

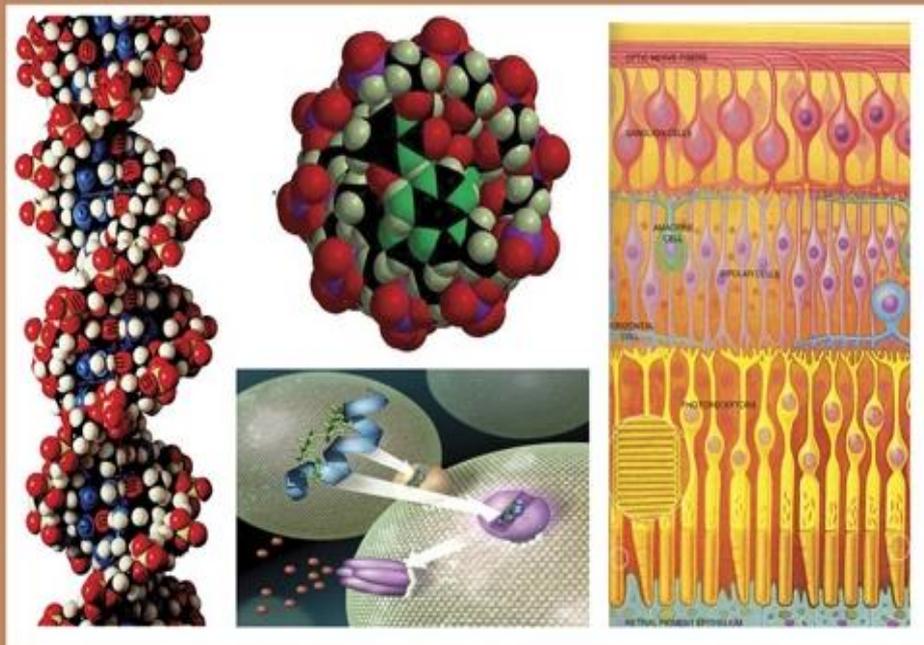


C

EGYPTIAN ACADEMIC JOURNAL OF

BIOLOGICAL SCIENCES

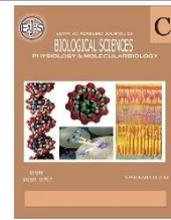
PHYSIOLOGY & MOLECULAR BIOLOGY



ISSN
2090-0767

WWW.EAJBS.EG.NET

Vol. 14 No. 2 (2022)



A novel *Niallia nealsonii* Bacteria Degrading Phenol Isolated From Oum Ghellaz Lake Shore Soil in Northern Algeria

**Maghnia Djamila¹, Adjoudj Fatma², Ait Abdeslam Arezki², Medouakh Linda¹,
Ammam Abdelkader³ and Belmamoun Ahmed Reda⁴.**

1-Laboratory for Experimental Biototoxicology of Biodepollution and Phytoremediation,
University of Oran Ahmed Ben Bella 1 . Algeria

2- Laboratory of applied microbiology university, University of Oran Ahmed Ben Bella 1 .
Algeria

3- University of Dr. Moulay Tahar, Saida; Algeria.

4- University of Djillali Eliabes, Sidi Belabbes; Algeria.

*E-mail: vetokadi@yahoo.fr

ARTICLE INFO

Article History

Received:10/9/2022

Accepted:20/12/2022

Available:24/12/2022

Keywords:

Biodegradation,
phenol, *Niallia
nealsonii*, Oum
ghellaz lake,
bioremediation,
contaminated soil.

ABSTRACT

A new indigenous soil phenol-degrading bacterium strain S2 was successfully isolated from Oum ghellaz lake shore soil in Oran in Algeria. Based on its morphological, physiological and biochemical characteristics, the strain S2 was characterized as a Gram-positive, facultatively anaerobic, forms endospores, and short rod-shaped bacterium that utilizes phenol as a sole carbon and energy source also other phenolic compounds. 16SrDNA sequence analysis revealed that this strain is belongs to *Niallia nealsonii* in the group of *firmicutes*. The strain was efficient in removing 91.6% of the initial 500 mg.L⁻¹ phenol within 48 h and had a tolerance of phenol concentration as high as 1500 mg.L⁻¹. These results indicated that *Niallia nealsonii* has promising bioremediation potential.

