Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. *84* (2) 225–236 (2019)
JSCS–5178
UDC 579.8+547.651:627.8.034.7+
556.53:615.9(540)
Original scientific paper

Characterization of some naphthalene using bacteria isolated from contaminated Cooum Riverine sediment of the Bay of Bengal (India)

SANCHO RAJAN[1], ANWESHA PATTANAIK[2], VENKATESH KUMARESAN[3], PRASANTH BHATT[3], SENTHILARASU GUNASEKARAN[2], JESU AROCKIARAJ[2], MUKESH PASUPULETI[4], VLADIMIR P. BEŠKOSKI[5#] and PAROMITA CHAKRABORTY[1,2]*

[1]*Department of Civil Engineering, SRM Institute of Science and Technology, Kattankulathur 603203, Tamil Nadu, India,* [2]*SRM Research Institute, SRM Institute of Science and Technology, Kattankulathur 603203, Tamil Nadu, India,* [3]*Division of Fisheries Biotechnology & Molecular Biology, Department of Biotechnology, Faculty of Science and Humanities, SRM Institute of Science and Technology, Kattankulathur 603203, Tamil Nadu, India,* [4]*Central Drug Research Institute, B.S. 10/1, Sector 10, Jankipuram Extension, Sitapur Road, Lucknow 226031, India and* [5]*University of Belgrade-Faculty of Chemistry, Studentski trg 12–16, 11000 Belgrade, Serbia*

(Received 24 July, revised 22 September, accepted 11 October 2018)

Abstract: Microorganisms capable of using naphthalene as the sole carbon source were isolated from the contaminated sediment of Cooum River. Twenty one isolates were recovered and nine were selected for enrichment due to differences in their morphological characteristics. Out of nine isolates, only four (NS3-SRMND14B, NS14-SRMND14A, NS15-SRMND14D and NS19--SRMND14E) were capable of completely utilizing naphthalene as the sole source of carbon in carbon free minimal medium (CFMM) supplemented with naphthalene. 16S rDNA sequencing showed that all the four isolates were distantly related to each other and belongs to *Bacillus* sp. (NS3-SRMND14B), *Pseudomonas* sp. (NS14-SRMND14A), *Cellulosimicrobium* sp. (NS15-SRMND14D) and *Sphingobacterium* sp. (NS19-SRMND14E), respectively. Based on the phylogenetic analysis of 16S rDNA sequencing, the isolate *Sphingobacterium* sp. (NS19-SRMND14E) has been identified as novel strain. Polymerase chain reaction (PCR) technique showed the presence of naphthalene dioxygenase (ndo) gene responsible for naphthalene degradation only in the Pseudomonas sp. (NS14-SRMND14A). We observed that the ndo gene is not the only gene responsible for naphthalene degradation. Based on our study, the indigenous microorganisms isolated from Cooum Riverine sediment can be used for bioremediation of the polluted sediment along the Bay of Bengal.

*Corresponding author E-mail: paromita.c@res.srmuniv.ac.in
[#] Serbian Chemical Society member.
https://doi.org/10.2298/JSC180724088R

Keywords: naphthalene; indigenous bacteria; ndo gene; 16S RNA; remediation; phylogenetic tree.

INTRODUCTION

Petroleum based hydrocarbons has been reported to be abundant in the coastal sediment of Chennai city.[1,2] Cooum River is an exceedingly polluted urban stream in Chennai city that flows into the Bay of Bengal. About 80 million litres of domestic sewage and 0.4 million L of industrial effluents flow into this river every day.[3] Further, within the city limits, about 158 sewage/storm water outlets bring raw sewage and silage water into the river, thus the primary source of freshwater quality has deteriorated. From our preliminary and ongoing studies, we observed that naphthalene is one of the most abundant 16 priority PAHs enlisted in USEPA in different environmental matrices monitored around Cooum River.[1] It is therefore utmost necessary to remove naphthalene from the environment particularly from the urban centres of Chennai.

Naphthalene has been used as a model compound in polycyclic aromatic hydrocarbons (PAHs) bioremediation studies.[4,5] It is the simplest and most abundantly occurring compound with the ability to induce cancer in humans and other animals owing to its lipophilic nature.[6–8] Recent years a new strategy called "bioaugmentation" has been proposed. Bioaugmentation involves the introduction of microbes into a contaminated site with the purpose of enhancing the biological activity of the existing population. Several experiments were conducted to introduce a high load of indigenous microorganisms, so that they can survive in the polluted area in higher numbers for degrading the pollutants available.[9–11] Bioaugmentation can be conducted using autochthonous – indigenous (originating from polluted location that need to be bioremediated) or allochthonous (originating from another polluted location) hydrocarbon degrading microorganisms, and various researchers had demonstrated that indigenous strains have many advantages (already adapted to present pollutants, nutrients, pH, temperature and redox conditions).[12,13]

Therefore, in this study our aim was to estimate the potential of indigenous microorganisms for bioaugmentation and bioremediation of naphthalene contaminated Cooum riverine sediment. The primary objectives of this study were to isolate indigenous bacteria capable of using naphthalene as sole carbon source from heavily contaminated surface sediment samples from the Cooum River estuary in the Bay of Bengal, followed by the characterization of them by both biochemical, as well as molecular methods such as 16S rDNA sequencing and phylogenetic analysis in order to further validate the presence and absence of ndo genes.

