Vol. 12(46), pp. 1032-1038, 14 December, 2018
DOI: 10.5897/AJMR2018.8860
Article Number: 6E49F9059669
ISSN: 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Isolation and characterization of phenol degrading *Bacillus* species from a Southeast Brazilian mangrove sediment

Anna Paula Santos Almeida Rotta[1], Bruno Francesco Rodrigues de Oliveira[2], Renan de Souza Soares[1], Igor Daniel Alves Ribeiro[3], Ariana Alves Rodrigues[1], Danns Pereira Barbosa[4], Matheus Maitan Vieira[5] and José Daniel Gonçalves Vieira[1*]

[1]Environmental Microbiology and Biotechnology Laboratory, Department of Microbiology, Immunology, Parasitology and Pathology, Tropical Pathology and Public Health Institute, Federal University of Goiás, Goiânia, Goiás, Brazil.
[2]Molecular and Marine Bacteriology Laboratory, Department of Medical Microbiology, Institute of Microbiology Paulo de Góes, Federal University of Rio de Janeiro, Rio de Janeiro, Rio de Janeiro, Brazil.
[3]Agriculture Microbiology Group, Department of Genetics, Institute of Biosciences, Federal University of Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil.
[4]Chemistry Laboratory, Institute of Chemistry, Federal Institute of Goiás, Goiânia, Goiás, Brazil.
[5]Laboratory of Bioprocesses and Sustainable Products, Department of Biotechnology, School of Engineering of Lorena, University of São Paulo, Lorena, São Paulo, Brazil.

Received 3 April, 2018; Accepted 5 December, 2018

Phenolic compounds are common pollutants in industrial effluents and pose a significant risk to the environment. The microbial capacity to degrade these molecules makes the prospection of bacteria with this ability very strategic for bioremediation processes in contaminated areas. This study was carried out to isolate bacteria with phenol degrading capacity from Brazilian mangrove soil. Microorganisms were evaluated for tolerance and the ability to use this substance as a single source of carbon. Two bacterial isolates were obtained: MF-1 and MF-2. The microorganisms were identified, respectively, as *Bacillus cereus* and *Bacillus circulans* by sequencing the 16S rRNA gene. The isolates exhibited tolerance to phenol at concentrations up to 600 mg.L^{-1} when grown in Bushnell-Haas Agar (BHA) and 1,500 mg.L^{-1} in nutrient agar (NA). As for the use of phenol as a single carbon source, the microorganisms were grown in concentrations of 100, 200 and 300 mg.L^{-1} of the compound. The isolate MF-1 presented a percentage consumption of 22.43, 11.52 and 3.33%, respectively, for each tested concentration. Although there was a decrease in phenol consumption in response to the increased concentration of this substance, bacterial growth was not affected. The best results were obtained for the isolate MF-2, in which consumption of phenol was, respectively, 21.54, 20.54 and 28.85% for these concentrations. Likewise, the increase in phenol concentration caused no reduction in the growth of this isolate.

Key words: Bioremediation, xenobiotic, phenol degrading bacteria, mangrove.

INTRODUCTION

Phenol and its derivatives comprise an important class of environmental contaminants, primarily by their presence in effluents from different industries, as in the manufacturing of agriculture inputs, as pesticides and

fungicides, in the bleaching of cellulose, in pharmaceutical and textile industries, oil refineries, among others (Gami, 2014; Senthilvelan et al., 2014; Banerjee and Ghoshal, 2016; Patil and Gena, 2016).

It is a pollutant with high stability and toxicity, even in small concentrations. This pollutant may be fatal if ingested and when in contact with skin and respiratory tract, is quickly absorbed and may cause severe irritation (Sarwade and Gawai, 2014; Mohanty et al., 2018). Due to its toxicity, the phenol should be removed from the environment. The available methods for phenol removal include physical, chemical and biological techniques. However, physico-chemical methods are expensive and tend to form equally toxic intermediate compounds, such as hydrocarbons. Thus, biological processes of phenol degradation are preferable because of their economy and low probability of formation of hazardous subproducts (Arutchelvan et al., 2006; Silva and Souza, 2015). The biodegradation of complex molecules normally involve the interactive effect of mixed communities that constitutes the microbiota of soil, water and sediments. When those environments are contaminated, there is an adaptation of certain microbial populations that recognize phenol and other aromatic compounds as carbon sources, and, therefore, are able to survive (Lehtinen et al., 2014; Bajaj and Singh, 2015). In this regard, the isolation and identification of microorganisms fitted for these environmental conditions commonly found in certain Brazilian ecosystems, such as mangroves, could be an alternative for the improvement of pollutant degradation bioprocesses.

