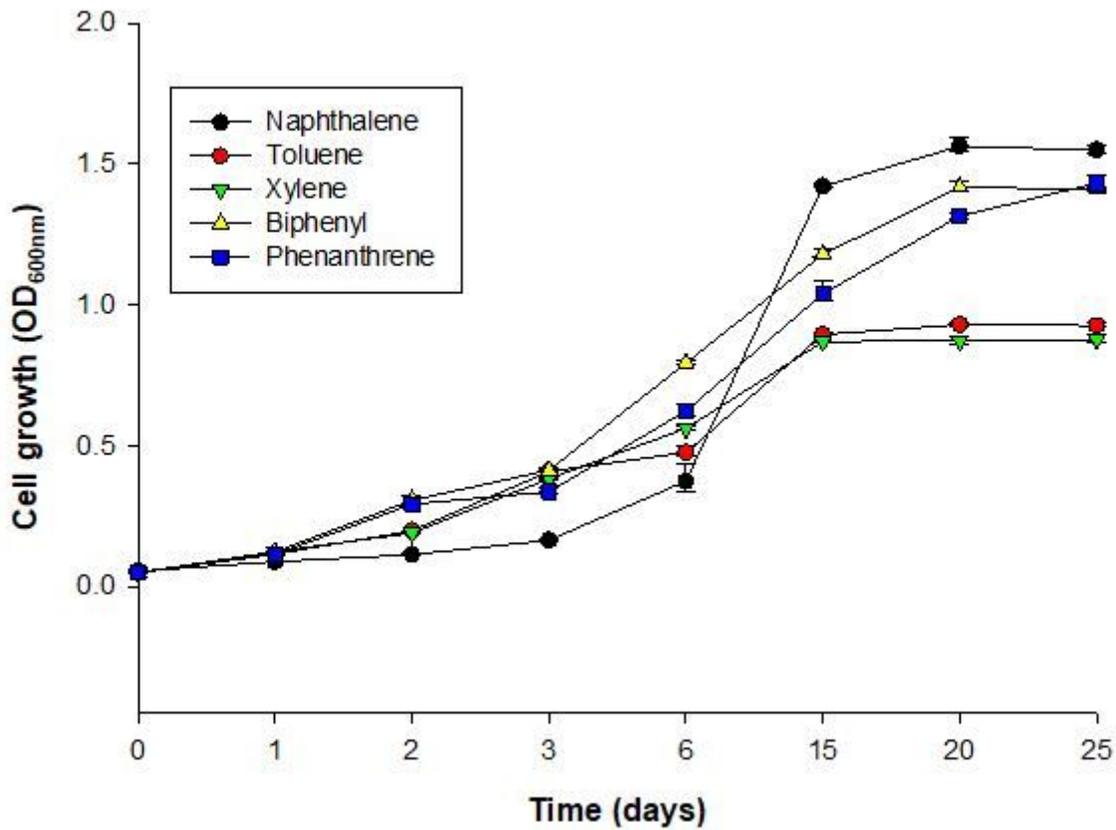


Figure 1

Growth of *S. yanoikuyae* S72 .Bacteria were measured as optical density (600nm) in liquid Bushnell Haas medium supplemented with each of the five different hydrocarbons (100 µg/mL) as sole source carbon for 25-day incubation.

