# UNVERSITY OF DELHI

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# FINAL REPORT

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2. **PROJECT TITLE** : Isolation of Novel Bacteria From Garbage Disposal Landfills in Delhi, Screening for Antibiotics or Secondary Metabolites and Genome Sequencing

3. **NAME OF COLLEGE/INSTITUTION:** SRI VENKATESWARA COLLEGE
   Benito Juarez Road, Dhaula Kuan, New Delhi, Delhi 110021

# DETAILS OF PI'S

| NAME | DEPARTMENT | CONTACT NO. | EMAIL ID |
|---|---|---|---|
| DR. AJAIB SINGH | ZOOLOGY | 9871158500 | ajaibmolbio@gmail.com |
| DR. V.V.S.N RAO | ZOOLOGY | 9911118304 | svelvada@yahoo.com |
| MR. AMIT KUMAR GAUTAM | CHEMISTRY | 8860183172 | akg@svc.ac.in |

# DETAILS OF MENTOR

**PROFESSOR RUP LAL**

MOLECULAR BIOLOGY LAB,

DEPARTMENT OF ZOOLOGY,

UNIVERSITY OF DELHI, DELHI-7

Phone Number : 9810785766

E mail: ruplal@gmail.com

Website: http://people.du.ac.in/~rlal/

# DETAILS OF STUDENTS

| NAME | COURSE | CONTACT NO | EMAIL ID |
|---|---|---|---|
| SAHIL YADAV | B.Sc LIFE SCIENCE 2ND YEAR | 9871094956 | yadavsahil146@gmail.com |
| VIDIT GAUR | B.Sc LIFE SCIENCE 2ND YEAR | 9953094454 | gaur.vidit8@gmail.com |
| PAYAL THAPA | B.Sc LIFE SCIENCE 2ND YEAR | 9953017726 | payalthapa1995@gmail.com |
| RAJATH R. PRABHU | B.Sc LIFE SCIENCE 2ND YEAR | 9971172210 | rajathprabhu@hotmail.com |
| AYUSH AGNIHOTRI | B.Sc LIFE SCIENCE 2ND YEAR | 9643490607 | pratyush17d@gmail.com |
| KOMAL | B.Sc LIFE SCIENCE 2ND YEAR | 8826934571 | masounkomal1996@gmail.com |
| AKRITI JAIN | B.Sc LIFE SCIENCE 1ST YEAR | 8059774999 | aakritij0@gmail.com |
| PALLAWI CHOUBEY | B.Sc LIFE SCIENCE 1ST YEAR | 8826603063 | pallawi.choubey9686@gmail.com |
| PIYUSH RAJPUT | B.Sc LIFE SCIENCE 1ST YEAR | 8826172050 | pkrajput1717@gmail.com |
| PRIYAANK SHARMA | B.Sc LIFE SCIENCE 1ST YEAR | 9953482059 | priyaanksharma.37@gmail.com |

# PROJECT : SVC-306
# (Final Report)

Isolation of Novel Bacteria From Garbage Disposal Landfills in Delhi, Screening for Antibiotics or Secondary Metabolites and Genome Sequencing.

# ABSTRACT

Bacteria are known to produce many kind of secondary metabolites with antibiotics or antibacterial agents as the most important group. Different kind of antibiotics has been isolated from different kind of bacteria. But with time bacteria evolve and develop multi drug resistance. Thus there is a need to find new antibiotics which can combat such bacteria. New bacteria and thus new antibiotics can be isolated from new habitats. Landfills are one such new habitat since many kind of pollutants are dumped there. An attempt was made to isolate bacteria from landfills situated at Mukarba Chowk, Delhi followed by their screening for production of antibacterial agents.