Several authors have cited the application of microorganisms, predominantly bacteria, targeting the removal of phenolic compounds in industrial wastewater as, for instance, *Acinetobacter* species (Jiang et al., 2013), *Alcaligenes faecalis* (Moghadam et al., 2016), *Pseudomonas* (Ahmad et al., 2014), *Ochrobactrum* species, *Kocurica camiphilia* (Hamedo et al., 2014) and *Bacillus* species (He et al., 2013).

The common route of phenol degradation by microorganism is by dihydroxylation of the benzene ring which generates a catechol derivate. From that point, the benzene ring can be opened by meta or ortho oxidation. In meta-oxidation, the catechol is oxidized by the enzyme catechol-2,3-dioxygenase, whereas in ortho oxidation, the reaction is conducted by catechol-1,2-oxygenase. Both routes generate products that can enter into the tricarboxylic acid cycle (Mahiudddin et al., 2012; Sridevi et al., 2012; Hasan and Jabeen, 2015).

In particular, the *Bacillus* genus have been recovered from various environments, and are capable of degrading phenol at various temperatures, pH and concentrations by ortho and meta catechol pathways (Banerjee and Ghoshal, 2010; Sarwade and Gawai, 2014). Several phenol degrading species has been reported, such as *Bacillus cereus* (Banerjee and Ghoshal, 2010; Zhang et al., 2013), *Bacillus brevis* (Arutchelvan et al., 2006), *Bacillus badius* (Sarwade and Gawai, 2014), *Bacillus thuringiensis* (Ereqat et al., 2017) and *Bacillus stearothermophilus* (Kim and Oriel, 1995). The genus was already recovered from mangroves (Yateem and Al-Sharrah, 2011; Auta et al., 2017) and reported as phenol tolerant and degrading (Sivasubramanian et al., 2016). Due to its ubiquitous presence and ability to tolerate and degrade phenol by both pathways, the *Bacillus* genus is extremely interesting for the bioprospecting of phenol degrading bacteria, especially those derived from phenol impacted environments, such as the Brazilian mangroves.

Biodegradation has been one of the multiple facets of microbial catabolism extensively explored in recent years, especially in bioremediation. Therefore, the aim of the present work was to evaluate, in lab-scale setup, the biodegradation of phenol by bacterial strains isolated from a Brazilian mangrove soil, watching out for the influence of previous bacterial adaptation, the effects of growth parameters in different phenol concentrations and tolerance to this compound.

MATERIALS AND METHODS

Sample collection and isolation of bacteria

Four 250 g sediment samples were collected from a mangrove area located in the city of Guarapari, ES, Brazil (20°39'565''S, 40°30'023''W). All samples were transferred aseptically to sterile plastic bags, kept at low temperature (0-4°C) and transported to the lab in styrofoam containers. A total of 10 g of each sample were initially inoculated and homogenized in 250-mL Erlenmeyer flasks containing 90 mL of Bushnell-Hass (BH) mineral medium ($g.L^{-1}$: KH_2PO_4, 1.0 g; K_2HPO_4, 1.0 g; NH_4NO_3, 1.0 g; $MgSO_4$, 0.2 g; $CaCl_2$, 0.2 g; $FeCl_3$, 0.05 g) (Bushnell and Hass, 1941) supplemented with phenol (Merck) as sole carbon source at a final concentration of 300 $mg.L^{-1}$ and of synthetic marine salts (18 $g.L^{-1}$) (Dias et al., 2009). The flasks were incubated under orbital agitation (130 rpm) at 30°C. After 72 h of growth, a 10 mL aliquot of each sample was collected and transferred to new BH broth (90 mL) added with phenol and synthetic marine salts at the previously described concentrations, succeeded by incubation under agitation as formerly specified. This procedure was repeated four more times. Subsequently, each sample was serially diluted from 10^{-1} to 10^{-10} in phosphate buffer (pH 8.0) and proper dilutions (10^{-4} to 10^{-10}) were plated on BH Agar (BHA), supplemented with glucose (1.0 $mg.L^{-1}$) and of synthetic marine salts (18 $g.L^{-1}$). The material was then incubated at 30°C for 24/48 h.