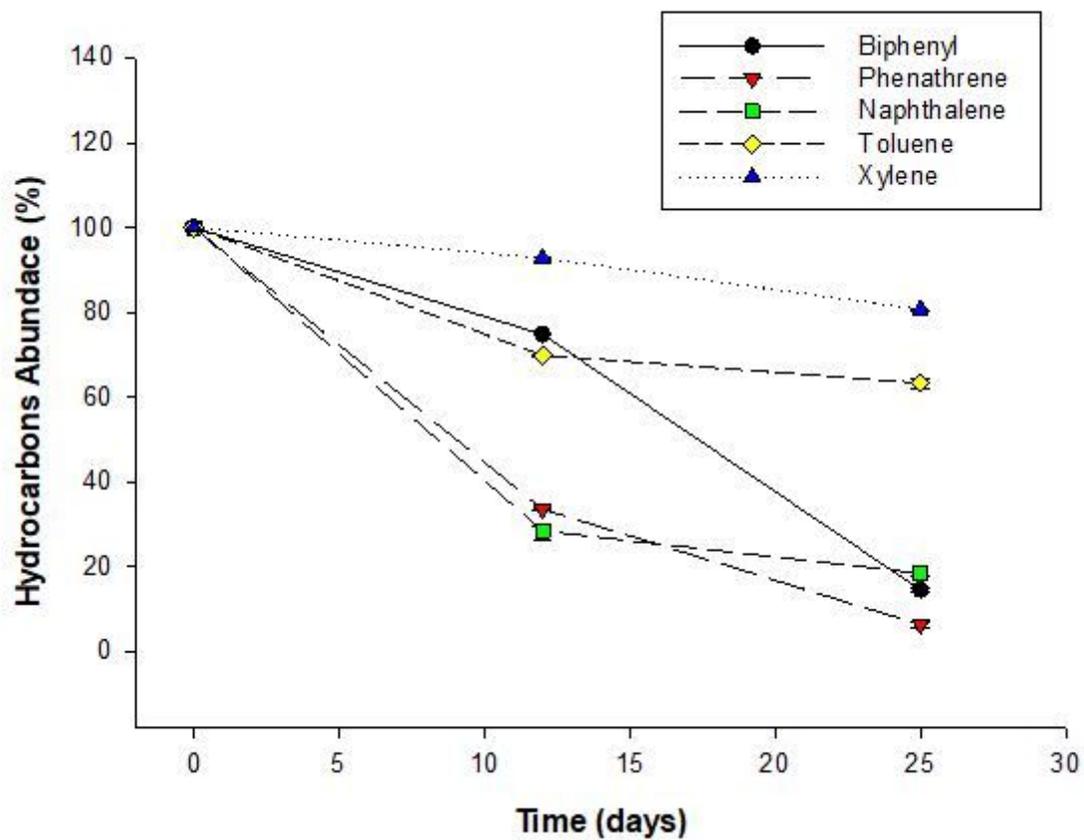


Figure 2

Degradation of hydrocarbons by *S. yanoikuyae* S72.. Hydrocarbon abundance (%) of five polycyclic aromatic hydrocarbons (PAHs) in liquid Bushnell Haas medium supplemented with each of the five different hydrocarbons (100 µg/mL) as sole source carbon for 25-day incubation.

