CHAPTER- II
ISOLATION AND IDENTIFICATION
OF ALKALIPHILIC AND NEUTROPHILIC BACTERIA
2.1 Introduction

The organic xenobiotic waste like nitro aromatics, polycyclic aromatic hydrocarbons (PAHs), household products, industrial disposal like pharmacy, textile, leather, refinery etc. are continuously discharged into environment and contaminating the flora and fauna [3]. Hence, soil and ocean is remaining a sink of organic xenobiotic compounds. Soil contains the wide range of microbes, in quantity and quality, receives the chemicals in various forms and acts as a scavenger of harmful substances. Because of toxicity of organic xenobiotics, due to their carcinogenic and mutagenic properties [175], has led to considerable research efforts to understand the microbial degradation of contaminated sites. Until recently, research on the isolation of organic xenobiotic compounds-degrading microorganisms has been attempted in the last few decades to gain a detailed understanding of the metabolism of xenobiotics biological degradation with pure cultures.

Organic xenobiotic compounds degrading microorganisms require an environmental habitat that has a sufficient and preferably sustainable source of nutrients, water, air, mild ambient temperature, and a moderate pH. Therefore, soil is a perfect source of microorganisms for isolation [176]. Bacteria are the primary driving force for degradation of organic contaminates in soil [177]. Bacteria possess numerous biotransforming enzymes like azo reductase, cytochrome P-450 monooxygenase, dioxygenases etc, by utilizing this enzyme machinery towards the xenobiotic compounds degradation to simple environmentally benign metabolites further consuming them as a carbon and energy source. These significant contaminated sites are now the reservoir of the well adapted microorganisms towards the different organic xenobiotic compounds such as PAHs, azo dye, nitroaromatic. Moreover, it is
widely recognized that contaminated land is a potential threat to human health, and its continual discovery over recent years has led to international efforts to remedy many of these sites. Therefore, it is necessary work to isolate the pure adapted microbial strain for the degradation as well as biotransformation studied of these organic xenobiotic compounds in laboratory. The authentication of several of the soil bacteria is still unknown; therefore it is always necessary to have an authentication of the microbial strain which is used for the degradation of xenobiotic compounds. In past two decade, it was a difficult task to identify and characterize bacteria by conventional phenotype methodology because of their difference in phenotypic characteristics [178]. Among the isolation techniques, the methods using zones of clearing on agar plates, developed have been widely used to screen PAH-degrading microorganisms under aerobic conditions [179-181].

In aerobic conditions, the conventional serial dilution and pour plating methods have been generally utilized to obtain pure cultures. Now, amplification of smaller subunit of ribosomal 16S rRNA genes and their specific differentiated regions has helped in identification and phylogenetic tree of the strain [182]. Based on 16S rRNA gene sequence classification, phylogenetic relation and novelty is determined by Felsenstein, J. [183], the studies are planned to identify the alkaliphilic bacterial strains obtained from pristine Crater Lake of Lonar and neutrophilic bacterial strain from soil obtained from the dye disposal area.

2.2 Materials and methods

2.2.1 Chemicals

All chemicals were of Analytical grade the highest grade of purity available. NaCl, Na₂CO₃, AgNO₃, HNO₃, NaOH, MgSO₄, MnSO₄, FeCl₃, CuSO₄, KH₂PO₄, Na₂HPO₄, (NH₄)₂SO₄, MgSO₄, MgO, FeSO₄, CaCO₃, ZnSO₄, CoSO₄ and H₃BO₃
were obtained from SRL (Mumbai, India), Nutrient agar, yeast extract, peptone, (Hi-
media, India), DNAzol (Invitrogen, USA), ethanol (Gebsen & Gessen, GmbH &
Co., Germany), dNTP (Vivantis, USA), 20F forward and 1540R primers (Integrated
DNA Technologies, USA), Nuclease free water, Taq polymerase enzyme, Taq
polymerase buffer A, Gel loading buffer, Agarose, TE buffer, TAE buffer, 500bp
DNA ladder, Proteinase K (Bangalore Genei, India). Anthracene was purchased from
sisco research laboratory (SRL) Phenanthrene was obtained from the organic
chemistry lab of our Department, fluoranthene and pyrene was purchased from Acros
Organics, New jersey, USA. Dichloromethane (DCM), ethyl acetate, ethanol,
benzene, and hexane were purchased from S.D. Fine chemical, India.