Bacterial colonies were isolated according to their morphology and selected morphotypes were purified by repeated streaking on BH agar supplemented with synthetic marine salts (18 $g.L^{-1}$). After completing the purification, the two recovered isolates were grown on Brain Heart Infusion Broth (BHI) (Oxoid) to which synthetic

*Corresponding author. E-mail: jdgvieira62@gmail.com.

marine salts (18 g.L^{-1}) was added. After incubation at 30°C for 24-48 h, bacterial strains were stored as 30% glycerol stocks at -20°C for further studies. Isolates were designated by the initials "M" (refereeing to the environment of origin, "mangrove") and "F" (related to the word phenol in Portuguese, "fenol") followed by a number: MF-1 and MF-2.

Molecular identification and phylogenetic analyses

Genomic DNA was extracted following the methodology described by Van Soolingen et al. (1994). Total genomic DNA (25-50 ng) was used as template for the amplification of the 16S ribosomal RNA (16S rRNA) coding region with the universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1541R (5'-AAGGAGGTGATCCAGCC-3') (Weisburg et al., 1991). The Polymerase Chain Reaction (PCR) was carried out using a reaction mixture of 50 µL containing 35.5 µL of water, 5.0 µL of PCR buffer 10 X (Invitrogen), 1.5 µL of $MgCl_2$ (50 mM) (Invitrogen), 1.0 µL of each primer (10 mM), 4.0 µL of deoxynucleoside triphosphate solution (2.5 mM) (Invitrogen) and 1.0 µL of Taq DNA polymerase (5.0 U) (Invitrogen). Amplification conditions were adjusted on a thermocycler (Bio-Rad T100™ Thermal Cycler) as follows: initial denaturation step at 94°C for 3 min; 30 cycles of 94°C for 1 min, 55°C for 30 s, 72°C for 30 s; and a final elongation step at 72°C for 10 min. PCR products were analyzed by electrophoresis on a 1.2% agarose gel and purified with the E.Z.N.A.® Cycle Pure Kit (Omega Bio-tek).

DNA sequencing was performed on ABI 3130xl platform (Applied Biosystems) with the primers: 27F, 530F (5'–GTGCCAGCMGCCGCGG –3'), 519R (5'–GWATTACCGCGGCKGCTG –3'), 907R (5'–CCGTCAATTCMTTTRAGTTT–3'), 926F (5'-AAACTYAAAKGAATTGACGG-3') and 1541R. All retrieved sequences had their quality checked, were merged into contigs with the CodonCode Aligner software version 8.0.1 (CodonCode Aligner), and compared with bacterial 16S rRNA gene sequences deposited in the GenBank database from the National Center for Biotechnology Information (NCBI) using Basic Local Alignment Search Tool (BLAST) algorithm (Altschul et al., 1990).

Representative sequences were selected from the GenBank database for phylogenetic inference that was conducted using the Molecular Evolutionary Genetics Analysis (MEGA) program version 7.0.212 (Kumar et al., 2016). ClustalW tool (Larkin et al., 2007) was applied to align sequences and phylogenetic trees were constructed using the neighbor-joining (NJ) method and the Jukes-Cantor model, with their reliability guaranteed by 1000 bootstrap replicates. The 16S rRNA gene sequences of both isolates, MF-1 and MF-2, were submitted to GenBank under the accession numbers NR_074540.1 and NR_112632.1.

Screening of phenol tolerance and utilization

Phenol tolerance was assessed by cultivation of the isolated strains on BHI medium containing phenol in crescent concentrations (0 to 1,600 mg.L^{-1}). Microorganisms were previously grown on BHI broth at 30°C for 48 h and seeded onto the surface of the phenol-containing Nutrient Agar (NA) with a Steer's replicator (Steers et al., 1959). The development of a single bacterial colony within 24-48 h at 30°C was considered as a tolerant strain for the evaluated phenol concentration.

The capacity to consume phenol was determined similar to the analysis of the tolerance ability, with the assays performed in BHA also supplemented with phenol in gradual concentrations (0 to 1,600 mg.L^{-1}). Glucose (1.0 g.L^{-1}) was incorporated in the medium with no addition of the aromatic compound (0 mg.L^{-1}). Microbial inoculation and interpretation of results were carried out as formerly detailed to the determination of tolerance. In this screening stage, both media (NA and BHA) were added with synthetic marine salts (18 g.L^{-1}).