The soil samples were collected from the landfill site at a depth of 20 cm and processed in the lab. Bacterial isolation was done by direct serial dilution. From different dilutions, inoculum was plated on LB plates and incubated at $28^0$C for 48 hrs. Many different kind of bacterial colonies were observed in the plates. From the colony size, color and other morphological and physiological characteristics different colonies were picked and streaked on fresh plates. The process was repeated multiple times until pure culture were obtained. Pure cultures were preserved by making glycerol stock and storing at $-80^0$C. The remaining cultures of bacteria were used for screening of antibacterial producing isolates. The three classical bacteria *E. coli*, *S. aureus* and *B. subtilis* were used for this purpose. Lawns of these bacteria were made on LB plate and the desired isolates were streaked in the centre. After proper incubation the plates were observed to see halo or inhibitory zone formation which is an indication of antibacterial activity by the isolate. Out of many bacteria assayed two were found to give positive results. Zone of inhibition was founded to be formed around isolates PK1 and YO1. These two bacteria were then chosen for genotypic and biochemical characterization by performing series of tests.

Genomic DNAs of these isolates were isolated. 16s rRNA genes were then amplified by PCR, separated on agarose gel electrophoresis, and purified by gel elution

method. The genes were then sequenced by Sanger's dideoxy method with sequencing machine.

The obtained gene sequences were assembled and the closest neighbours of the required strains were determined by web server sites like NCBI-BLAST and EzTaxon-e Server. Alignment of sequences of 16SrRNA genes was performed by CLUSTAL X alignment software. Phylogenetic analysis was performed with MEGA version 5.2.2 using the neighbour-joining method with bootstrap analyses as 100 replications. Strain PK1 was found to be a strain of *Bacillus filamentosus* and is now named as strain *Bacillus filamentosus* PK1. Similarly strain YO1 was found to be strain of *Pseudomonas parafulva* and is named as *Pseudomonas parafulva* YO1.

Further biochemical characterization of both of these strains was also done. Strain *Bacillus filamentosus* PK1 grew well under aerobic conditions on LB $28^0$C within 24-48 h of incubation. It's colonies were pink colored, entire, smooth, circular and varied in size. Gram staining revealed it to be Gram positive. Susceptibility towards antibiotics was determined which revealed that strain PK1 was sensitive to penicillin but resistant to vancomycin, tetracycline, rifampicin, ciprofloxacin, chloroamphenicol, nalidixic acid and polymixin B and kanamycin. *Pseudomonas parafulva* YO1 also grow well in aerobic conditions on LB at $28^0$C within 24-48 h of incubation. The colonies of *Pseudomonas parafulva* YO1were yellow colored, entire, smooth, circular and varied in size Gram staining revealed the strain YO1 to be Gram negative.

# INTRODUCTION

Bacteria are known to produce various kinds of products useful for the mankind. They find their use in industries like textile, food, pharmaceutical, agriculture, environment, and what not. Bacteria not only produce these useful products by primary metabolism but secondary metabolism also plays a major role in the synthesis of these products.

A primary metabolite also called as central metabolite is a metabolite that is directly involved in growth, development, and reproduction of the organism. Almost all the species contain conserved genetic pathways of primary metabolism. The primary metabolite plays major physiological role in the organism without which organism will die. Common examples of primary metabolites are pyruvic acid, ethanol, lactic acid, amino acids and fatty acids.

However secondary metabolite is not vital for life as such and only plays ecological role. It is beneficial to the organism but not mandatory. Only some species get specialized in the production of secondary metabolites. Examples of secondary metabolites are alkaloids, antibiotics, naphthalenes, nucleosides, phenazines, etc.

## SECONDARY METABOLITES

Bacteria have been great resource for secondary metabolite production which have immense commercial applications to mankind. Secondary metabolites are small molecules secreted by bacteria under stress conditions which although are not essential for life still confer evolutionary advantage to the producer organism (Jenke-Kodama., 2008, Metlen.,2009).

Whereas primary metabolic processes (e.g., glycolysis, amino acid biosynthesis, fatty acid synthesis etc.) are found in almost all species of life, the pathways for secondary metabolites production are specialized. In bacteria there are many such pathways which exhibit enormous structural variation and consequently display a

wide range of biological activities. The various secondary metabolites which are produced by these pathways can be categorised into different classes: terpenes, antibiotics, polyketides, alkaloids, non-ribosomal peptides, cytochalasin etc.