INTRODUCTION

Phenolic compounds are organic pollutants that are very harmful to both humans and the environment. Indeed, some of them can be highly toxic due to their mutagenic and/or carcinogenic properties (Das *et al.*, 2011). Phenols are produced annually at a rate of 7 million tons in the world. They are discharged by various industries such as oil refineries, coking plants, pharmaceutical industry , chemical industry, etc. (Senthivelan *et al.*, 2014). Due to its high water solubility, phenol in wastewater reaches downstream water sources and can harm life in aquatic environments (Deng *et al.*, 2018). Due to the limits of physicochemical treatment which are costly, non-ecological, complex, and can lead to the destruction of the texture, the characteristics of the soil, of the waters, bioremediation by using microorganisms is recognized as a valuable alternative for detoxification and elimination of the toxic substance, due to their cost-effectiveness, respect for the environment and their technological simplicity (Ren *et al.*, 2017).it is an evolutionary method for the removal and degradation of many environmental pollutants, including phenols.

Many phenol-degrading microorganisms, including bacteria, yeast and algae have been isolated from environment, among which the bacteria are studied extensively, like *Pseudomonas putida* (Kumar *et al.*, 2005), *Burkholderia* sp. (Arora and Jain, 2012), *Kocuria* sp. (Wu *et al.*, 2018), *Acinetobacter* sp. (Jiang *et al.*, 2013; Iqbal *et al.*, 2018), *Arthrobacter* sp. (Wong *et al.*, 2015), *Bacillus* sp. (Banerjee and Ghoshal, 2010; Hasan and Jabeen, 2015; Iqbal *et al.*, 2018), *Halomonas* sp. (Haddadi and Shavandi, 2013), *Pseudomonas aeruginosa* (Hasan *et al.*, 2015), *Citrobacter* sp. (Deng *et al.*, 2018), *Raoultella* sp (Jayachandran K *et al.*, 2018) and *Pseudomonas* sp. ATR208 (Sephehr *et al.*, 2019). However, there is no information available regarding phenol degradation activity within the genus *Niallia*. This paper describes the isolation and identification of a novel phenol-degrading Gram-positive bacterium of *Niallia* species Oum ghellaz lake shore soil in Oran in Algeria and to determine the kinetics of biodegradation.

MATERIALS AND METHODS

Chemicals, Media and Soil Sample:

All chemicals used were analytical reagents. The minimal salt media (MSM) and Luria-Bertani (LB) media were used in this study. The MSM contained KH_2PO_4 0.5 g, K_2HPO_4 0.5 g, CaCl_2 0.1 g, NaCl 0.2 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.5 g, $\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$ 0.01 g, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.01 g, NH_4NO_3 1.0 g per liter. The LB media was composed of tryptone 10 g, yeast extract 5 g and NaCl 5 g per liter. Deionized, distilled water was used for the experiments. The soil samples were collected from Oum ghellaz lake shore soil in Oran in Algeria by carefully scraping the soil using a sterile spatula. The soil was sieved under aseptic conditions to a particle size of approximately 2–4 mm, and then 10g of soil was added to 90ml of mineral salts medium (MSM) for enrichment culture.

Isolation and Enrichment of Aerobic Bacteria That Degrade Phenol: The sample was collected from Oum ghellaz

lake shore soil in the north of Algeria, 10 g of soil was inoculated into flasks containing 90 ml of MSM for the enrichment culture. Phenol was supplemented in the media as the sole carbon source, and the various concentrations of phenol were 200, 500, 800, 1100, 1400 and 1700 mg.L^{-1} . The enriched culture observed with more biomass (in the flask with 500 mg.L^{-1} phenol) was further transferred into a freshly prepared enrichment media with higher concentrations of phenol (increased from 500 mg.L^{-1} to 1500 mg.L^{-1}). The final enriched media were diluted serially and spread on LB agar plates supplemented with phenol (500 mg.L^{-1}). The plates were incubated at 30°C and single colonies with morphological differences were selected and streaked on new plates. The resulting isolates were stored at 4°C for further study.