Growth assessment and phenol degradation

To study the capacity of the bacterial strains to use phenol as a sole carbon source under stationary conditions, microorganisms were firstly grown in BH broth added with 1.0% glucose and 1.8% synthetic marine salts at 30°C for 48 h. Consecutively, a 10% aliquot of starved cultures was used as inoculum into 250-mL Erlenmeyer flasks with 50 mL of BH broth. The considered phenol concentrations were 100, 200 and 300 mg. L^{-1}, respectively. Flasks were incubated at 30°C under orbital agitation (130 rpm) and 5.0 mL aliquots were collected at two-time intervals, 0 and 96 h, for the determination of bacterial growth and phenol degradation under stationary culture. Microbial growth was assessed spectrophotometrically (BIO-RAD/Smart Spec PLUS) by measuring absorbance at 600 nm. Initial (0 h) and final residual (96 h) phenol concentrations were determined by YL9100 High Performance Liquid Chromatography (HPLC) SYSTEM. Briefly, 1.0 mL aliquot of cultures was centrifuged at 4,000 rpm for 15 min under low temperature (4°C). HPLC analysis was performed with C-8 phenonmenex column, methanol-water (70:30; v/v) as mobile phase and a flux of 1.0 mL.min^{-1}. Compounds derived from phenol biotransformation were monitored by YOUNLIN INSTRUMENTO MODELO NO-acme 9000 and phenol was analyzed using a UV-Vis detector at a wavelength of 264 nm.

RESULTS AND DISCUSSION

Despite being considered as stressed environments, primarily due to anthropogenic action, mangroves are recognized as tolerant and adaptive ecosystems in the presence of wastewaters and other pollutants, as heavy metals. This can be partially explained by the complex mechanisms in roots controlling contaminant uptake and processing (Lewis et al., 2011). Particularly in sediments, low molecular weight aromatic derived compounds can be notoriously hazardous due to its phytotoxicity and capacity to affect plants during all their development. In this context, the associated microbiota in these environments has a pivotal role in persistence of mangroves even in highly impacted areas, especially polycyclic aromatic hydrocarbon (PAH)-degrading bacteria. Beyond their presence, these microorganisms are also capable of adapting to contamination which can explain their extensive attenuation ability in polluted regions (Gomes et al., 2010; Guo et al., 2012).

Two bacterial isolates were recovered from the mangrove sediment samples based on colony morphology. Once the present work aimed to evaluate the degradation potential of the isolated bacteria rather than the characterization of the phenol-degrading culturable bacterial diversity, some factors could explain the isolation of only two strains. Firstly, the applied phenol concentration (300 mg.L^{-1}) may have been excessively high and inhibited bacteria capable of metabolizing the aromatic compound even in higher

Table 1. Phenol tolerance and utilization by bacterial isolates in different culture media added with gradual concentrations of the aromatic compound.

Phenol concentration (mg.L^{-1})	Bacterial isolates			
	MF-1		MF-2	
	NA	BHA	NA	BHA
0 - 500	+	+	+	+
600 - 1,000	+	+	+	+
1,100 - 1,500	+	–	+	–
1,600	–	–	–	–

+ = positive; - = negative.

Table 2. Phenol degradation and growth assessment by bacterial isolates in different concentrations of the compound (mg.L^{-1}) after 96 h of incubation in BH broth.

Bacterial isolate	Consumption rate (%)			Microbial growth (OD_{600nm})		
	100	200	300	100	200	300
MF-1	22.43	11.52	3.33	1.54	1.82	1.68
MF-2	21.64	20.54	28.85	1.28	1.30	1.14

concentrations, that should be initially isolated in a lesser selective environment. Secondly, and in relation to the first reason, levels of local phenol and other aromatic hydrocarbons levels could not be sufficient enough to select more tolerant and/or degrading bacterial members of microbial communities associated with sediment at the time of sampling, eventually reflecting as a low number of isolated bacteria. It could not be discarded also, the time interval between sampling and the beginning of sample processing at the laboratory. This may have influenced microbial viability for cultivation purposes. The preliminary screening strategy was essential to assess the extent of degradation and tolerance abilities for these strains. When studying fungal and bacterial isolates from culture collections regarding their biodegradation potential, Passos et al. (2009) and Monteiro (1998) suggests as an initial step the utilization of rich and minimum culture media added with the aromatic compound. This addresses a primary selection of tolerant microbial strains, mainly when a high number of isolates are being verified, which was not our case. These strains could be further evaluated directly with respect to the phenol consumption in stationary growth, for instance. Supplementation of culture medium with various concentrations of phenol (Table 1) evidenced the better adaptability of strains when investigated with NA, once the culture medium provided a feasible environment for bacterial growth due to its higher nutrient content in contrast with the mineral medium (BH). This is expressed by the presence of colonies up to concentration of 1,500 mg.L^{-1} in NA and 600 mg.L^{-1} in BHA.