Terpenes are derived from five carbon precursors dimethylallylpyrophosphate (DMAPP) and isopentylpyrophosphate (IPP) (Kitaoka et al., 2015). Polyketides are synthesized from acetate units (McDaniel et al., 2005). Non ribosomal Peptide metabolites are built from amino acids using non-ribosomal peptide synthetases (Wenzel., 2005, Murrel., 2005). Alkaloids covers a broad range of metabolites all of which possess a basic nitrogen atom (Ashihama et al., 2008, Duge de Bernonville et al., 2014, Xu et al., 2014, Ziegler.,2008, Facchini., 2008).

Since past many centuries scientists and researchers across the world have tried to isolate bacteria which would yield secondary metabolites of human interests Secondary metabolites have been used for pharmaceutical, cosmetic, and agrochemical purposes for thousands of years. Today these secondary metabolites account for more than one-third of all therapeutic compounds (Newman and Cragg., 2012). In the present project we have specifically targeted bacteria producing antibacterial agents or antibiotics.

**ANTIBIOTICS FROM BACTERIA**

Bacteria have been a big source of many antibiotics. Since the discovery of penicillin in 1929 (Fleming., 1929), many more effective antimicrobials have been discovered. In nature bacteria uses antibiotics for it’s own survival. However man has manipulated the bacterial genetic machinery for his own benefit –to survive the diseases caused by various pathogens.

The various antibiotics isolated from bacteria can be classified based on the cellular component or system they affect, in addition to whether they induce cell death (bactericidal drugs) or merely inhibit cell growth (bacteriostatic drugs). In general Most of the antibiotics work by inhibiting DNA, RNA, cell wall or protein synthesis (Walsh., 2003).

Various classes of antibiotics along with their mode of action have been enlisted in Table 1.

**Table: 1 Table showing classes of drugs with examples and their mode of action (Kohanski et al., 2010)**

| TYPE | DRUG | MODE OF ACTION |
|---|---|---|
| ***Fluoroquinolones*** | Nalidixic acid, ciprofloxacin, levofloxacin and gemifloxacin | DNA synthesis inhibitor |
| ***Trimethoprim–sulfamethoxazole*** | Co-trimoxazole (a combination of trimethoprim and sulfamethoxazole in a 1:5 ratio | DNA synthesis Inhibitor |
| ***Rifamycins*** | Rifamycins, rifampin and rifapentine | RNA synthesis inhibitor |
| ***β-lactams*** | Penicillins (penicillin, ampicillin, oxacillin), cephalosporins (cefazolin, cefoxitin ceftriaxone, cefepime) and carbapenems (imipenem) | Cell wall synthesis inhibitors |
| ***Glycopeptides and glycolipopeptides*** | Vancomycin; teicoplanin | Cell wall synthesis inhibitor |
| ***Lipopeptides*** | Daptomycin and polymixin B | Cell wall synthesis inhibitors |

| | | |
|---|---|---|
| ***Aminoglycosides*** | Gentamicin, tobramycin, streptomycin and kanamycin | Protein synthesis inhibitors (30S ribosome) |
| ***Tetracyclines*** | Tetracycline and doxycycline | Protein Synthesis inhibitors (30S ribosome) |
| ***Macrolides*** | Erythromycin and azythromycin | Protein Synthesis inhibitors (50S ribosome) |
| ***Streptogramins*** | Pristinamycin, dalfopristin and quinupristin | Protein synthesis inhibitor (50S ribosome) |
| ***Phenicols*** | Chloramphenicol | Protein Synthesis inhibitor (50S ribosome) |

**A complete timeline of all the antibiotics can be accessed here - https://en.wikipedia.org/wiki/Timeline_of_antibiotics**

**MULTIPLE DRUG RESISTANCE**

Although there are so many antibiotics as discussed above, however many bacteria have developed resistance to many of them and may develop resistance to others also in future. So there is a need for constant discovery of new bacteria which may produce new antibacterial agents for which no resistance has been developed yet.