To test the ability of the isolate to use other phenolic compounds as a sole source of carbon and energy, the strain S2 was spread onto MSM plates and the carbon source was supplemented via vapor phase by adding it to the sterile Eppendorf plastic tip (50 μl) and placing the tip in the lid of the Petri dish. After 2–4 days of incubation at 30 °C, the plates were screened for the presence of colonies. Growth was confirmed by comparison with control plates without substrate and 20 mM glucose as carbon source, respectively. (Djokic, 2011)

Taxonomic Identification and Characterization of Phenol degrading Bacteria:

One strain capable of achieving high biomass yields with phenol as a sole carbon and energy source was selected for further phenotypically characterization. The morphological properties of the isolated colonies were observed by optical microscopy. The typical physiological and biochemical characteristics of the phenol-degrading bacteria strains, such as Gram's staining, motility, starch hydrolysis, and gelatinase (Kloos *et al.*, 1974) were

systematically performed according to Bergey's manual of determinative of bacteriology (Holt *et al.*, 1998) Indole test, methyl red test was also analyzed (Zeinat *et al.*, 2008)

This strain was also identified by sequence analysis of 16S rRNA genes. The genomic DNA of bacterial strains was extracted by using the Promega Genomic DNA Extraction kit. The quantity and the quality of DNA extracts are monitored by the NanoDrop spectrophotometer (thermo scientific, USA). The extracted DNA was used as a template to amplify bacterial 16Sr DNA with universal primers 27F and 1492R (5'-AGAGTTTGATCMTGGCTCAG-3') and 1392R (5'-ACGGGCGGTGTGTGTRC-3') with a Biorad cyler thermo- cyler (Biorad, USA). The amplification PCR was performed under the following conditions: An initial denaturation step of 5 min at 94°C was conducted, followed by 35 cycles of 94 °C for 30 s, 55°C for 45 s and 72 °C for 90s. The procedure was completed with a final elongation step at 72 °C for 10 min. The PCR products were sequenced and the sequences were compared with bacterial 16Sr DNA sequences in GenBank (National Centre for Biotechnology Information, Rockville Pike, Bethesda, MD), (<http://www.ncbi.nlm.nih.gov/>) (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). (Altschul S.F *et al.* , 1997) were constructed using the Molecular Evolutionary Genetics Analysis (MEGA version 6) (Tamura. K *et al.*, 2011). The reliability of phylogenetic reconstructions was estimated through bootstrap analysis (1000 replicates).

Phenol Degradation:

The culture of strain was prepared and adjusted to an optical density at 600 nm

(OD₆₀₀) of 1.0, then the final concentration of 2% (v/v) inoculums was inoculated into the flasks containing MSM media with phenol as sole carbon source (katarzynna. H *et al.* ,2012) the range of phenol concentrations was increased from 100 to 1500 mg·L⁻¹. The flasks were incubated at 30 °C with 150 rpm for 3 days. Samples were collected periodically to measure the biomass and the phenol degradation. The biomass contents were monitored spectrophotometrically by measuring absorbance at 600 nm. The phenol concentrations were determined by using 4-amino antipyrine in the colorimetric assay, according to standard methods reported by (APHA .,2005).

RESULTS AND DISCUSSION

Isolation and Characterization of Phenol-Degrading Strains:

After three weeks of enrichment and one week of strain isolation, a total of 10 isolates were obtained after 24 h growth on the LB agar plates with 100 µL of a 10⁻⁵ – 10⁻⁶ fold dilution of enrichment culture. All these stains utilized phenol as the sole carbon source and energy, and 1 of the 10 isolates exhibited more growth in phenol-containing media than the others. The outstanding isolate was named S2 and was applied in the following study. The selected strain was tested for its ability to grow on a range of additional 10 phenolic substrates as the sole source of carbon and energy on a solid MSM medium (Table 1). These substrates included o-crésol , m- crésol etc ..The isolate S2 were capable of degrading 8 aromatic compounds, including o-crésol , m- crésol , p-crésol , 3,4-DMP, Ethylbenzène ,benzene, toluene, Xylène (Table 1).