Once the phenol tolerance was determined, microbial isolates were submitted to the evaluation of their efficiency in consuming the compound by HPLC method (Table 2). It was verified that the phenol concentration was associated with the metabolic efficiency of the strain MF-1, that is, the higher the phenol concentration the lesser was the capacity of the strain to consume the substance during its growth. However, although the isolate MF-1 have not exhibited the most prominent consumption profile, it presented a better growth; in other words, the increase of the chemical agent concentration did not inhibit microbial development. Conversely, the MF-2 strain was able to consume phenol even in higher concentrations, though this ability was not proportional to bacterial growth. A suitable explanation for this observed behavior is not only a matter of bacterial metabolic pathways for biotransformation of phenolic compounds; the substrate specificity and regulation of key enzymes are also involved in these processes (Nešvera et al., 2015). One forthcoming goal of this study comprises the molecular screening for mono- and dioxygenases coding genes commonly responsible for the degradation and detoxification of phenol in microbial cells so as to clarify more this differential profile between these two strains.

16S rRNA gene sequencing evidenced that both strains belonged to the genus *Bacillus* (Table 3), which was already suspected by macro and micro morphological examination. As demonstrated in the generated phylogenetic tree (Figure 1), the MF-1 isolate was grouped with several *Bacillus* spp. beyond *B. cereus*, while the MF-2 isolate remained well clustered with *B. circulans*, which was supported by bootstrap analyses.

Besides the fact that most studies related to biodegradation of phenolic compounds and other xenobiotics involves bacteria from *Pseudomonas* genus

Table 3. Molecular identification of bacterial strains based on BLASTn analyses of 16S rRNA gene sequences.

Bacterial isolate	BLASTn best hit	Identity (%)	Accession number
MF-1	*Bacillus cereus*	96	NR_074540.1
MF-2	*Bacillus circulans*	97	NR_112632.1

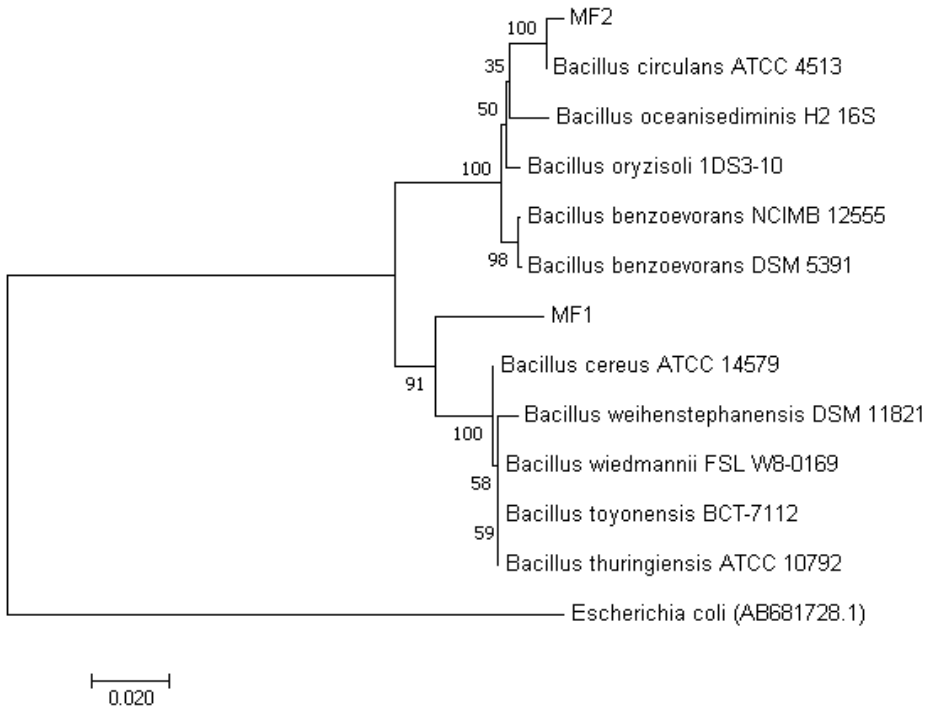

Figure 1. NJ tree of the phenol-degrading *Bacillus* strains based on sequence similarity of 16S rRNA genes from the GenBank database. Bootstrap (1000 replicates) values are shown above or below tree branches. *Escherichia coli* K-12 was used as outgroup.