EXPERIMENTAL

Collection of sediments

Heavily contaminated surface sediment samples (0–20 cm) were collected from five different locations, CN1 (N 13°06′91, E80°28′57), CN2 (N 13°06′89, E80°28′61), CN3

(N13°06′81, E80°28′37), CN4 (N13°06′81, E80°28′39) and CN5 (N 13°06′81, E 80°28′39) from the Cooum River estuary along the Bay of Bengal. Previous published data indicate that the concentration of PAHs containing two aromatic hydrocarbon rings in Cooum sediment was around 9371 and 1044 ng g^{-1}, respectively (Fig. S-1 of the Supplementary material to this paper).[1] The primary source of PAH pollution in urban estuarine environments are the dense urban settlements and industries surrounding the Cooum river as well as the presence of coal-fired thermal power station and the oil spillage from motorized fishing boats.[1] Details related to the study area are given in the Supplementary material.

Enrichment and isolation

For enrichment, carbon free minimal medium (CFMM) broth was used as reported elsewhere.[14] All the five sediment samples were pooled together and 20 g of pooled sample was suspended in 200 mL of CFMM broth (K_2HPO_4, 2.2 gm L^{-1}, KH_2PO_4, 0.8 gm L^{-1}, NH_4NO_3, 3.0 gm L^{-1}, $MgSO_4 \cdot 7H_2O$, 0.5 gm L^{-1}, of trace element solution Fe_2SO_4, 1 %, $CaCl_2$, 1 %. The medium was supplemented with 1 mL of 10 % naphthalene solution in DMSO to obtain 5000 mg L^{-1} and the flasks were incubated at 28±1 °C under shaking condition for 10 days. At the end of the enrichment process, the bacterial strains in the microbial consortium were isolated by spreading 10-fold serially diluted consortium on 20 mL CFMM media plate with 500 µg g^{-1} of naphthalene. The plates were incubated at 28±1 °C for 7 days and the bacterial colonies grown on plates were purified by repetitive streaking on nutrient agar plates. Colonies which showed maximum growth and different morphologies were selected, purified and maintained on slants containing 10 % naphthalene. Each isolate was named by the code SRMND followed by a numerical.

Biochemical tests

Biochemical tests were carried out using a commercially available kit purchased from Hi-Media India. The cells were harvested from overnight culture grown in CFMM and subjected to Indole test, methyl red (MR) test, Voges Proskauer (VP) test and citrate test. Gram staining was performed for all the colonies incubated in the CFMM broth. Growth was confirmed by the colony formation on agar plates containing the naphthalene as sole carbon.

Utilization of naphthalene

The flask containing 1L CFMM broth with an initial naphthalene concentration of 500 µg g^{-1} was inoculated with 10^7 cells mL^{-1} of each isolate. The samples were incubated at 28±1 °C for 10 days. The broth containing 500 µg g^{-1} naphthalene concentrations without inoculum was maintained as a negative control. For quantification of bacterial growth, the samples were aseptically collected in alternate days and assayed for the optical density (OD_{600}) using UV/Vis spectrophotometer (ELICO SL159). Quantification of the residual naphthalene, present in the media inoculated by the bacterial strains, was carried out in high performance liquid chromatography (HPLC, Shimadzu, LC-6AD) using C18 reversed-phase column (150X 4.6 mm, 5µm). Twenty µL of sample was injected and 0.1 % formic acid and acetonitrile were used as mobile phase. Two standards, 10 and 1 µg g^{-1} of naphthalene, were kept as positive control and DMSO was used as negative control. The retention time of naphthalene was observed and the concentration was calculated according to the standard curve.

16S rDNA sequencing and phylogenetic analysis

The genomic DNA was extracted and amplified using the universal forward primer 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and reverse primer 1492R (5'-TACGGYTACCTTGTTACGACTT-3').[15,16] Briefly, the reaction mixture contained 10X assay buffer, 2 µl,

$MgCl_2$, 2 µl, each dNTPs, 2 µl, each primers, 1 µl, Taq DNA polymerase, 0.1 µl, template DNA, 2 µl and the final volume was made up to 20 µl. Amplification was performed in Agilent thermocycler with following conditions: initial denaturation at 94 °C for 2 min followed by 35 amplification cycles of 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 1 min and a final polymerization for 10 min at 72 °C. The amplified products were eluted, purified and sequenced. The DNA sequences obtained in Fasta format were subjected to BLAST analysis (http://www.ncbi.nlm.nih.gov). All the sequences were deposited in the Gene Bank (NS14--SRMND14A, NS3-SRMND14B, NS15-SRMND14D and NS19-SRMND14E).