2.2.2 Isolation of bacteria

The *Bacillus badius strain D1* used in this work was collected from alkaline
Crater Lake of Lonar, Maharashtra state, India, and *Lysinbacillus sphaericus DL8*
isolated from dye finishing industry disposal site, Ichalkaranji, India. Isolation of
pure strains of microorganisms was done by serial dilution and pure plate methods,
during serial dilution of soil sample ten small sterile test tubes labeled. The tubes 1 to
10 and then add 4.5 mL saline to each test tube. Saline solution is a physiological
buffered, bacteria will not grow in this saline but will remain in a state of stasis until
the diluted cells are plated on media containing a carbon source. By the pipette 0.5
mL of the original solution transfer into test tube 1, this bacterial suspension should
be mixed thoroughly before proceeding to the next step. With a clean pipette and
withdraw 0.5 mL of the diluted bacterial suspension from the first test tube
and pipette that into the second test tube. Continue in this proceed until diluted the
original bacterial suspension into test tube 10. In test tube 1 the bacteria diluted 10
fold, a 1:10 or 1 x 10^{-1} and the dilution, in test tube 5 diluted the bacteria from the
original tube to obtain a $1 \times 10^{-5}$ dilution, in test tube 10 diluted the bacteria from the original tube to obtain a $1 \times 10^{-10}$ dilution. Using the pipette, 1 ml was drawn from the last tube into two petri plates. These plates should be labeled with initials and the dilution factor. In this case we will plate the following dilutions: $1 \times 10^{-1}$, $1 \times 10^{-2}$, $1 \times 10^{-3}$, $1 \times 10^{-4}$, $1 \times 10^{-5}$, $1 \times 10^{-6}$, $1 \times 10^{-7}$, $1 \times 10^{-8}$, $1 \times 10^{-9}$ and $1 \times 10^{-10}$ For the cell suspension that will be plated onto the agar plate labeled $1 \times 10^{-1}$, pipette 0.5 mL of the diluted suspension from the appropriately diluted test tube onto the surface of the plate. Dip the hockey stick into the alcohol solution and flame the stick until the alcohol has burned off for sterilizing the hockey stick. After sterilizing the stick, use the hockey stick to spread the bacterial suspension evenly over the entire surface of the plate. Allow the plate to dry. Continue this process with the remainder of the bacterial dilutions. Tape all of plates together and incubate the plates, upside down, at $37^\circ C$ for 24 hours and then stored in the refrigerator.

After 24 hrs of incubation, the number of colonies has been counted and the pure colonies of *Bacillus badius D1*, and *L. sphaericus DL8* strains were selected from agar plates and grown in liquid media. Growth curve of the isolated bacterial strains is checked over the time period of 0 to 40 hours. The nutrient medium was inoculated with the pure culture of these strains and growth of the cells was monitored by checking its optical density at 600 nm with JASCO V- 630 UV-visible spectrophotometer.
2.2.3 Growth conditions for *Bacillus badius D1*.

*Bacillus badius D1* bacterial strain was grown in 500 ml conical flask on static condition. The cultivation medium contained the following (g / litre): 5.0 yeast extract, 5.0 peptone, 5.0 NaCl, Trace elements, KH$_2$PO$_4$ 170 mg, Na$_2$HPO$_4$ 980 mg, (NH$_4$)$_2$SO$_4$ 100 mg, MgSO$_4$ 0.87 mg, MgO 0.1 mg, FeSO$_4$ 0.05 mg, CaCO$_3$ 0.2 mg, ZnSO$_4$ 0.08 mg, CuSO$_4$ 0.016 mg, CoCl$_3$ 0.015 mg, boric acid 0.006 mg. The pH was adjusted to 9.0 by addition 5-10 drops of NaOH to the media. Flasks containing 100 ml of medium were inoculated with loop full of microorganism culture. After 24 hrs of incubation 50 mg of PAHs (ANT, PHE, FLU and PYR) were added. The alkaliphilic strain *Bacillus badius D1* which was found to have a higher ability to mineralized and degrade the PAHs under aerobic conditions, was grown aerobically at 37°C for 24 hrs in nutrient broth medium.

2.2.4 Morphological study by scanning electron microscopy

The bacterial cells were collected by centrifugation of culture at 9000 x g for 10 min in cold condition. The suspension of bacterial sample was mixed with 4% glutaraldehyde using sterile distilled water and specimen was fixed for SEM as per the protocol of Grekova-Vasileva et al [185] thereafter the cells were dehydrated
with gradient alcoholic solution. After drying and mounting of specimen on an aluminium stub gold-coating was done for 5 min and image was examined using the JEOL-JSM-5510 scanning electron microscope [186].