(Nair et al., 2008), there are several recent reports in the literature with *Bacillus* strains as potential phenol degraders isolated from various environmental sources. Banjeree and Ghoshal (2010) conducted the isolation of two *Bacillus* strains, *B. cereus* MTCC 9817 strain AKG1 and *B. cereus* MTCC 9818 strain AKG2, from wastewater samples of one refinery site and exploitation in India, adopting a similar approach of the present study. Furthermore, the authors evaluated a series of parameters (pH, temperature and initial phenol concentration) impacting the survival of the strains under minimal medium with the aromatic compound. Both strains grew well in a temperature of 37°C and neutral pH and in concentrations of phenol of 600 $mg.L^{-1}$ for AKG1 and 1000 $mg.L^{-1}$ for AKG2, which corresponds with the phenol concentration adopted in the microbial isolation phase: 500 $mg.L^{-1}$. In this research, it was preferable to adopt a lower phenol concentration range (0 to 300 $mg.L^{-1}$) in degradation assays under stationary growth once the concentration of the compound added to the BH medium at the isolation stage was 300 $mg.L^{-1}$. Additionally, both strains were able to tolerate phenol up to the range between 600 to 1000 $mg.L^{-1}$ in the BHA. Likewise, at this concentration, the microbial growth was not severely affected. Kafilzadeh and Mokthari (2013) isolated *Pseudomonas putida*, *Acinetobacter* species, *Bacillus thuringiensis*, *Brevibacterium iodinum*, and *Staphylococcus aureus* from nine mangrove sediment samples in the Persian Gulf and studied their degradation ability in association with growth assessment. The *B. thuringiensis* strain was able to grow up to 1,000 $mg.L^{-1}$ of phenol, removing it from the culture medium in 91% for 7 days of incubation in mineral medium.

Aburas (2016) effectuated the isolation of twenty bacterial strains from wastewater samples at Jedah city and following a similar screening strategy of our work. They selected a *Bacillus stearothermophilus* ABO11 which grew in minimal media supplemented with 0.8 $g.L^{-1}$ of phenol. The most appropriated conditions for phenol degradation was growth after 10 days, at 40°C, pH 8 and

using NH_4Cl as nitrogen source. From a total of seven strains isolated from Palestinian olive mill wastes, only one denominated J20, later identified as *B. thuringiensis*, was assessed regarding its ability to tolerate and degrade phenol in the work led by Ereqart et al. (2017). Apart from the fact that the bacilli strains grew in minimal salt medium with phenol as a sole carbon and energy source up to a concentration of 700 $mg.L^{-1}$, the authors investigated phenol degradation of free and immobilized cells (in alginate), with a better performance of the strain in this last state after 96 h of incubation. Chris Felshia et al. (2017) verified the efficiency of encapsulation for a *Bacillus licheniformis* strain SL10 isolated from sludges samples of effluent treatment station in India. It was found out that encapsulated strains were able to degrade 2,000 ppm of phenol only after 72 h of incubation in comparison with the free cells took 96 h. It is remarkable that optimization of culture conditions is a common experimental step and one of this study's main future perspectives relies on the evaluation of some growth conditions, as time of incubation, temperature, pH along with the effect of medium salinity on the removal of phenol by these strains considering their autochthonous origin.

Conclusion

From the mangrove soil, two isolates of *Bacillus* with phenol degrading ability were obtained. Bacteria had a maximum tolerance of 600 $mg.L^{-1}$ using this substance as the sole source of carbon. The isolate MF-2 showed a maximum consumption of 28.85% phenol for the initial concentration of 300 $mg.L^{-1}$. A better characterization of the capacity for metabolizing phenol by these isolates may provide further insight into the dynamics of the use of this substance as well as the potential application of these microorganisms in bioremediation processes.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Aburas MMA (2016). Removal of phenol using spore forming *Bacillus* ABO11 isolated from waste water treatment plant. Advances in Microbiology 6:898-908.