For phylogenetic analysis, 39 additional sequences related to our taxa were obtained in FASTA format from NCBI nucleotide sequence database (Table S-I of the Supplementary material to this paper). The sequences were manually curated for contig readings and aligned by CLUSTAL W1.6 module in the MEGA v.6.0 software. Maximum Parsimony tree was constructed with the bootstrap value of 1000 replicates. The maximum Parsimony tree was generated using the subtree-pruning-regrafting algorithm with search level three, in which the initial trees were obtained by the random addition of sequences with five replicates and the gaps that were treated as missing data were eliminated.

PCR amplification for ndo genes

The bacterial colonies were suspended in 20 µl of Millipore water, kept at 98 °C for 5 min in thermo-cycler and centrifuged at 10000 rpm for 2 min. The supernatant was subjected to polymerase chain reaction (PCR) using ndo coding primers NarAaF (AGAATGTCTGACTCGAGAAGG) and NarAbR (AGATGAGTCTGGAATCCGAGG) for Gram-positive, and NahAcF (CACCTGATTCATGGCGATGAA), NahAdR (ACCATCAGATTGTGCGTCTGA) for Gram-negative bacteria.[15] *Pseudomonas putida* (ATCC 12633) was used as a positive control as it was well studied for naphthalene degradation with ndo gene. Twenty µl reaction mixture containing 2 µl of 10X assay buffer, 2 µl of $MgCl_2$, 2 µl of each dNTPs, 1 µl of each primer, 0.1 µl of Taq DNA polymerase, 2 µl of template DNA and Molecular Biology grade MQ water was added to make up the volume of the reaction. The thermal cycling conditions included initial denaturation at 94 °C for 2 min followed by 30 cycles of 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 1 min and a final polymerization for 10 min at 72 °C. Amplification was observed through 1.2 % agarose gel electrophoresis. All the samples were analyzed in triplicates.

QA/QC (quality assurance/ quality control)

All the samples consisting naphthalene were diluted with water (1:1), before incubating at 28 °C for 48 h to ensure that the naphthalene degrading bacteria was enumerated. The mean colony count value was obtained from the triplicate experiments. Isolation and PCR amplification were also done in triplicates. One procedural blank followed by a control sample was injected in HPLC, prior to injection of each of the triplicate. Isotope labelled naphthalene was used as surrogate standard. Surrogate recovery varied from 91 to 108 %.

RESULTS AND DISCUSSION

Screening and biochemical characterization

Isolation techniques employed in this study were unbiased and could select both Gram-negative and Gram-positive bacteria. After plating the serially diluted samples on CFMM agar plate supplemented with naphthalene and incubation for 7 days, twenty one morphologically different colonies were selected and res-

treaked on CFMM agar with naphthalene to remove false positive strains. After 24 hrs of incubation at 30 °C, nine morphologically different microbial colonies were observed. These nine isolates referred as NS3-SRMND14B, NS6, NS9, NS10, NS11, NS14-SRMND14A, NS15-SRMND14D, NS19-SRMND14E and NS21 were further cultured in CFMM media with naphthalene to check their growth. Meanwhile, Gram staining and basic biochemical tests for the strains were carried out for better clarity. Details of the Gram staining and biochemical tests are presented in Table I. Out of the 9 isolates recovered from Cooum River sediment, three isolates were Gram-positive (NS3-SRMND14B, NS15--SRMND14D and NS21) and the rest were Gram-negative (NS6, NS9, NS10, NS11, NS14-SRMND14A and NS19-SRMND14E).

TABLE I. Biochemical characteristics of the isolates; (+) positive results, (–) negative results

TEST	Shape	Gram's staining	Indole	MR	VP	Citrate
NS3 (SRMND14B)	Rod	+	+	+	–	+
NS6	Coccus	–	–	–	–	+
NS9	Rod	–	+	+	–	+
NS10	Rod	–	–	+	–	+
NS11	Coccus	–	–	+	–	+
NS14 (SRMND14A)	Coccus	–	–	–	–	+
NS15 (SRMND14D)	Coccus	+	–	–	–	–
NS19 (SRMND14E)	Coccus	–	–	–	–	+
NS21	Rod	+	–	+	–	–

Enrichment and isolation

Four isolates, NS3-SRMND14B, NS14-SRMND14A, NS15-SRMND14D, and NS19-SRMND14E, were further cultured in CFMM supplemented with naphthalene at 28±1 °C due to their efficient growth at 500 μg g^{-1} naphthalene as sole carbon source. Capability of all the four isolates for using naphthalene as sole carbon source was further correlated with the increasing growth optical density (*OD*). We could observe that all four isolates showed drastic increase in growth from day 1 to day 7 and then started decreasing from day 8 and ultimately stopped on day 10 due to depletion in the substrate concentration (Fig. 1).