2.2.5 Identification of microorganisms by 16s rRNA method

2.2.5.1 Identification of Bacillus badius D1 by 16s rRNA method

2.2.5.1.1 Extraction of bacterial DNA

The DNA from the bacterial cells was extracted using a typical DNAzol method [187]. Approximately 9 colonies from petri plate were suspended in 1x TE buffer and washed twice in 500 µl TE by vortex mix followed by centrifuge at 10000 x g for 10 minutes at room temperature. Bacterial pellets then suspended to 100 µl Proteinase K (at the final concentration of 325µg/ml) at 65°C for 1 hour and 30 minutes. Lysis was done by DNAzol treatment as 500µl for bacterial pellets with Proteinase K mixture at 65°C for 5 minutes. Upper DNAzol phase was carefully separated onto 500µl of absolute chilled ethanol resulting in bacterial DNA precipitation followed by two washing of 70% cold ethanol and DNA pellets were collected at 10000 x g for 10 minutes at room temperature. The resulting pallet was solubilized in 100µl of TE buffer. For each 100 PCR reaction 500ng templates DNA was used.

2.2.5.1.2 16S rRNA sequence amplification by PCR.

DNA extracted from sub cultured colonies by DNAzol method was subjected to 16S rRNA PCR. Following primers were used for PCR and DNA sequencing [188], Forward primer 20F 5’ATGTTGATCATGGCTCA3’ Reverse primer 1540R 5’AAGGAGGTGATCATGGCTCA3’ generate a PCR product of approximately 1.5 kbp (E. coli numbering system [189]. PCR mix was prepared by adding 10 µl 10X Taq polymerase buffer, 4 µl of 10 mM dNTP mix, 40 picomoles each reverse and
forward primers, 1 U of Taq Polymerase enzyme to 67 µl of nuclease free water. To this 90 µl master mix, 10 µl of DNA template was added, mixed, and immediately spinned and divided into five equal aliquots in 200 µl capacity PCR tubes. PCR was carried out with 400 µM final dNTP mix, 40 picomoles each primers. Following conditions were used for thermal cycling. Initial denaturation at 94ºC for 3 min, followed by 30 cycles of denaturation at 94ºC for 1 min, primer annealing at 50.2ºC for 1 min and primer extension at 72ºC for 2 min, with final extension at 72ºC for 5 min. An approximately 1.5 kbp amplicon was observed in 1% agarose gel.

2.2.5.1.3 DNA electrophoresis for visualization of PCR products

Briefly, 0.5 g agarose was added to 50 ml of 1X TAE buffer for preparing 1% gel, mixed without shaking and placed for boiling under microwave oven for 1 min. When temperature of the boiling gel was lowered up to 65 - 70°C, 20 µl of 1mg/ml Ethidium bromide was added and mixed to 50 ml warm gel solution, allowed to cool further and casted with 10 comb mini gel casting mechanism, without forming bubbles in a gel. Submarine gel electrophoresis for DNA visualization was performed in Bio-Rad mini sub cell GT submarine electrophoresis systems using 1X TAE buffer at 75 volts for 45 minutes with Bio-Rad universal power pack. When the tracking dye migrates to anodal end of the gel, electrophoresis was stopped and gel was observed and documented in gel doc XR system.

2.2.5.1.4 DNA sequencing and BLAST tree analysis

PCR product was sequenced by Sanger’s Dideoxy chain termination method and subjected to electrophoresis by automated DNA Sequencer Applied Biosystem ABI 3100 genetic analyzer. After DNA sequence data interpretation, nucleotide data subjected to NCBI BLAST analysis. The phylogenic analysis was constructed by using a Neighbor-Joining (NJ) method in MEGA4.1 software [190-192]. The
bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed by 16S rRNA sequence analysis. The culture was deposited in National Collection of Industrial Microorganisms (NCIM), Pune, India with the accession number NCIM 5330. The sequence of the 16S rDNA gene of the strain *Bacillus badius D1* is available under the GenBank accession number HQ015711.