Till date a number of bacteria have been reported which have developed resistance to multiple drugs. *M.tuberculosis* strains has been reported to show resistant against isoniazid and rifampicin (Shah et al., 2007, Sotgiu et al., 2009). Similarly multiple drug resistance has been reported in many Gram-negative pathogens like *Escherichia coli*, *Salmonella enterica*, and *Klebsiella pneumoniae*, against lactam

class of antibiotics (Bush and Jacoby., 2010, Jacoby., 2009, Livermore et al., 2007, Queenan and Bush., 2007).

**Table: 2 Table showing modes of resistances used by bacteria against different classes of drugs (Davies and Davies., 2010).**

| Antibiotic class | Examples | Modes of resistance |
|---|---|---|
| β-Lactams | Penicillins (ampicillin), cephalosporins (cephamycin), penems (meropenem), monobactams (aztreonam) | Hydrolysis, efflux, altered target |
| Aminoglycosides | Gentamicin, streptomycin, spectinomycin | Phosphorylation, acetylation, nucleotidylation, efflux, altered target |
| Glycopeptides | Vancomycin, teicoplanin | Reprogramming peptidoglycan biosynthesis |
| Tetracyclines | Minocycline, tigecycline | Monooxygenation, efflux, altered target |
| Macrolides | Erythromycin, azithromicin | Hydrolysis, glycosylation, phosphorylation, efflux, altered target |
| Lincosamides | Clindamycin | Nucleotidylation, efflux, altered target |
| Streptogramins | Synercid | C-O lyase (type B streptogramins), acetylation (type A streptogramins), efflux, altered target |
| Oxazolidinones | Linezolid | Efflux, altered target |
| Phenicols | Chloramphenicol | Acetylation, efflux, altered target |

| | | |
|---|---|---|
| Quinolones | Ciprofloxacin | Acetylation, efflux, altered target |
| Pyrimidines | Trimethoprim | Efflux, altered target |
| Sulfonamides | Sulfamethoxazole | Efflux, altered target |
| Rifamycins | Rifampin | ADP-ribosylation, efflux, altered target |
| Lipopeptides | Daptomycin | Altered target |
| Cationic peptides | Colistin | Altered target, efflux |

*Pseudomonas aeruginosa* which causes nosocomial infections has become resistant to various lactams and aminoglycosides class of antibiotics (Horrevorts.,1990).
The Gram-positive superbug *Staphylococcus aureus* earlier was treated with penicillin however it developed resistant against lactam antibiotics. Further it was tackled with methicillin in 1959 which was the first designer antiresistance antibiotic. However within three years the bacteria developed resistance to methicillin to become methicillin-resistant *S. aureus* (MRSA). The strain has acquired new pathogenicity genes, such as cytotoxic Panton-Valentine leukocidin encoding gene (DeLeo and Chambers., 2009) and is difficult to cure. *Vibrio cholerae* is also developing resistance to cephalosporins, the newer penicillins, and fluoroquinolones and can become another major superbug (Lipp et al., 2002).

The MDR can be achieved by a wide range of biochemical and physiological mechanisms briefly listed in Table 2. According to a recent database there exists more than20,000 potential resistance genes (r genes) which belong to 400 different types (Liu and Pop., 2009). The major mechanisms of antibiotic resistance have been summarized in table 2. For more details two good reviews are by Levy and Marshall (Levy and Marshall., 2004) and White et al. (White et al., 2005).

From the above discussion it is clear that multiple drug resistance bacteria are the major threats in the modern world. Hence there is a need for constant exploration of bacteria which produce new antibiotic or antibacterial agents. If pathogens can acquire or create new genetic machinery to become resistant against antibiotics by the same process bacteria can evolve pathways to produce new antibiotics.

Since different environment provide different kind of stress where such evolution can take place, researchers have explored varied habitat to isolate antibiotic producing bacteria. This theme forms the core of this project and an attempt was made to isolate antibacterial agent producing bacteria from landfill sites.

In further sections of the report objectives, material and methods, results and discussion have been discussed in detail.