Molecular characterization

A BLASTn search comparing the 1200 bp nucleotide sequences of the strain NS14-SRMND 14A showed 98 % sequences similarity with the 16s rDNA sequences of *Pseudomonas putida* (KC952984), *P. plecoglossicida* (KC345028, GQ301534), *P. monteilii* (HM060245, GU191931), and different strains of *Pseudomonas* species (KC294049, KR063184, KJ534491, KR023988).

The nucleotide sequences of NS3-SRMND 14B comprising of 1472 bp showed maximum sequence (97 %) similarity with the sequences of *Bacillus cereus* (CP009641, AY129651), *B. thuringiensis* (CP009335, GU120652), *B. anthracis*

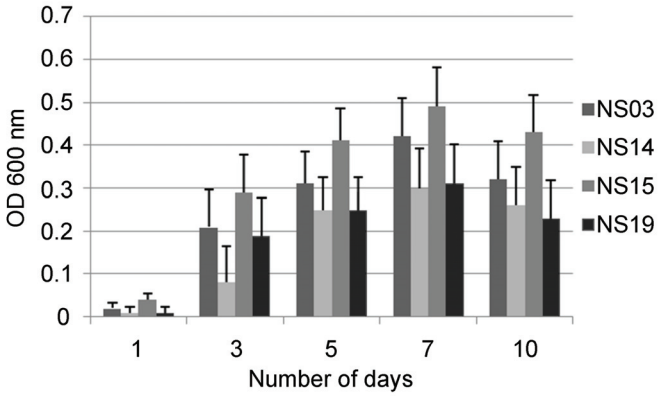

Fig. 1. Optical Density (OD600) values for a span of 10 days.

(VCP009328), and a *Bacillus* strain (KF479557). The 16S rDNA sequences of NS15-SRMND 14D comprising of 1439 bp showed 98–97 % sequence similarity with the sequences of *Cellulosimicrobium funkei* (JQ659856, NR_042937, JQ659850), *C. cellulans* (GU012422, NR119095, NR_115251, EU287931) and a *C.* strain (KR906518). The partial sequences comprising of 1206 bp of NS19--SRMND 14E have 93 % sequence similarity with *Sphingobacterium alimentarium* (FN908504, NR108489), and 92 % similarity with *S. composti* (NR112559), *S. nematocida* (NR122101), *S. psychroaquaticum* (NR108297), *S. hotanense* (NR108440) and different strains of *Sphingobacterium* (FJ999951, KC009698, AB680845.1, KJ152099.1). In a phylogenetic tree constructed by maximum likelihood method, the sequences of SRMND isolates fall out into four distinct Clades. Clade I comprising of species of *Pseudomonas* and Clade II includes *Bacillus* species. Clade III and Clade IV are represented by the species of *Cellulosimicrobium* and *Sphingobacterium*, respectively. The strain NS14-SRMND 14A clusters with Clade I and hence identified as a species of *Pseudomonas*. Similarly, the strain NS3-SRMND 14B falls out in the Clade II and identified as *Bacillus* species. The strains NS15-SRMND 14D and NS19-SRMND 14E nested with Clade III and Clade IV, respectively. The strain NS15-SRMND 14D taxonomically assigned to the species *Cellulosimicrobium*.

Although the strain NS19-SRMND14E falls in the Clade IV comprising of *Sphingobacterium* species, the considerable branch length differentiates our strain from other closely related taxa. Hence, we suspected this could be a novel species and our future research will confirm or refute this issue.

Isolates NS14-SRMND14A, NS15-SRMND14D, NS19-SRMND14E and NS3-SRMND14B belong to Gammoproteobacteria (*Pseudomonas* sp.) Terrabacteria (*Cellulosimicrobium* sp., *Bacillus* sp.) FCB group and Bacteroidetes group (*Sphingobacterium* sp.) respectively. It has been reported that the soil microbe

isolates with PAH degrading ability mostly belong to Sphingomonadaceae family.[8,17,18]

Isolate NS14-SRMND14A is closely related to *Pseudomonas* spp. in the group Gammaproteobacteria. *Pseudomonas* sp. has been found to uptake naphthalene completely. Our results clearly showed that the isolated *Pseudomonas* sp. is able to use naphthalene as the sole source of carbon. The products of naphthalene biodegradation were thoroughly searched using non-target analysis, but based on our results in the study with *Pseudomonas* sp. NS14-SRMND 14A, the uptake of naphthalene did not leave any byproducts as shown in Fig 2C.