### 2.2.6 Growth conditions for *Lysinibacillus sphaericus* DL8

*Lysinibacillus sphaericus* DL8 bacterial strain was grown in 500 ml conical flask on static condition. The cultivation medium contained the following in (g/litre) 5.0 yeast extract, 5.0 peptone, 5.0 NaCl, Trace elements, KH$_2$PO$_4$ 170 mg, Na$_3$HPO$_4$ 980 mg, (NH$_4$)$_2$SO$_4$ 100 mg, MgSO$_4$ 0.87 mg, MgO 0.1 mg and FeSO$_4$ 0.05 mg. The pH was adjusted to 7.0. Flasks containing 100 ml of medium were inoculated with loop full of microorganism culture. After 24 hrs PAHs were added.

### 2.2.7 Identification of *Lysinibacillus sphaericus* DL8 by 16s rRNA method

#### 2.2.7.1 Bacterial culture, DNA extraction and 16S rRNA PCR

The bacterial strain was grown aerobically on nutrient agar. DNA was isolated by using the DNAzol method already described in section 2.2.5.1.1

#### 2.2.7.2 16S rRNA sequence amplification by PCR

DNA was isolated by using the DNAzol method already described in section 2.2.5.1.2.

PCR mix was prepared by adding 10 µl 10X Taq polymerase buffer, 4 µl of 10 mM dNTP mix, 40 picomoles each reverse and forward primers, 1 U of Taq Polymerase enzyme to 67 µl of nuclease free water. To this 90 µl master mix, 10 µl of DNA template was added, mixed, and immediately spinned and divided into five equal aliquots in 200 µl capacity PCR tubes. PCR was carried out with 400 µM final dNTP
mix, 40 picomoles each primers. Following conditions were used for thermal cycling. Initial denaturation at 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 1 min, primer annealing at 49°C for 1 min and primer extension at 72°C for 2 min, with final extension at 72°C for 5 min. An approximately 1.5 kbp amplicon was observed in 1% agarose gel.

2.2.7.3 DNA electrophoresis for visualization of PCR products

1 gm of agarose was added to 100 ml of 1X TAE buffer for preparing 1% gel, mixed without shaking and placed for boiling under microwave oven for 1 min. When temperature of the boiling gel was lowered up to 65-70°C, 20 µl of 1mg / ml ethidium bromide was added and mixed to 100 ml warm gel solution, allowed to cool further and casted with 10 comb mini gel casting mechanism, without forming bubbles in a gel. Submarine gel electrophoresis for DNA visualization was performed according to already described method in section 2.2.5.1.3.

2.2.7.4 DNA sequencing and BLAST tree analysis

PCR product was sequenced by Sanger’s Dideoxy chain termination method as per described in section 2.2.5.1.4

The sequence of the 16S rRNA gene of the strain *Lysinibacillus sphaericus DL8* is available under the Gen Bank accession number HQ829965.
2.3 Result and Discussion

2.3.1 Isolation and identification of bacterial strain *B. badius* D1

The pure colonies of *B. badius* strain *D1* were selected from agar plate and grown in liquid media. The growth of the bacterial strain *B. badius* *D1* by taking its optical density is plotted against the time. The maximum bacterial growth is after 24 hrs and it started declining after 36 hrs. *B. badius* has 0 to 5 hrs lag phase and from 5 to 25 hrs long exponential phases followed by static phase for around 11 hrs (*Fig.15*). For molecular identification of this strain *B. badius* *D1*, the 16S rRNA gene amplified by PCR with 1.5 kbp product using the conserved region primers in the 16rRNA gene, and its nucleotide sequence was determined by Sanger’s Dideoxy Chain Termination method (*Fig.16*).

2.3.2 Morphological study by scanning electron microscopy (SEM)

SEM the purified isolate *B. badius* *D1* was taken in higher magnification. Serially dilution of culture and repeated study of SEM confirms that the strain *D1* (*Fig.17A*) and *L. sphaericus* *DL8* (*Fig.17.B*) are having typical morphology of rod shaped Bacillus with spongy appearance at magnification x 25,000 separately spread having the size of 1.0 to 4.0 µm as shown in (*Fig.17*). The strain *B. badius* *D1* is optimized at 37 °C at pH-9.0 and used further experiment.

![Fig.15 Growth curve of *Bacillus badius* *D1*](image-url)
Fig. 16 PCR product from 16S rRNA of *B. badius D1*,
Lane 1: Mol. wt. marker (500 base pair DNA ladder),
Lane 2: 1.5 kb desired PCR product.