# OBJECTIVES

Objectives of this project were as under:

- Survey and selection of garbage disposal landfill sites in Delhi
- Collection of soil samples from Mukarba Chowk landfill site
- Isolation of bacterial strains from soil sample
- Screening of antibiotic producing strains
- Screening of secondary metabolite (pigments) producing strains
- Phylogenetic analyses of novel isolates by 16S rRNA gene amplification
- Genotypic characterisation of the novel isolates
- Biochemical characterisation of the novel isolates.

# METHODOLOGY

## Collection of soil samples

For isolation of antibiotic or antibacterial agent producing bacteria soil samples were collected from Mukarba Chowk based landfill. Samples were collected from a depth of 20 cm under sterile conditions and immediately transported to laboratory. For bacterial isolation samples were processed immediately and for other purposes stored at 4°C.

## Isolation of bacteria producing antibacterial agents

Bacteria were isolated from soil samples by serial dilution method. One gram of soil was taken to make serial dilution in normal saline (0.85% NaCl). Dilutions ($10^{-2}$, $10^{-4}$ and $10^{-6}$) were plated on LB plates supplemented nystatin (30 µg/ml). Plates were incubated at 28°C for two days. Different kind of bacteria were streaked and restreaked to obtain pure cultures based on different colony morphology, shape and size. The bacteria were then subjected for screening to see antibacterial activity by assays against *E. coli*, *S. aureus* and *B. subtilis*.

## Assay for antibacterial activity

To test the isolated bacteria for any antibacterial activity assays were performed against *E. coli*, *S. aureus* and *B. subtilis*. For this a lawn of these bacteria were made on LB plates with 2-4 µL of the inoculums. The newly isolated strains were streaked above the lawn. The plates were incubated at 28°C for two days and observed for the inhibitory zone if any.

## Genomic DNA isolation

100ml of 24-30h grown culture of  strain was pelleted by centrifugation at 6,500 rpm for 10 min.The cells were resuspended in 15ml of 10mM NaCl, 20mM Tris-HCl (pH 8.0) and 1mM EDTA (pH 8.0). To it 100µg/ml proteinase K and 0.5% (*w/v*) SDS (pH

7.2) was added.The suspension was mixed gently by inversion and incubated for 6h at $50^0$C. Equal volume of phenol-chloroform was added when the solution became clear and mixed by gentle inversion for around 10 min. at room temperature. The mix was centrifuged the tube at 4,000 rpm for 20min at room temperature. The upper aqueous layer was transferred carefully with a wide-bore pipet to a fresh tube. The DNA sample was adjusted to 0.3M sodium acetate (pH 5.5) and gently 2 volumes of ethanol was layered on top of the DNA solution. The DNA was spooled out at the aqueous ethanol interphase using a sterile glass rod, washed with 70% (*v/v*) ethanol, touching the DNA to the side of a sterile tube to drain the ethanol and air-dried. The DNA was dissolved in 5-8ml of TE buffer (pH 8.0), stored at $4^0$C overnight without shaking and allowed up to 2 days to resuspend the sample. The quality of DNA was confirmed by subjecting it to agarose gel electrophoresis and subsequent staining with ethidium bromide.

**Purification of genomic DNA**

5ml of extracted DNA was taken in a sterilized centrifuge tube and diluted by adding the sterile MQ or TE. To it 10μl of RNase (10 mg/ml) was added and incubated at 37°C for 1 h. After incubation with RNase, proteinase–K was added and re incubated at 37°C in order to remove the residual protein. An equal volume of tris equilibrated phenol- chloroform was added and extracted as previously (twice). After phenolization content was re-extracted with equal volume of chloroform in order to remove the residual phenol. Aqueous phase was carefully transferred in a new falcon tube, added 0.1V of 3.0M Sodium acetate, pH 4.8 and finally DNA was precipitated with two volume of absolute alcohol. Tube was incubated overnight at -20°C for better precipitation of the DNA. DNA was re-spooled out on glass rod, washed with 70% alcohol, air dried, dissolved in MQ and stored at -80°C for further use.