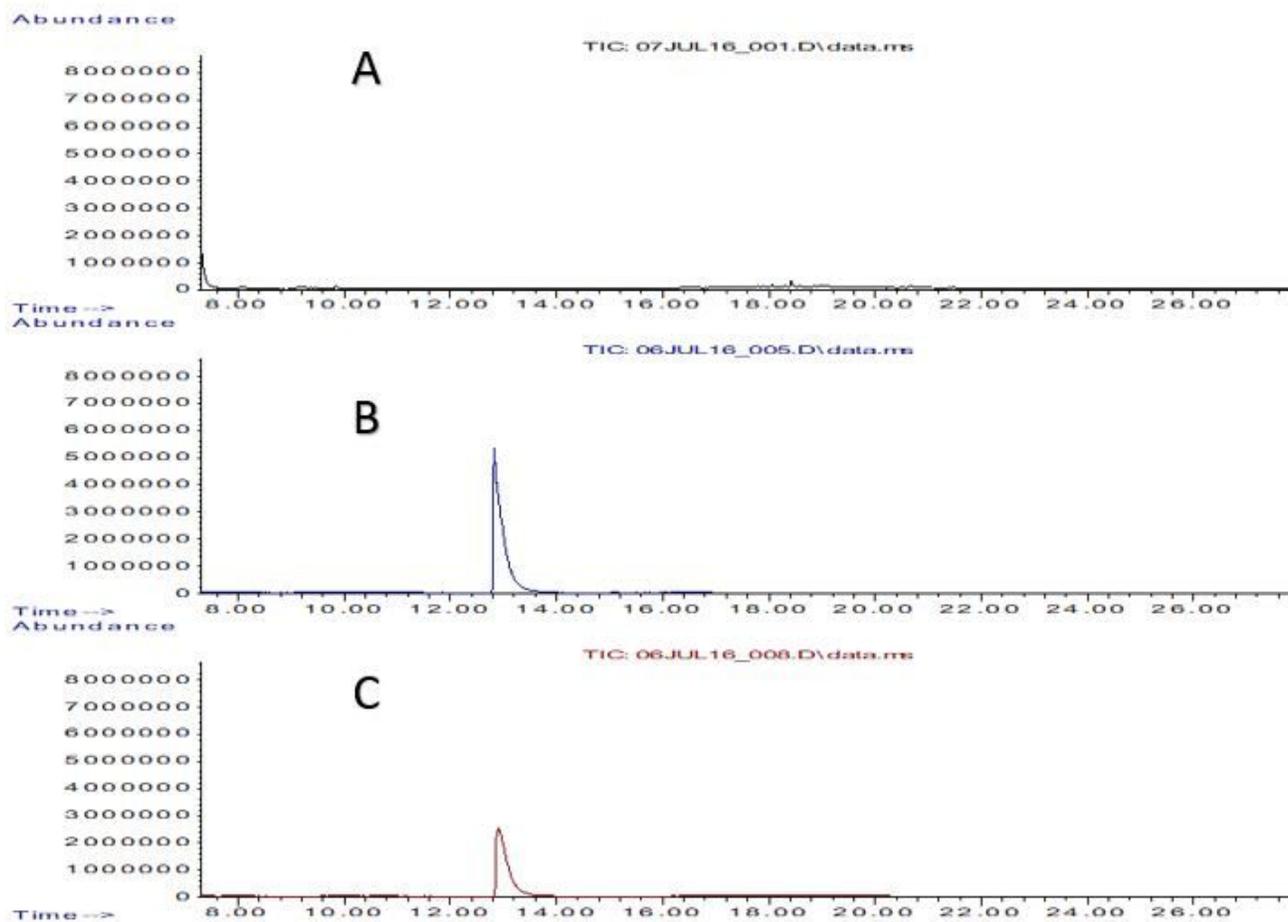


Figure 3

Gas column chromatography analysis of Naphthalene before and after degradation by *Sphingobium yanoikuyae* S72 A: Uninoculated culture medium without Naphthalene B: Naphthalene recovered from medium without S72 C: S72 treated naphthalene containing medium