Fig. 17 Scanning electron micrograph (SEM) of *Bacillus badius D1* (A) and
*Lysinbacillus sphaericus DL8* (B).

*Bacillus badius* strain D1 16S ribosomal RNA gene, partial sequence
(HQ015711.1)

TAACCTGCCTGTAAGACTGGGATAAATCCCGGAAAAACCGGGGCTAATACCGG
ATTCTTTTTCTTCGCATGAAGAAGAATGGAAAGGCGGCTTTAGCTGTCA
TACAGATGGACCCCGGCATATAGCTAGTTGGTGAGTTAAGTGCTACCCAA
GGCAACGATGCGTGACCTGAAAAAAAAAGAGGGGCACTGGAATTATTGGGCG
TGACTTAAATCTCTAGTGAGAAGGGACATCCACGTGACACATGCTACAC
TGATGGTTTAGCTGAGGCGCGAAGCGTGTGGAGCGAACAGGATTAGA
CCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTGGAGGGTTTCC
GGAGTACGGC
AAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCAT
GTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCCG
GACCGGTCTGGAGACAGGCCTTTCCCTTCGGGACAGCGGTGACAGGTGGTG
CATGGTTGTGCAGCAGCTAATGCTAGCAGAGTAGTGGTGTTGTTAAGTGCGAC
CGCAACCCCTTATCTTATGGTCGCAGCATTACGGTGGCCTCTAAGTGTA
CCCGTGACAAAAAGGGAGGAGGAGGTGAGTTAATATCAGACAGACGC
ATGACCTGGGTGACTACACAGCTGTCAGAATGGATATGGTACAAAAAGGGAGT
CCCGAAGTGTAGCGTACTACCCCAATAAAGCCATTCTCAGTGGATTTGGCGAC
AATCTGCGTGAAGCCCGAATCGTACTGTAATCCCGGATCAGCATGCGC
GGTGAATACGTTCCCGGCGCTTAAGTACCGCGCAGCTACCGAGAGTTT
GCAACACCCGAAGTGGGTGAGTA
The phylogenetic tree was constructed by using a neighbor-joining (NJ) method [190]. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site [190,191,192]. Based on 16S rRNA gene NCBI BLAST search analysis, the isolated species is *B. badius*, which is found to be 99 percent identical to *Bacillus badius D1* ATCC 14574 (X77790) among selected taxonomic group of *bacillus* species (Fig.18).

![Phylogenetic tree](image)

**Fig.18** Phylogenetic tree 11taxa a neighbor-joining method based on 16S rRNA gene sequences showing the relationships between *Bacillus badius D1* Lonar Lake and other relatives within the genus *Bacillus*.

### 2.3.3 Isolation and identification of neutrophilic bacterial strain *L. sphaericus DL8*.

The pure colonies of *L. sphaericus* strain *DL8* were selected from agar plates of various concentrations and grown in liquid media. The growth of the bacterial strain *L. sphaericus* is judged by increase in optical density of media. The graph is plotted by taking optical density verses time and it is showing maximum after 20 hrs and it
started declining after 27 hrs. *L. sphaericus DL8* has 0 to 4 hrs lag phase and from 5 to 25 hrs long exponential phase followed by static phase for around 7 hrs (Fig.19).

![Fig.19](image1.png)  
**Fig.19** Growth curve of *L. sphaericus* DL8.

![Fig.20](image2.png)  
**Fig.20** PCR product from 16S rRNA of *Lysinibacillus sphaericus DL8*: 100 base pair DNA ladder on both sides (Line 1 and 5) and single band in lane 3 at the position of 1.5 kb suggest perfect amplification of product from *L. sphaericus DL8* has 0 to 4 hrs lag phase and from 5 to 20 hrs long exponential phase followed by static phase for around 7 hrs.
The molecular identification of this strain, *L. sphaericus DL8*, and the 16S rRNA gene was amplified by PCR with 1.5 kbp product using the conserved region primers in the 16S rRNA gene, and its nucleotide sequence was determined by Sanger’s Dideoxy Chain Termination method (Fig. 20).

**Fig. 21** Phylogenetic tree of 10 taxa: Neighbor-Joining Phylogenetic tree based on 16S rRNA gene sequences showing the relationships between *Lysinbacillus sphaericus DL8* and other relatives within the genus Bacillus.
Bootstrap values (%) are based on 500 replicates. Bar indicates 100 substitutions per nucleotide position. Accession number is mentioned before each *Lysinibacillus* species.