**Spectrophotometric quantitation of DNA**

DNA was diluted either in TE or Millipore water in a micro centrifuge tube. $OD_{260}$ was taken for the diluted sample against blank. DNA concentration was calculated as

µg/ml $=OD_{260}$ X dilution factor X 50. OD was also taken at 280 nm. At ratio of 260/280 = 1.8, DNA was considered highly pure.

**Agarose gel electrophoresis**

Prepared 0.8% (*w/v*) agarose solution by adding 0.8g agarose to 100ml of 0.5 X Tris-Borate-EDTA buffers. The mix was heated in a microwave oven to form a clear transparent solution. Horizontal gel electrophoresis tank was level adjusted and the gel-comb was positioned over the platform such that 0.5-1.0 mm gap remained between the base of the platform and teeth of the comb. The warm agarose solution (50-60$^0$C) was poured into the platform to form 3-5mm thick layer avoiding the air bubbles. The gel was allowed to solidify at room temperature. The comb was carefully removed and electrophoresis buffer (0.5X TBE) added to tank so as to completely submerge the gel in buffer. Mixed the sample DNA with 1/10$^{th}$ gel loading dye and loaded into the well of the submerged gel. Marker DNA containing known-sized DNA fragments was also loaded so

as to determine the size of DNA fragments in the samples and to confirm the smooth occurrence of electrophoresis. The electrodes present at two sides of the tank (platinum wires-that act as cathode and anode) were connected to the power supply and 70-80V direct current was applied. When the dye migrated to 2/3$^{rd}$ of the gel, the electric current was turned off and the gel was stained in ethidium bromide solution for 30-40 minutes. DNA was visualized under UV.

**Amplification of specific DNA sequences by Polymerase Chain Reaction**

Specific oligonucleotides primers were synthesized commercially from Microsynth GmbH (Balgach, Switzerland). In a thin-walled 0.2 ml PCR tube kept on ice, a standard reaction mixture of desired volume was prepared by adding the following components as listed:

**Reaction mixture components for 50 µl final volume**

| | |
|---|---|
| MQ water | to make up final volume |
| Reaction buffer (10X) | 5 µl (1X final concentration) |
| dNTPs mix (25mM) | 3 µl (2.5 mM each dNTP) |
| Dimethylsulphoxide (DMSO) | 5 µl |
| Sense primer | 2 µl (50 pmol) |
| Antisense primer | 2 µl (50 pmol |
| Template DNA | <10 ng/µl in final mixture |
| *Taq* DNA polymerase (1unit/□l) | 1 µl |

The contents were mixed gently by pipetting. The tubes were centrifuged briefly (10-20 sec) to collect the contents at the bottom of the tube. Amplification was done using PCR machine (Robocycler® Gradient 96 Temperature Cycler, Stratagene, USA) at conditions given below.

| | **Block 1** | **Block 2** | **Block 3** | **Block 4** |
|---|---|---|---|---|
| Set temperature | **96$^0$C** (Denaturation) | **55$^0$C** (Annealing) | **72$^0$C** (Extension) | **6$^0$C** |
| Window 1 Cycle 1 | 5 min. | 0 min. | 0 min. | 0 min. |
| Window 2 Cycle 2-30 | 5 min. | 1 min. | 1 min. | 0 min. |
| Window 3 | 0 min. | 0 min. | 10 min. | 0 min |

After amplification an aliquot (10 µl) of the final PCR product was analyzed by standard agarose gel electrophoresis.

## 16S rDNA sequencing and phylogenetic analysis

Sequencing of 16S rRNA gene of bacterial strains was done and the universal primers used were; 8f [5′AGAGTTTGATCCTGGCTCAG3′], and 1542r [5′AAGGAGGTGATCCAGCCGCA3′] (Lane, 1991). Sanger sequencing was carried out by ABI 3700/373061 sequencer with the mentioned primers. The facility of sequencing was available at South Campus, University of Delhi.