Ahmad N, Ahmed I, Shahzad A, Khalid N, Mehboob F, Ahad KAGM (2014). Molecular identification and characterization of *Pseudomonas* sp. NCCP-407 for phenol degradation isolated from industrial waste. Journal of the Korean society for Applied Biological Chemistry 57:341-346.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990). Basic local alignment search tool. Journal of Molecular Biology 215:403-410.

Arutchelvan V, Kanakasabai V, Elangovan R, Nagarajan S, Muralikrishnan V (2006). Kinetics of high strength phenol degradation using *Bacillus brevis*. Journal of Hazardous Materials 129:216-222.

Auta HS, Emenike CU, Fauziah SH (2017). Screening of *Bacillus* strains isolated from mangrove ecosystems in Peninsular Malaysia for microplastic degradation. Environmental Pollution 231:1552-1559.

Bajaj S, Singh DK (2015). Biodegradation of persistent organic pollutants in soil, water and pristine sites by cold-adapted microorganisms: mini review. International Biodeterioration and Biodegradation 100:98-105.

Banerjee A, Ghoshal AK (2010). Phenol degradation by *Bacillus cereus*: pathway and kinetic modeling. Bioresource Technology 101:5501-5507.

Banerjee A, Ghoshal AK (2016). Biodegradation of real petroleum wastewater by immobilized hyper phenol-tolerant strains of *Bacillus cereus* in a fluidized bed bioreactor. 3Biotec 6:137.

Bushnell LD, Haas HF (1941). The utilization of certain hydrocarbons by microorganisms. Journal of Bacteriology 41:653-673.

Chris Felshia S, Aswin Karthick N, Thilagam R, Chandralekha A, Raghavarao K, Gnanamani A (2017). Efficacy of free and encapsulated *Bacillus lichenformis* strain SL10 on degradation of phenol: a comparative study of degradation kinetics. Journal of Environmental Management 197:373-383.

Dias ACF, Andreote FD, Dini-Andreote F, Lacava PT, Sá ALB, Melo IS, Azevedo JL, Araújo, WL (2009). Diversity and biotechnological potential of culturable bacteria from Brazilian mangrove sediment. World Journal of Microbiology and Biotechnology 25:1305-1311.

Ereqat S, Abdelkader A, Nasereddin A, Al-Jawabreh A, Zaid T, Letnik I, Abdeen Z (2017). Isolation and characterization of phenol degrading bacterium strain *Bacillus thuringiensis* J20 from olive waste in Palestine. Journal of Environmental Science and Health Part A 53:39-45.

Gami AA (2014). Phenol and its toxicity. Journal of Environmental Microbiology and Toxicology 2:2014.

Gomes N, Flocco C, Costa R, Junca H, Vilchez R, Pieper D, Krögerrecklenfort E, Paranhos R, Mendonça-Hagler L, Smalla K (2010). Mangrove microniches determine the structural and functional diversity of enriched petroleum hydrocarbon-degrading consortia. FEMS Microbiology Ecology 74:276-290.

Guo C, Ke L, Dang Z, Tam N (2012). Effect of exposure time, contamination level, and type of PAH compound on biodegradation capacity of mangrove sediment. Soil and Sediment Contamination 21:291-304.

Hamedo HA, El-Shamy AR, Awad HM (2015). Molecular identification and characterization of phenol-degrading bacteria from oil-contaminated sea water. *Journal* of Policy Analysis and Management 9:71-78.

Hasan SA, Jabeen S (2015). Degradation kinetics and pathway of phenol by *Pseudomonas* and *Bacillus* species. Biotechnology and Biotechnological Equipment 29:45-53.

He XP, Liu J, Liu HJ, Wang SS, Xu WH, Huang ZC (2013). Effect of conventional carbon sources on phenol degradation by *Bacillus* sp. CDQ. Advanced Material Research 726:301-304.

Jiang L, Ruan Q, Li R, Li T (2013). Biodegradation of phenol by using free and immobilized cells of *Acinetobacter* sp. BS8Y. Journal of Basic Microbiology 53:224-30.

Kafilzadeh F, Mokhtari S (2013). Isolation and identification of phenol degrading bacteria from mangrove sediments in the Persian Gulf (Asaluyeh) and their growth kinetics assay. Biomedical and Pharmacology Journal 6:189-196.

Kim IC, Oriel PJ (1995). Characterization of the *Bacillus stearothermophilus* BR219 phenol hydroxylase gene. Applied and Environmental Microbiology 61:1252-1256.