The isolate NS3-SRMND 14B is closely related to *Bacillus* sp. in the group Firmicutes. Previous studies showed that *Bacillus* sp. were capable of using one or more hydrocarbon species due to their ability to colonize the environments contaminated with hydrocarbons.[8,19] According to Kosaric *et al. Bacillus pumilus* strain 28-11, showed a high emulsion capacity of 100 % after 3 days of static storage with other microorganism.[20]

The isolate NS15-SRMND 14D is closely related to *Cellulosimicrobium* sp. in the group Actinobacterium. *Cellulosimicrobium cellulans* is an indigenous bacterium in tarball which is capable of using hydrocarbon mixtures in diesel oil as their sole carbon source through aerobic or anerobic pathways.[9,21]

Identification of ndo gene

It is reported in the literature that the bacterial strains which show bioremediation phenomenon usually exhibit genes related to degradation activity.[22,23] Simon *et. al* reported the naphthalene degradation ability of *Pseudomonas putida* strains G7 and NCIB 9816-4 ability to degrade the naphthalene is due to the presence of naphthalene dioxygenase, ndo gene on pDTG1 plasmid. Given this information, we investigated the presence of naphthalene dioxygenase, ndo gene in the isolates by using standard PCR. Of the four bacterial strains screened for ndo genes, the amplification was observed only in strain NS14-SRMND 14A, which revealed a band at 1.8 kb, whereas the other three strains (NS3-SRMND 14B, NS15-SRMND 14D and NS19-SRMND 14E) did not show any amplification in all the triplicate samples (Fig. 3). In order to rule out the possibility of false positive results and experimental limitations associated with PCR method, the presence of the ndo gene was also explored by using the degenerate primers and altering PCR conditions. Based upon our results, it is very intriguing to speculate the specific pathways or enzymes involved in the degradation of naphthalene by the other three strains (NS3- SRMND 14B, NS15-SRMND 14D and NS19-SRMND 14E) as we did not find any signs of amplifications.

Sphingomonas strain F199 contains 13 gene clusters which are predicted to encode enzymes associated with the degradation of aromatic compounds.[25] On the other hand, *Sphingobium yanoikuyae* strain B1 has a 40-kb plasmid which has two dioxygenase genes.[26] In contrast to Gram-negative bacteria like *Pseudomonas*