Table 1: Capability of isolate S2 to use different phenolic compounds.

Strains	Phenolic compounds										
	phénol	o-crésol	m-crésol	p-crésol	<i>p</i> -nitrophénol	<i>p</i> -chlorophénol	3,4-DMP	benzène	Toluène	Ethylbenzène	Xylène
PSA	+	+	+	+	-	-	+	+	+/-	+	+/-
PSP	+	+	+	+	-	-	+	+	+	+	+
S2	+	+/-	+	+	-	-	+	+	+/-	+	+

Good growth (+) Moderate growth (+/-) No growth (-), PSA: *Pseudomonas aeruginosa* ATTC 27853 and PSP: *Pseudomonas putida* ATTC12633.

The strain S2 was a gram-positive, short rod-shaped bacterium, facultatively anaerobic and forms endospores, colonies on LB agar are irregular, rough, umbonate with undulate edges and beige in colour. The biochemical characteristics of strain S2 were determined, and biochemical tests

showed that Catalase is produced, but oxidase, arginine dihydrolase, lysine and ornithine decarboxylases, uree and indol are not. (Table 2), This strain could grow at temperatures range of 30 °C –45 °C and a wide range of pH 5–11. The optimum growth was at the condition of 30°C -35°C.

Table 2: Morphological and biochemical characteristics of S2 strain.

Characteristics	Strain S2
Color Of Colony	White
Morphology	Short rod
Motility	-
Gram Straining	+
Aerobic Growth	Facultatively anaerobic
Catalase	+
Oxydase	-
Glucose	+
Mannitol	-
Methyl Red	-
Arginine Dihydrolase	-
Ornithine Decarboxylase	-
Lysine Decarboxylase	-
Uree Production	-
Indol Production	-
+ : Positive Result, -: Negative Result	

The 16S rRNA gene sequences of S2 were sequenced and used to construct a phylogenetic tree for further analysis. The partial sequence of 16S rRNA gene was a continuous stretch of 1180 bp. The similarities between the S2 sequence and the bacterial sequences deposited in the

GenBank databases were calculated, and the S2 sequence showed 100% similarity to that of *Niallia nealsonii* strain MMAPL-X 1 previously named *Bacillus nealsonii*. The phylogenetic analysis revealed that the strain was classified in the *Niallia* genera, which belongs to the family of *Bacillaceae*

from the order *Bacillales*. Based on neighbor-joining methods, a phylogenetic tree was constructed which indicated that the closest relative of strain S2 was *Niallia nealsonii* (Fig. 1). Therefore, the strain S2

was identified and affiliated to *Niallia nealsonii*. 1,393 bp. The obtained sequence was deposited in the Gene-Bank with accession number OQ428241.