## Phylogenetic analysis

With the help of software Sequencing Analysis 5.1.1 which does base calling and manual assembly of raw data obtained from Sanger sequencing the 16S rRNA gene sequence was assembled. The closest neighbors of the required strains were determined by various web servers like GenBank (Altschul et al., 1990), EzTaxon-e Server (Kim et al., 2012) and RDP II (Maidak et al., 2001) Alignment of sequences of 16SrRNA genes was performed by CLUSTAL X alignment software (Thompson et al., 1997) and at 5´ and 3´ ends the gaps were removed consecutively. Phylogenetic analysis was performed with MEGA version 5.2.2 (Tamura et al., 2011) using the neighbour-joining method (Jukes & Cantor, 1969) with bootstrap analyses as 1000 replications. Maximum-likelihood method (Goldman, 1990) validated the tree topology previously obtained.

## PHENOTYPIC AND BIOCHEMICAL CHARACTERIZATION

### Morphological features

The colony size, colour, form, elevation and type of the margin were observed by streaking the bacterial culture on LB agar and incubating the plates at 28°C for 48 h.

**Gram-staining**

Gram staining was performed using HiMedia's Gram stain kit. A thin smear of culture was made, air dried and fixed by gentle heating. The smear was flooded with crystal violet (primary stain) for one minute and then rinsed off with water. To this Gram's iodine (mordant) was added and after one minute rinsed off with water. Smear was washed with 95% ethanol (decolorizer) until all the violet color was removed from the smear. Smear was again rinsed with the water for 5 seconds and safranin (the counter stain) was applied for one minute. Finally slide was washed with water, air dried and watched under light microscope at 100X with immersion oil. Blue-violet color of the cells indicated Gram-positive reaction while red colored cells showed negative one.

**Catalase test**

Few drops of 3% hydrogen peroxide were added on a clean glass slide which was then inoculated with bacterial colonies with the help of loop. Production of air bubbles indicated positive test (McCarthy and Cross, 1984).

**Oxidase test**

A smear of bacterial culture in log phase was made on paper towels placed inside Petri plates and flooded with few drop of freshly prepared oxidase reagent (N, N', N'-tetramethyl-p-phenylenediamine dihydrochloride). Oxidase-positive organisms produced a purple or dark purple color within 20 seconds.

**Urease production test**

A loopful of bacterium was inoculated in the urea broth and incubated at desired temperature. Change in the color of culture broth with bacterial growth was observed. Pinkish red color of culture broth indicated positive test while colorless reaction indicated negative test (Christensen, 1946).

**Nitrate reductase test**

Bacterium was inoculated in 10 ml nitrate broth in 20 ml capacity test tubes and incubated at desired temperature. After 24 h of growth added 5.0 drop of sulphanilic acid and $\alpha$-naphthylamine respectively with the help of pasture pipette and shaked vigorously in order to mix the regents. Red color of the culture broth indicated positive test while colorless reaction could be negative or positive. Therefore to colorless reaction added a pinch of zinc dust if color of broth turned red then the test was recorded as negative and if colorless it was considered positive.

**Gelatin Utilization**

Nutrient broth containing gelatin (10%) was prepared in test tubes and inoculated with bacterial isolates. The tubes were incubated at 28$^{o}$C and observed for liquefaction. The tubes were then incubated at 4$^{o}$C for 15min and checked again for the liquefaction of the nutrient gelatin media. Retained liquefaction was a positive test (McCarthy and Cross, 1984).

**Tween Hydrolysis**

Tween agar plates were prepared and inoculated with bacterial isolates. Incubated at 28°C and growth was observed to check the release of $Ca^{2+}$ions. An opaque zone formed around the area of the growth indicated a positive test (Collins et al., 1989).

**Aesculin Decomposition**

Tubes with sterile aesculin broth were inoculated with bacterium. The tubes were observed for a black colour development, indicating a positive test (Gordon et al., 1974)

**Starch hydrolysis**

Starch hydrolysis was carried out as described by Cowan and Steel (1965), to examine the hydrolysis of starch to dextrins, maltose and glucose. The nutrient agar media was prepared and autoclaved, to which 2 % starch (autoclaved separately for 5 min) was added and the plates were poured.