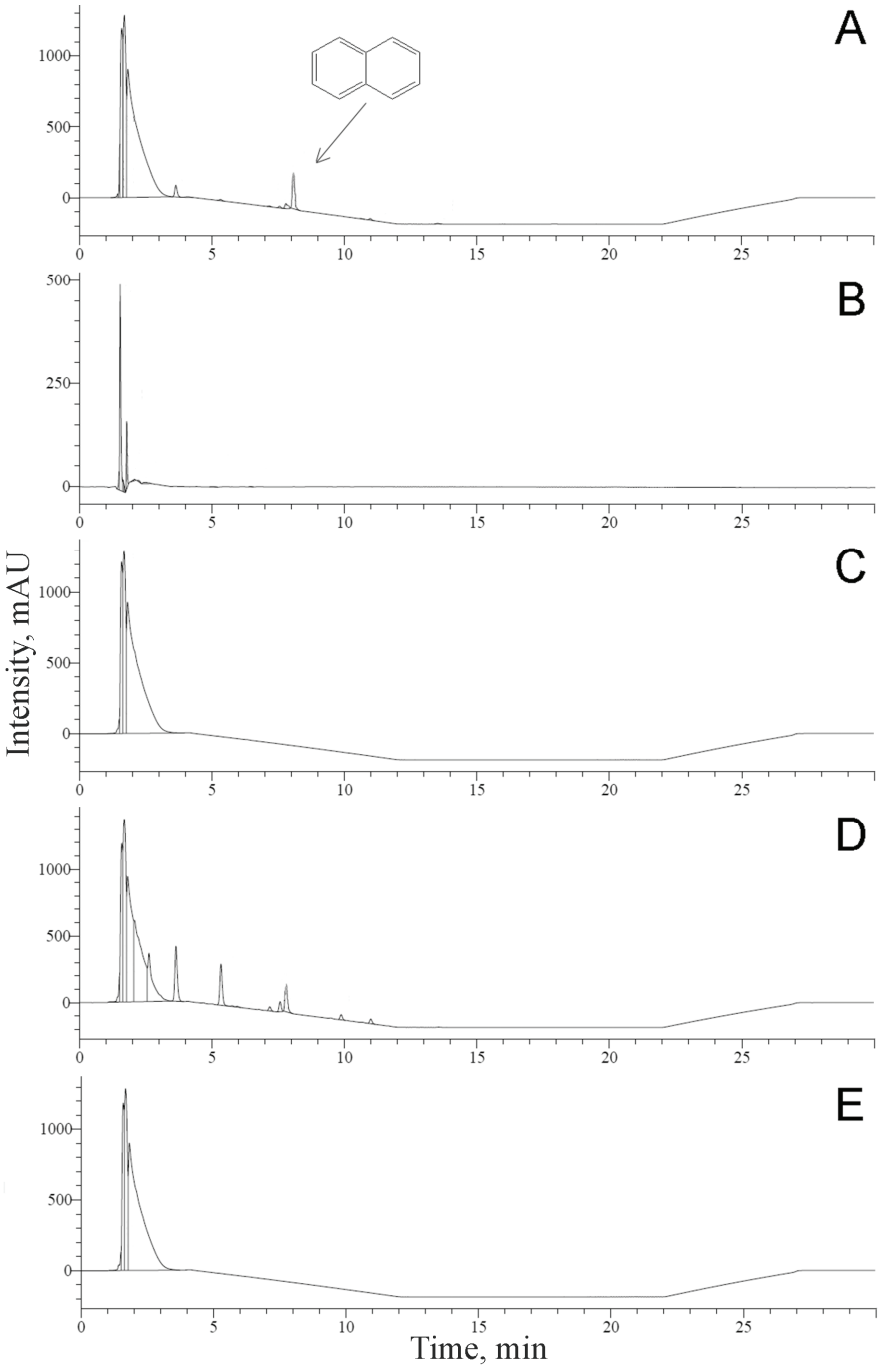

Fig. 2. Biodegradation of naphthalene: control (A), NS3 *Bacillus* sp. (B), NS14 *Pseudomonas* sp. (C), NS19 *Sphingobacteruim* sp. (D), NS15 *Cellulosimircobium* sp. (E).

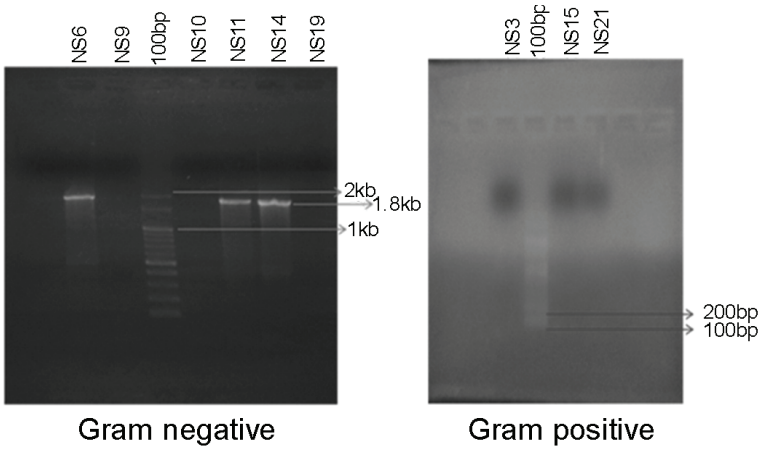

Fig. 3. Amplification of ndo gene in Gram-negative and Gram-positive bacteria using PCR.

and *Sphingobium*, Gram-positive bacteria *Rhodococcus* strains use three structural genes (*narAa*, *narAb* and narB) for naphthalene utilization. Even though narAa and narAb encode for enzymes with activity against naphthalene, they share only 30% identity with the Pseudomonas NDO subunits at amino acid level.[27] Some *Sphingobium* sp. have multiple ring-hydroxylating oxygenase (RHO) genes which have the putative genes responsible for the degradation of various aromatics in this bacterial strain.[28] The members of the *Sphingomonas* genus are often isolated from petroleum-contaminated soils, on selective media, due to their unique abilities to degrade polycyclic aromatic hydrocarbons (PAHs), which are important for in situ bioremediation.[29] However, other isolates *Cellulosimicrobium* sp., *Bacillus* sp. and *Sphingobacterium* sp. were capable of utilizing naphthalene as the sole carbon source like *Pseudomonas* sp. Various gram negative bacteria also contain extra plasmid which contains an array of genes particularly ndo gene, responsible for degradation of aromatic compounds as in the case of *Pseudomonas* sp. Our results are consistent with other observations which reported that ndoB genes are responsible for PAH degradation in *Pseudomonas* strains.[30,31]

CONCLUSION

In the present study, the indigenous naphthalene degrading bacteria were isolated from Cooum Riverine sediment and identified as *Pseudomonas* sp., *Cellulosimicrobium* sp., *Bacillus* sp. and *Sphingobacterium* sp based on 16S rDNA sequence characteristics. The results of this study indicate that the indigenous bacteria isolated from the contaminated sites of Cooum River sediment actively used naphthalene as sole carbon source. These isolates could be useful for site remediation studies. Further, we observed that the ndo gene is not the only gene responsible for naphthalene degradation. However, more research is

needed to explain the degradation mechanism with other genes the same as with ndo gene. Based on our study, when naphthalene is used as the only source of carbon, these bacteria may use the entire carbon source without leaving any secondary compounds.

SUPPLEMENTARY MATERIAL

Additional data are available electronically at the pages of journal website: http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

Acknowledgment. This work was supported by the start-up grant provided by SRM University to Dr. Paromita Chakraborty and project funded by Ministry of Environment and Forest and Climate Change, India, File No.Q-14011/ 43/2013-CPW (EHC). Authors would also like to thank those graduate students of SRM Institute of Science and Technology who extended their help in sample collection.