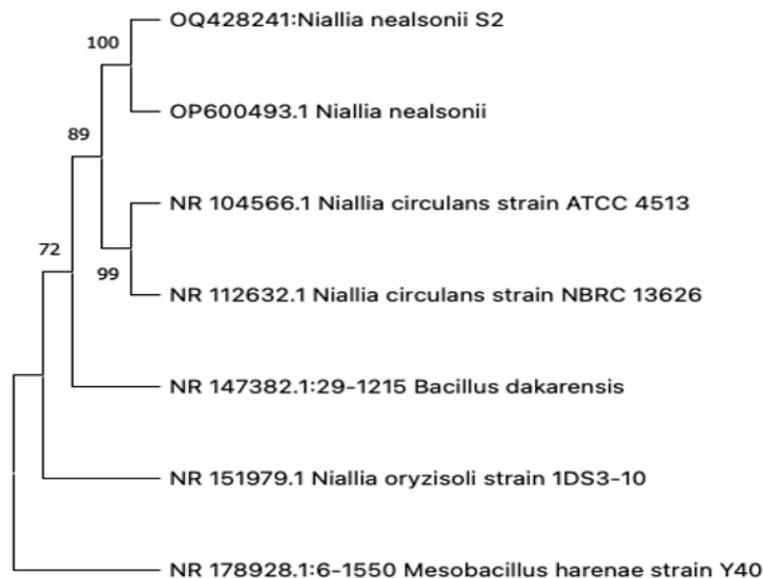


Fig. 1 Phylogenetic relationship based on the 16S rRNA gene sequences of strain S2 and related organisms from the GenBank database. Bootstrap values were calculated from 1000 replications of Kimura 2.- parameter, and bootstrap values higher than 70% were shown. the scale bar represents 0,002 changes per sequence position.

Extensive biodegradation studies have described the effectiveness of bacillus species in removing many environmental pollutants from contaminated sites including *Bacillus brevis* (Arutchelvan *et al.*, 2006), *Bacillus cereus* (Banerjee and Ghoshal, 2010), *Bacillus stearothermophilus* (Dong *et al.*, 1992; Gurujey- alakshmi and Oriel, 1989) and *Bacillus subtilis* (Tam *et al.*, 2006). Banerjee *et al.* (2010) isolated two *Bacillus* strains from the oil refinery and exploration sites which could grow on phenol (1000 mg.L⁻¹). Nevertheless, no studies have been reported on *Niallia nealsonii* degrading phenol or aromatic compounds.

The isolation of native microbial species from locally polluted environments has been reported to be more adaptive and efficient than the biodegradation of non-indigenous microorganisms. Therefore, the

isolation of new phenol-degrading bacteria is recommended for the bioremediation of phenol-contaminated sites in various regions (Liu *et al.*, 2016).

The Phenol Biodegradation by S2 *Niallia nealsonii* :

The phenol-degradation characteristics and biomass of S2 *Niallia nealsonii* at various initial concentrations of phenol (100–1500 mg.L⁻¹) were determined by monitoring phenol concentration cell growth at OD600 periodically. The maximum biomass and degradation of phenol were observed at the initial phenol concentration of 500 mg.L⁻¹ (Fig.2). An inhibitory effect showed that the biomass growth and the degradation of phenol declined with the elevated initial phenol concentration higher than 500 mg. L⁻¹. The removal rates of phenol were above 79% at the initial phenol concentration ranging

from 200 to 600 mg. L⁻¹. When the initial phenol concentration was higher than 1500 mg, there was no growth of bacteria. L⁻¹.

The strain could grow on phenol up to a concentration of 1000 mg. L⁻¹ with a degradation rate of 40%.