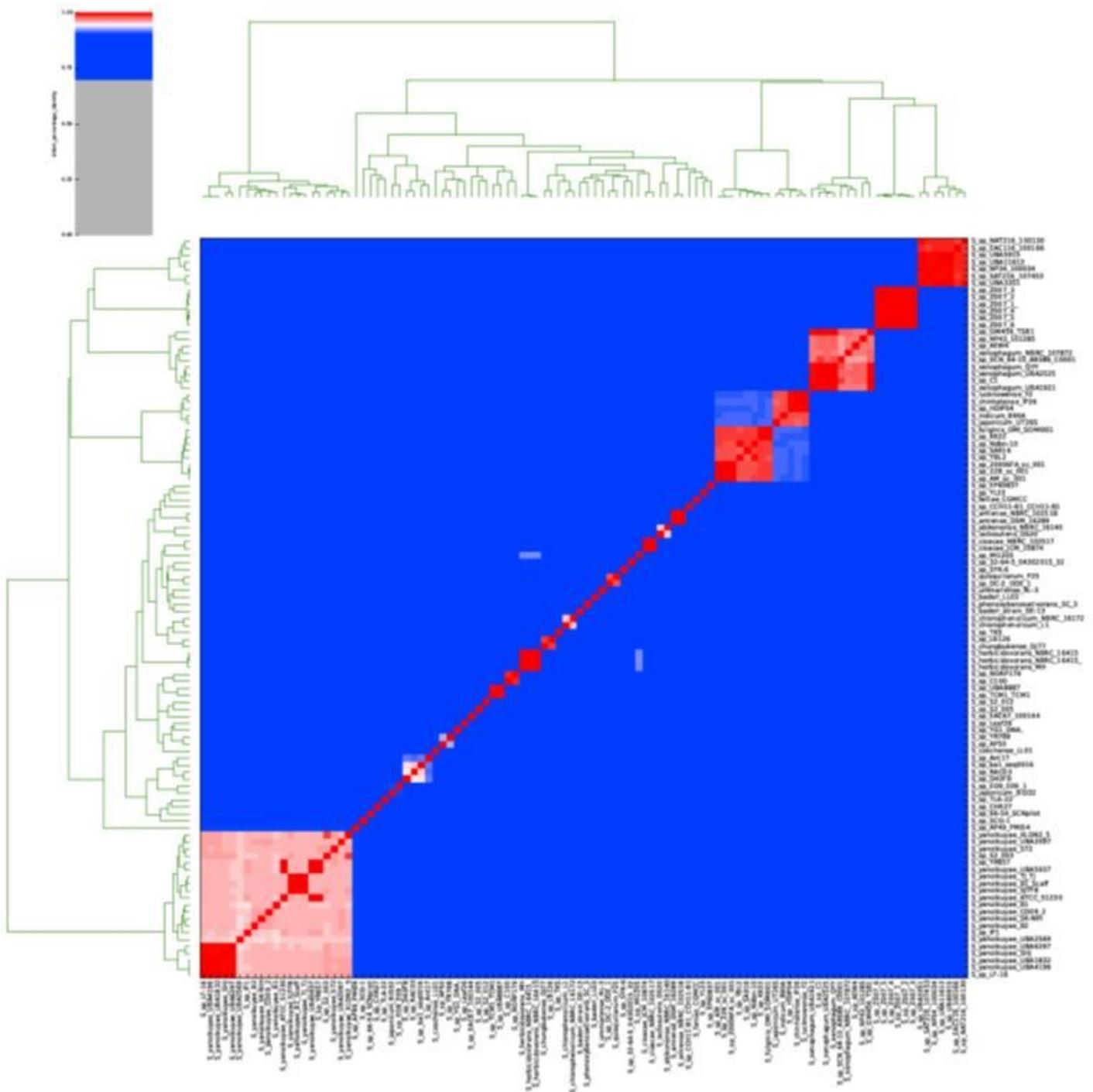


Figure 4

ANIm % Identity of 106 Spingobium Strains. ANI analysis based on MUMmer alignment of the genome sequences was performed using PYANI.

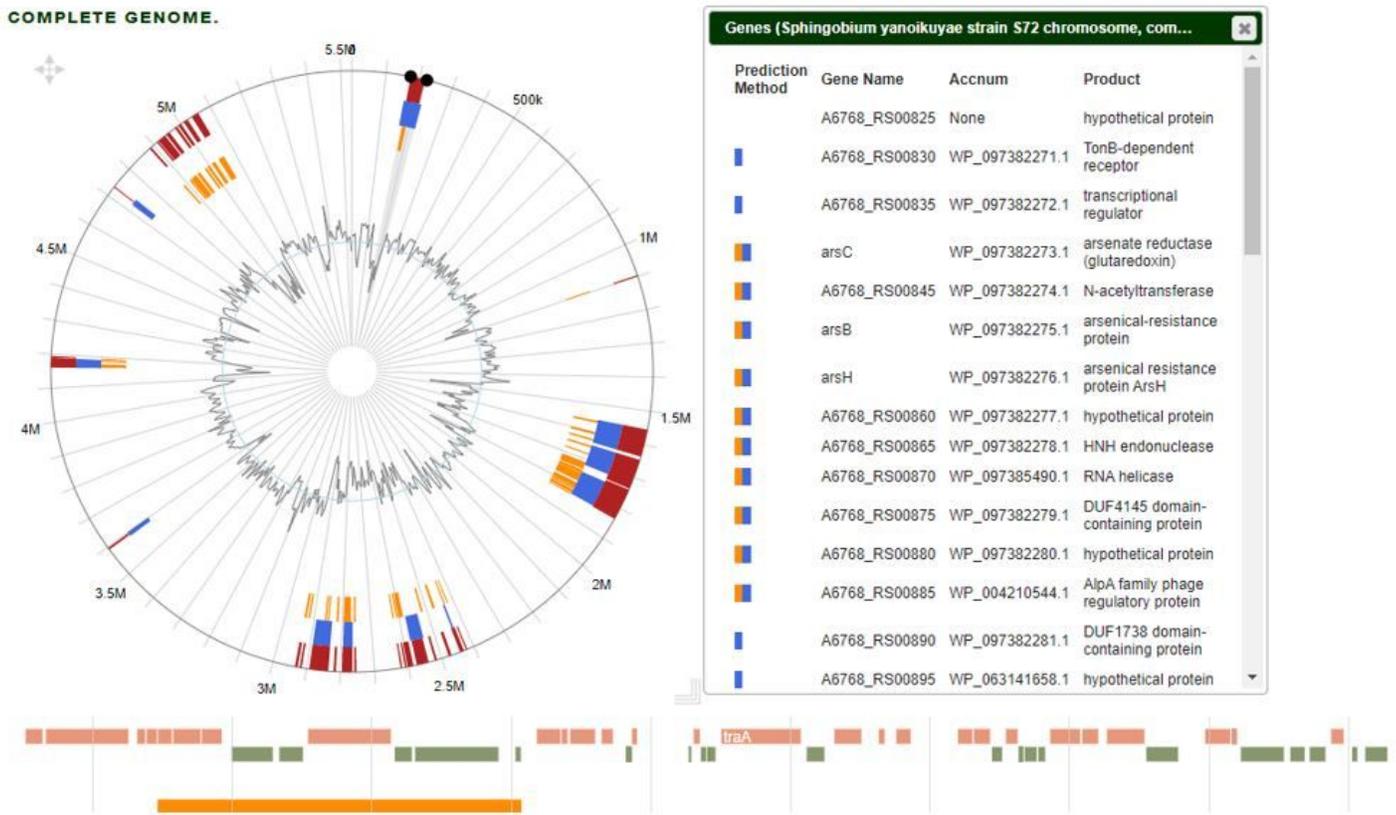


Figure 5

Genomic Island predicted in S72 with Island viewer 4

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