И З В О Д

КАРАКТЕРИЗАЦИЈА НАФТАЛЕН-ДЕГРАДИРАЈУЋИХ БАКТЕРИЈА ИЗОЛОВАНИХ ИЗ ЗАГАЂЕНОГ СЕДИМЕНТА РЕКЕ COOUM У БЕНГАЛСКОМ ЗАЛИВУ (ИНДИЈА)

SANCHO RAJAN[1], ANWESHA PATTANAIK[2], VENKATESH KUMARESAN[3], PRASANTH BHATT[3], SENTHILARASU GUNASEKARAN[2], JESU AROCKIARAJ[2], MUKESH PASUPULETI[4], VLADIMIR P. BESKOSKI[5] и PAROMITA CHAKRABORTY[1,2]

[1]Department of Civil Engineering, SRM Institute of Science and Technology, Kattankulathur 603203, Tamil Nadu, India, [2]SRM Research Institute, SRM Institute of Science and Technology, Kattankulathur 603203, Tamil Nadu, India, [3]Division of Fisheries Biotechnology & Molecular Biology, Department of Biotechnology, Faculty of Science and Humanities, SRM Institute of Science and Technology, Kattankulathur 603203, Tamil Nadu, India, [4]Central Drug Research Institute, B.S. 10/1, Sector 10, Jankipuram Extension, Sitapur Road, Lucknow 226031, India и [5]Универзитет у Београду–Хемијски факултет, Студентски трг 12–16, 11000 Београд

Микроорганизми способни да користе нафтален као једини извор угљеника изоловани су из контаминираног седимента реке Cooum. Селектован је двадесет један изолат и девет је изабрано за даљи рад и обогаћивање услед разлика у њиховим морфолошким карактеристикама. Од девет изолата, само су четири (NS3-SRMND14B, NS14-SRMND14A, NS15-SRMND14D, NS19-SRMND14E) била у стању да у потпуности искористе нафтален у минералној подлози са нафталеном као јединим извором угљеника. Секвенцирање 16S rDNA показало је да су сва четири изолата еволутивно удаљена један од другог и да припадају родовима *Bacillus* sp. (NS3-SRMND14B), *Pseudomonas* sp. (NS14-SRMND14A), *Cellulosimicrobium* sp. (NS15-SRMND14D), и *Sphingobacterium* sp. (NS19-SRMND14E). На основу филогенетске анализе секвенце 16S rDNA, изолат *Sphingobacterium* sp. (NS19--SRMND14E) је идентификован као нови соj. Техника ланчане реакције полимеразе показала је присуство гена нафтален-диоксигеназе (ndo) одговорног за деградацију нафталена само у изолату *Pseudomonas* sp. (NS14-SRMND14A). Уочили смо да ndo ген није једини ген одговоран за деградацију нафталена. На основу наше студије, аутохтони микроорганизми изоловани из седимента Коум реке могу се користити за биоремедијацију загађеног седимента дуж Бенгалског залива.

(Примљено 24. јула, ревидирано 22. септембра, прихваћено 11. октобра 2018)