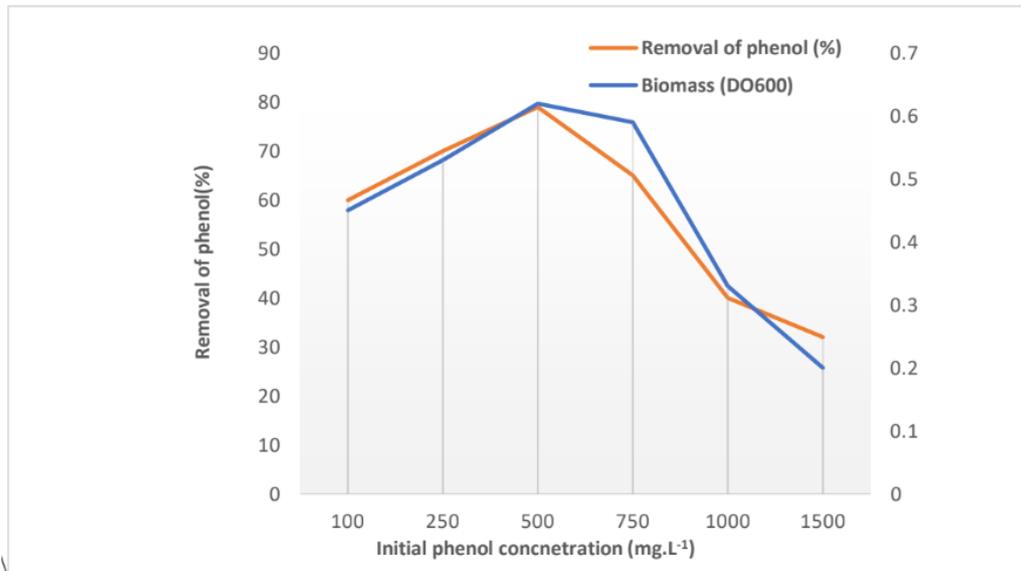


Fig. 2: Profile of bacterial cell growth and phenol degradation at various initial concentrations.

The effects of factors such pH values and temperature on the degradation were investigated. bacterial strain could grow within a range of pH 5–11 (Fig. 3a), and the degradation of phenol was above 75% in the range of pH 7–9. The optimum pH for phenol degradation was 7.0. These results showed that the bacterial growth of strain S2 and the degradation of phenol (above 70%) were favored at temperatures of 30°C –35°C (Fig 3b). The biomass and

phenol degradation reached the maximal values at a temperature of 30 °C. On the contrary, the phenol degradation declined sharply when the temperature reached 40°C and over. Therefore, the optimal temperature for the growth of strain S2 was 30°C. These growth conditions of *Niallia nealsonii.S2* for phenol degradation were similar to those of *Bacillus* species Strain *Bacillus sp.*

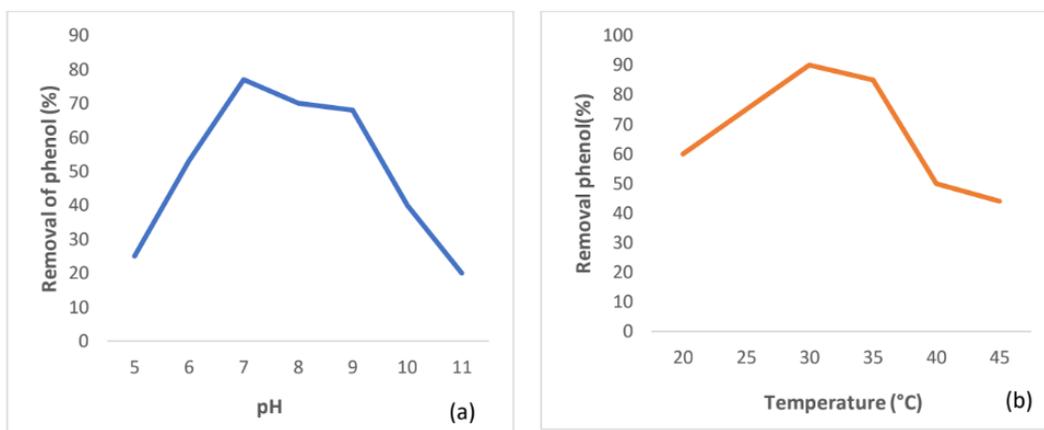


Fig. 3: Effect of pH and temperature on phenol biodegradation (initial phenol concentration 500 mg·L⁻¹). (a) Effect of pH on the removal of phenol; (b) effect of temperature on the removal of phenol.

PS11 was isolated and characterized as a strain that utilizes a high amount of phenol (up to 1400 mg.L⁻¹) in liquid culture without apparent inhibition of growth and it performed well in the initial soil microcosm experiment have been reported by Djokic (2011).

Several *Bacillus* strains have been recognized to grow by using phenol as the sole carbon and Several energy sources. However, no study has been done on the biodegradation of phenol by *Niallia* species. In our study, the inhibition limit of strain *Niallia nealsonii* was as high as the phenol concentration of 1500 mg.L⁻¹.