Kumar S, Stecher G, Tamura K (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33:1870-1874.

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007). ClustalW and ClustalX version 2. Bioinformatics 23:2947-2948.

Lehtinen T, Mikkonen A, Sigfusson B, Ólafsdóttir K, Ragnarsdóttir KV, Guicharnaud R (2014). Bioremediation trial on aged PCB-polluted soils-a bench study in Iceland. Environmental Science and Pollution Research 21:1759-1768.

Lewis M, Pryor R, Wilking L (2011). Fate and effects of anthropogenic

chemicals in mangrove ecosystems: A review. Environmental Pollution 159:2328-2346.
Mahiudddin MD, Fakhruddin ANM, and Abdullah-Al-Mahin (2012) Degradation of Phenol via Meta Cleavage Pathway by *Pseudomonas fluorescens* PU1. ISRN Microbiology 2012:1-6.
Moghadam MS, Safaei N, Ebrahimipour GH (2016). Optimization of phenol biodegradation by efficient bacteria isolated from petrochemical effluents. Global Journal of Environmental Science and Management 2:249-256.
Mohanty MP, Brahmacharimayum B, Ghosh PK (2018). Effects of phenol on sulfate reduction by mixed microbial culture: kinetics and bio-kinetics analysis. Water Science and Technology 77: 1079-1088.
Monteiro AAMG (1998). Degradação biológica de fenol com bactérias imobilizadoras num suporte de poros largos. PhD, UFG.
Nair CI, Jayachandran K, Shashidhar S (2008). Biodegradation of phenol. African Journal of Biotechnology 7:4951-4958.
Nešvera J, Rucká L, Pátek M (2015). Catabolism of phenol and derivatives in bacteria: genes, their regulation, and use in the biodegradation of toxic pollutants. Advances in Applied Microbiology 93:107-60.
Passos CT, Burkert JFM, Kalil SJ, Burkert CAV (2009). Biodegradação de fenol por uma nova linhagem de *Aspergillus* sp. isolada de um solo contaminado do sul do Brasil. Quim. Nova 32:950-954.
Patil SS, Jena HM (2016). Isolation and characterization of phenol degrading bacteria from soil contaminated with paper mill wastewater. Indian Journal of Biotechnology 15:407-411.
Sarwade VD, Gawai KR (2014). Biodegradation of phenol by alkaliphiic *Bacillus badius* D1. Journal of Environmental Science, Toxicology and Food Technology 8:28-35.
Senthilvelan T, Kanagaraj J., Panda RC, Mandal A (2014). Biodegradation of phenol by mixed microbial culture: an eco-friendly approach for the pollution reduction. Clean Technologies and Environmental Policy 16:113-126.
Silva CF, Souza CB (2015). Construção de protótipo de reator com sistema rotacional para isolar microrganismo com potencial para biorremediação de águas contaminadas por metais pesados. UNILUS Ensino e Pesquisa 12:66-67.
Sivasubramanian K, Ravichandran S, Vigneshwaran P (2016) Isolation of phenol-degrading bacteria from decapods mangrove crabs. SOJ Microbiology and Infectious Diseases 4:1-5.
Sridevi V, Lakshmi MVVC, Manasa M, Sravani M (2012). Metabolic pathways for the biodegradation of phenol. International Journal of Engineering Science and Advanced Technology 2:695-705.
Steers E, Foltz EL, Graaves VS (1959). An inocula replicating apparatus for continue testing of bacterial susceptibility to antibiotics. Antibiotics and Chemotherapy (Northfield) 9:307-311.
Van Soolingen D, De Haas PE, Hermans PW, Van Embden JD (1994). DNA Fingerprinting of mycobacterium tuberculosis. Methods in Enzymology 235:196-205.
Weisburg WG, Barns SM, Pelletier DA, Lane DJ (1991). 16S ribosomal DNA amplification for phylogenetic study. Journal of Bacteriology 173:697-703.
Yateem A, Al-Sharrah T (2011). Enhanced hydrocarbons degradation in the rhizosphere of mangrove plants by a halophilic *Bacillus subtilis* strain. Soil and Sediment Contamination 20:261-273.
Zhang Y, Lu D, Ju T, Wang L, Lin S, Zhao Y, Wang C, He H, Du Y (2013). Biodegradation of phenol using *Bacillus cereus* WJ1 and evaluation of degradation efficiency based on a graphene-modified electrode. International Journal of Electochemical Science 8:504-519.