REFERENCES

1. P. Goswami, T. Ohura, K. S. Guruge, M. Yoshioka, N. Yamanaka, M. Akiba, N. Munuswamy, *Ecotox. Environ. Safe.* **130** (2016) 113 (https://doi.org/10.1016/j.ecoenv.2016.04.016)
2. S. Veerasingam, R. Venkatachalapathy, S. Sudhakar, P. Raja, V. Rajeswari, *J. Environ. Sci.* **23** (2011) 1129 (https://doi.org/10.1016/S1001-0742(10)60524-4)
3. P. Nammalwar, P. Pakshirajan, in *Proceedings of the IV National Symposium on "Environment"*, 1995, Madras, Anna University, India, p. 52
4. B. Prajapati, G. Shiv, S. Gaherwal, K. Billore, R. Soni, *Int. J. Res. Chem. Environ.* **6** (2016) 26
5. S. K. Samanta, O. V. Singh, R. K. Jain, *Trends Biotechnol.* **20** (2002) 243 (https://doi.org/10.1016/S0167-7799(02)01943-1)
6. R. Nagabhushanam, P. Machale, R. Katyayani, P. Reddy, R. Sarojini, *J. Ecotoxicol. Environ. Monit.* **1** (1991) 185
7. E. C. Santos, R. J. Jacques, F. M. Bento, R. P. Maria do Carmo, P. A. Selbach, E. L. Sá, F. A. Camargo, *Bioresource Technol.* **99** (2008) 2644 (https://doi.org/10.1016/j.biortech.2007.04.050)
8. D. Ghosal, S. Ghosh, T. K. Dutta, Y. Ahn, *Front. Microbiol.* **7** (2016) 1369 (https://doi.org/10.3389/fmicb.2016.01369)
9. B. M. Dellagnezze, G. V. de Sousa, L. L. Martins, D. F. Domingos, E. E. Limache, S. P. de Vasconcellos, G. F. da Cruz, V. M. de Oliveira, *Mar. Pollut. Bull.* **89** (2014) 191 (https://doi.org/10.1016/j.marpolbul.2014.10.003)
10. M. Hassanshahian, G. Emtiazi, G. Caruso, S. Cappello, *Mar. Environ. Res.* **95** (2014) 28 (https://doi.org/10.1016/j.marenvres.2013.12.010)
11. A. Pawar, S. Ugale, M. More, N. Kokani, S. Khandelwal, *J. Bioremed. Biodeg.* **4** (2013) 203 (https://doi.org/10.4172/2155-6199.1000203)
12. G. Gojgic-Cvijovic, J. Milic, T. Solevic, V. Beskoski, M. Ilic, L. Djokic, T. Narancic, M. Vrvic, *Biodegradation* **23** (2012) 1 (https://doi.org/10.1007/s10532-011-9481-1)
13. V. P. Beškoski, G. Gojgić-Cvijović, J. Milić, M. Ilić, S. Miletić, T. Šolević, M. M. Vrvić, *Chemosphere* **83** (2011) 34 (https://doi.org/10.1016/j.chemosphere.2011.01.020)
14. N. Ceyhan, *Sci. Res. Essays* **7** (2012) 66 (https://doi.org/10.5897/SRE11.1146)
15. C. Alquati, M. Papacchini, C. Riccardi, S. Spicaglia, G. Bestetti, *Ann. Microbiol.* **55** (2005) 237
16. T.-T. Liu, Y. Xu, H. Liu, S. Luo, Y.-J. Yin, S.-J. Liu, N.-Y. Zhou, *Appl. Microbiol. Biot.* **90** (2011) 671 (https://doi.org/10.1007/s00253-010-3033-1)
17. T. Basta, A. Keck, J. Klein, A. Stolz, *J. Bacteriol.* **186** (2004) 3862 (https://doi.org/10.1128/JB.186.12.3862-3872.2004)
18. J. K. Fredrickson, D. L. Balkwill, M. F. Romine, T. Shi, *J. Ind. Microbiol. Biot.* **23** (1999) 273 (https://doi.org/10.1038/sj/jim/2900741)
19. W.-Q. Zhuang, J.-H. Tay, A. Maszenan, S. Tay, *Appl. Microbiol. Biot.* **58** (2002) 547 (https://doi.org/10.1007/s00253-001-0909-0)
20. N. Kosaric, H. Choi, *Tenside Surfact. Det.* **27** (1990) 294
21. B. M. Nkem, N. Halimoon, F. M. Yusoff, W. L. W. Johari, M. P. Zakaria, S. R. Medipally, N. Kannan, *Mar. Pollut. Bull.* **107** (2016) 261 (https://doi.org/10.1016/j.marpolbul.2016.03.060)
22. G. S. Sayler, S. W. Hooper, A. C. Layton, J. H. King, *Microb. Ecol.* **19** (1990) 1 (https://doi.org/10.1007/BF02015050)
23. C. M. Serdar, D. T. Gibson, *Biochem. Bioph. Res. Co.* **164** (1989) 772

24. M. J. Simon, T. D. Osslund, R. Saunders, B. D. Ensley, S. Suggs, A. Harcourt, S. Wenchen, D. L. Cruder, D. T. Gibson, G. J. Zylstra, *Gene* **127** (1993) 31
25. M. F. Romine, L. C. Stillwell, K.-K. Wong, S. J. Thurston, E. C. Sisk, C. Sensen, T. Gaasterland, J. K. Fredrickson, J. D. Saffer, *J. Bacteriol.* **181** (1999) 1585
26. E. Kim, G. Zylstra, *J. Ind. Microbiol. Biot.* **23** (1999) 294
27. L. A. Kulakov, S. Chen, C. C. Allen, M. J. Larkin, *Appl. Environ. Microb.* **71** (2005) 1754 (https://doi.org/10.1128/AEM.71.4.1754-1764.2005)
28. P. Khara, M. Roy, J. Chakraborty, D. Ghosal, T. K. Dutta, *FEBS Open Bio.* **4** (2014) 290 (https://doi.org/10.1016/j.fob.2014.03.001)
29. L. Zhou, H. Li, Y. Zhang, S. Han, H. Xu, *Braz. J. Microbiol.* **47** (2016) 271 (https://doi.org/10.1016/j.bjm.2016.01.001)
30. C. Hamann, J. Hegemann, A. Hildebrandt, *FEMS Microbiol. Lett.* **173** (1999) 255
31. D. Das, R. Baruah, A. S. Roy, A. K. Singh, H. P. D. Boruah J. Kalita, T. C. Bora, *Genomics* **105** (2015) 182 (https://doi.org/10.1016/j.ygeno.2014.12.006).