CONCLUSION

In conclusion, a novel bacterial strain capable of degrading phenol was isolated from Oum ghellaz lake shore soil in Oran in Algeria and it was identified as *Niallia nealsonii* S2 based on the 16S rDNA sequence and the phylogenetic analysis. *Niallia nealsonii* S2 has the ability to grow in a liquid medium with phenol at different concentrations as the sole carbon and energy and to use other phenolic compounds as substrates (including o-crésol, m- crésol, p-crésol, 3,4-DMP, Ethylbenzène, benzene, toluene, Xylène)

The strain was able to degrade 80% of the initial 500 mg.L⁻¹ phenol and grow at the phenol concentration of as high as 1500 mg.L⁻¹. The optimal growth conditions for phenol degradation of strain were at 30 °C and pH 7.0. Regarding that native microbial species were more adaptive than non-indigenous microorganisms in polluted environments, their predominance facilitated the bioremediation of the phenol-contaminated environments. *Niallia nealsonii* S2 isolated may be applied for the bioremediation of the phenol-contaminated environments in Algeria.

REFERENCES

- Altschul, S.F., Gish, W., Miller, W., Myers, E.W, and Lipman, D.J. 1990. *Journal of Molecular Biology*. 215:403–41
- APHA. 2005. Standard methods for the examination of water and wastewater, 21st edn. American Public Health Association, Washington. DC
- Arora, P. K., and Jain, R. K. 2012. Metabolism of 2-chloro-4-nitrophenol in a Gram-negative bacterium, *Burkholderia* sp. RKJ 800. *PLoS One*, 7:e38676
- Arutchelvan, V., Kanakasabai, V., Nagarajan, S. and Muralikrishnan, V. 2005. Isolation and identification of novel high-strength phenol degrading bacterial strains from phenol-formaldehyde resin manufacturing industrial wastewater. *Journal of Hazardous Materials*, 127:238-243
- Banerjee, A., and Ghoshal, A. K. 2010. Phenol degradation by *Bacillus cereus*: pathway and kinetic modeling. *Bioresources Technology*, 101:5501–5507.
- Das, N.; Chandran, P. 2011. Microbial Degradation of Petroleum Hydrocarbon Contaminants: An Overview. *Biotechnology Research In*. 1–13.
- Das, B., Mandal, T.K., Patra, S. 2016. Biodegradation of phenol by a novel diatom BD1IITG-kinetics and biochemical studies. *International Journal of Environmental Science and Technology*, 13:529–542
- Deng T, Chen Y, Liu B, Laguna MP, de la Rosette JJMCH, Duan X, Wu W, Zeng G. 2018. Systematic review and cumulative analysis of the managements for proximal impacted ureteral stones. *World Journal of Urology*, 1687-1701
- Djokic, L., Narancic, T., Nikodinovic-Runic, J., Savic, M., Vasiljevic, B. 2011. Isolation and characterization of four novel Gram-positive bacteria associated with the rhizosphere of two