The phylogenetic tree was constructed by using a neighbor-joining (NJ) method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches (Fig.21). The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site [190-192]. Based on 16S rRNA gene NCBI BLAST search analysis, the isolated species is *L. sphaericus*, which is found to be 99 percent identical to GU129144 *Lysinibacillus sphaericus* TMB5 among the selected taxonomic group of *bacillus* species.
2.4 Discussion

Different of PAHs are ubiquitously distributed in the environment, many of them possess mutagenic, carcinogenic and teratogenic properties. Increasing in PAHs during industrial activities, residential heating, power generation, incineration and vehicle emissions [193] could increase the risk of cancer and other adverse health outcomes [194]. Biological treatment system has been widely used in various aspects of remediation of environment, using suitable microorganisms; this treatment is an eco friendly, relatively simple and cost-effective alternative to physico-chemical clean up options. Degradation of PAHs in the soil involves microorganisms having specific metabolic capacities. In polluted environments, specific microorganisms are abundant because of the adaptation of microorganisms to pollutant. It has been shown that bacteria are the main agents responsible for the degradation of xenobiotics. In this study, we isolated, characterized, and identified bacterial strains to degradation of these xenobiotic compounds. An alkaliphilic bacterial strain *Bacillus badius D1* isolated from soil sediment sample from alkaline Crater Lake of Lonar, Buldana, MS India. The water is alkaline with pH of 9-12, checked with pH paper on the spot. This alkaliphilic isolate was identified as *Bacillus badius D1* by 16S rRNA sequence analysis of these representative strains allowed a first tentative species identification and was deposited in National Collection of Industrial Microorganisms (NCIM), Pune, India with the accession number HQ015711.1 The 16S rDNA sequencing and phylogenetic analysis indicated that the strain isolated was related to genera *Bacillus* shared more than 99 % identity with their closest phylogenetic relative analysis confirm the strain *Bacillus badius D1*.

On the other hand Isolation and identification of neutrophilic bacterial strain *L. sphaericus DL8*, isolated from dye finishing industry disposal site, Ichalkaranji,
India. Based on 16S rRNA gene NCBI BLAST search analysis, the isolated species is *L. sphaericus DL8*, which is found to be 99 percent identical to GU129144 *Lysinibacillus sphaericus* and was deposited in National Collection of Industrial Microorganisms (NCIM), Pune, India with the accession number HQ829965.2. Morphological character of both alkaliphilic and neutrophilic bacteria showed the gram positive strain with rod shape having motility. According to phylogenetic study, molecular identification and morphology examination isolated identified as non-pathology aerobic bacteria.

Number of investigators has reported the degradation potential of various microbial strains towards the pollutants [195]. In the present study a successful attempt has been made for new aerobic bacteria isolation and purification such bacteria as alkaliphilic bacteria *Bacillus badius D1* and neutrophilic bacteria *Lysinibacillus sphaericus DL8* were used for the degradation of polycyclic aromatic hydrocarbons (PAHs). The longer exponential phase of strain keeps its enzyme ability to perform the biocatalytic activity like di and monooxygenase by the action of cytochrome P-450. Both bacterial strains observed the potential degradation of PAHs; alkaliphilic bacteria *Bacillus badius D1* was shown the higher % degradation activity than neutrophilic bacterial strains *Lysinibacillus sphaericus DL8* which had checked in both static and shaking condition, in the shaking condition shown higher degradation of PAHs than static condition. Since the strains confirmed are wild sort, acclimatization results reciprocate and found strain is of immense use in of biotechnological process to clean the environment. Bacterial chemotaxis might have evolved as a selective advantage to bacteria for searching for the chemicals that could act as a source of carbon and energy to the cells [196]. The study showed a potential, very dependable and beneficial characteristic of strains for bioremediation.
and biotechnological application in the polycyclic aromatic hydrocarbons polluted area. Degradation potential of this bacterium increased the applicability of this microorganism for the PAHs removal applications particularly in industry waste. The microorganism is good candidate for the bioremediation of hypersaline environments and treatment of saline effluents. It is clearly evident from the study that considerable diversity exists among *Bacillus badius D1* and *Lysinibacillus sphaericus DL8* to degrade the various polycyclic aromatic hydrocarbons. Technological platforms can provide environmentally friendly and cost-effective solutions for effluent of contaminated environment.