- endemorelict plants capable of degrading a broad range of aromatic substrates. *Applied Microbiology Biotechnology*, 91: 1227–1238
- Dong, F.-M., Wang, L.-L., Wang, C.-M., Cheng, J.-P., He, Z.-Q., Sheng, Z.-J., Shen, R.-Q. 1992. Molecular cloning and mapping of phenol degradation genes from *Bacillus stearothermophilus* FDTP-3 and their expression in *Escherichia coli*. *Applied Environmental Microbiology*, 58: 2531–2535.
- Gurujeyalakshmi G., and P., Oriol .1989. Isolation of phenol-degrading *Bacillus stearothermophilus* and partial characterization of the phenol hydroxylase. *Applied Environmental Microbiology*, 55: 500-502
- Hasan, S. A., and Jabeen, S. 2015. Degradation kinetics and pathway of phenol by *Pseudomonas* and *Bacillus* species. *Biotechnology Equipment*, 29:45–53
- Haddadi, A., and Shavandi, M. 2013. Biodegradation of phenol in hypersaline conditions by *Halomonas* sp. strain PH2-2 isolated from saline soil. *International Biodeterioration and Biodegradation*, 85:29–34
- Holt, S.G.; Kriey, N.R.; Sneath, P.H.A.; Staley, J.T.; Williams, S.T. 1998. *Bergey's Manual of Determinative for Bacteriology*; Williams and Wilkins: New York, NY, USA
- Iqbal, A., Arshad, M., Hashmi, I., Karthikeyan, R., Gentry, T. J., and Schwab, A. P. 2018. Biodegradation of phenol and benzene by endophytic bacterial strains isolated from refinery wastewater-fed *Cannabis sativa*. *Environmental Technology*, 39: 1705–1714.
- Jayachandran K., Anoop M and Indu C. Nair. 2018. Growth kinetics of *Alcaligenes* sp d2 during phenol biodegradation in mineral salt phenol medium. *World Journal of Pharmaceutical Research*, 7: 1084-1092
- Jiang, L., Ruan, Q., Li, R., and Li, T. 2013. Biodegradation of phenol by using free and immobilized cells of *Acinetobacter* sp. BS8Y. *Journal Basic of Microbiology*, 53:224–230
- Kumar, A., Kumar, S., Kumar, S., 2005. Biodegradation kinetics of phenol and catechol using *Pseudomonas putida* MTCC 1194. *Biochemistry and Engeneering Journal*, 22:151–159
- Kloos, W.E.; Tornabene, T.G.; Schleifer, K.H. 1974. Isolation and characterization of *micrococci* from human skin, including two new species: *Micrococcus lylae* and *Micrococcus kristinae*. *International Journal of Systematic of Bacteriology*, 24:79–101
- Liu, Z.; Xie, W.; Li, D.; Peng, Y.; Li, Z.; Liu., S. 2016. Biodegradation of Phenol by Bacterium Strain *Acinetobacter calcoaceticus* PA Isolated from Phenolic Wastewater. *International journal of environmental research*, 13(3): 300
- Ren LF, Chen R, Zhang X, Shao J, He Y. 2017. Phenol biodegradation and microbial community dynamics in extractive membrane bioreactor (EMBR) for phenol-laden saline wastewater. *Bioresources and Technology*, 244(Pt1):1121-1128
- Senthilvelan, T., Kanagaraj, J., Panda, R.C., Mandal, A.B. 2014. Biodegradation of phenol by mixed microbial culture: an eco-friendly approach for the pollution reduction. *Clean Technology of Environnement*, 16: 113–126
- Sepehr, S.; Shahnavaz, B.; Asoodeh, A.; Karrabi, M. 2019. Biodegradation of phenol by cold-tolerant bacteria

- isolated from alpine soils of Binaloud Mountains in Iran. *Environnemental Science*, 54(4): 367-379
- Silva, I. S.; dos Santos, E. d. C.; de Menezes, C. R.; de Faria, A. F.; Franciscon, E.; Grossman, M.; Durrant, L. R. 2009. Bioremediation of a Polyaromatic Hydrocarbon Contaminated Soil by Native Soil Micro- biota and Bioaugmentation with Isolated Microbial Consortia. *Bioresources and Technology*, 100(20):4669–4675.
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2005. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular and Biology Evolution*, 28(10):2731-9
- Tam L.T., Eymann C., Albrecht D., Sietmann R., Schauer F., Hecker M., and H., Antelmann .2006. Differential gene expression in response to phenol and catechol reveals different metabolic activities for the degradation of aromatic compounds in *Bacillus subtilis*. *Environnemental Microbiology*, 8: 1408- 1427
- Wu, L., Ali, D. C., Liu, P., Peng, C., Zhai, J., Wang, Y. 2018. Degradation of phenol via ortho-pathway by *Kocuria* sp. strain TIBETAN4 isolated from the soils around Qinghai Lake in China. *PLoS One*. 13:e0199572.
- Wang, Y., Wang, C., Li, A., and Gao, J. 2015. Biodegradation of pentachloronitrobenzene by *Arthrobacter nicotianae* DH19. *Letters in Applied Microbiology*, 61:403–410
- Zeinat Kamal, M.; Nashwa, A.; Mohamed, A.I.; Sherif, E. N. 2008. Biodegradation and Detoxification of Malathion by of *Bacillus thuringiensis* MOS-5. *Australian Journal of Basic and Applied Science*, 2:724–73