**Citrate hydrolysis**

Citrate hydrolysis was performed using Simmons citrate agar (HiMedia, Mumbai, Pvt. Ltd). This test is done to detect the utilization of citrate as sole carbon source for the bacterium. Ammonium dihydrogen phosphate and sodium citrate serve as the sole nitrogen and carbon source respectively. Metabolism of these salts causes the medium to become alkaline, indicated by a change in colour of the pH indicator from green to blue. Bromothymol blue is the pH indicator.The media was autoclaved, poured in petri plates at 45°C and was allowed to solidify.Bacterial culture was streaked across the plates and the plates were incubated at 28°C for 4-5 days. A colour change from green to blue indicated positive test, whereas unchanged colour indicated negative test.

**H2S production**

The H2S production was estimated using Triple Sugar Iron agar (TSI) obtained from HiMedia, Mumbai, Pvt. Ltd. It is a differential media which detects both fermentation and hydrogen sulphide production. TSI contains a pH indicator, peptones, several sugars (for testing fermentation) and sulphur (for testing sulphur reduction that produces hydrogen sulphide gas).

**Antibiotic sensitivity test**

Sensitivity of bacterial strain towards antibiotics was checked on Muellor-Hinton II agar medium using readymade antibiotic discs (HiMedia, Mumbai, Pvt. Ltd.) with varying amount of antibiotics (between 5 to 30 µg per disc). Mueller Hinton II agar

medium (Appendix II) was prepared, autoclaved and theplates were poured. 100 µl of freshly grown bacterial culture was plated on the Mueller Hinton II agar plates. Readymade antibiotic disc with varying amount of antibiotic (between 5 to 30 µg/disc) were placed on the Mueller Hinton II agar plates and incubated in upright positions at 28° C for 2-4 days.Following incubation with appearance of a zone of clearance around the antibiotic disc indicated sensitivity of bacterial strain to the tested antibiotics, while full growth suggested resistance to the antibiotic.

**Urea utilization**

Urease activity was detected as described by Christensen (1946). Urease catalyzes the conversion of urea into ammonia and carbon dioxide. Ammonia produced upon hydrolysis is alkaline, which increases the pH of the medium To confirm the production of urease, strains were inoculated in urea broth (Appendix II) which contains pH indicator, phenol red Urea broth was dispensed in tubes and autoclaved. A 20% stock solution of urea was prepared, filtered (through Millipore filter) and was added to each tube to get a final concentration of 2%. Cultures were inoculated and a tube without urea was kept as negative control. Change in the colour of culture broth was observed. Pinkish colour of culture broth indicated positive test, while colourless reaction indicated negative test.

**Acid production from carbohydrates**

Various carbohydrates tested were Glucose, Arabinose, Maltose, fructose, sorbose, lactose, maltose, galactose, sucrose. To 5.0 ml basal medium in 20 ml test tubes filter sterilized carbohydrates solution to a final concentration of 1% was added. Tubes were inoculated with bacterium and incubated at desired temperature. Positive test for acid production was reported by change in color of the indicator from blue to yellow (Gordon *et al.*, 1974).

# RESULTS AND DISCUSSION

## COLLECTION OF SOIL SAMPLES

The soil samples were collected from landfill located at Mukarwa Chowk, Delhi. The landfill contrary to it's name has outvolumed itself, came out from the underground and has turned into a big hill (Fig1).

The landfill acts as a source of dump of domestic waste collected from households of Delhi. The landfill is quite old and could be a playground for the evolution of new bacterial strains. With this hypothesis in mind we chose this site for soil sample collection and isolation of secondary metabolite mainly antibiotics/antibacterial agents producing bacteria thereafter.

The soil samples were collected from different sites of the landfill at a depth of upto 20 cm under sterile conditions. The collected samples were transported to lab and were immediately processed for further studies.

**Fig 1- Photograph showing landfill at Mukarba Chowk, Delhi from where soil samples were collected**

**Isolation of Bacterial Strains**

From the soil sample collected from Mukarba Chowk, Delhi landfill we tried to isolate different bacteria. The soil samples were serially diluted and plated on Lysogenic Broth (LB) agar plates. Different colonies of varied morphology appeared within 36 h of incubation at 28 °C. (Fig 2).

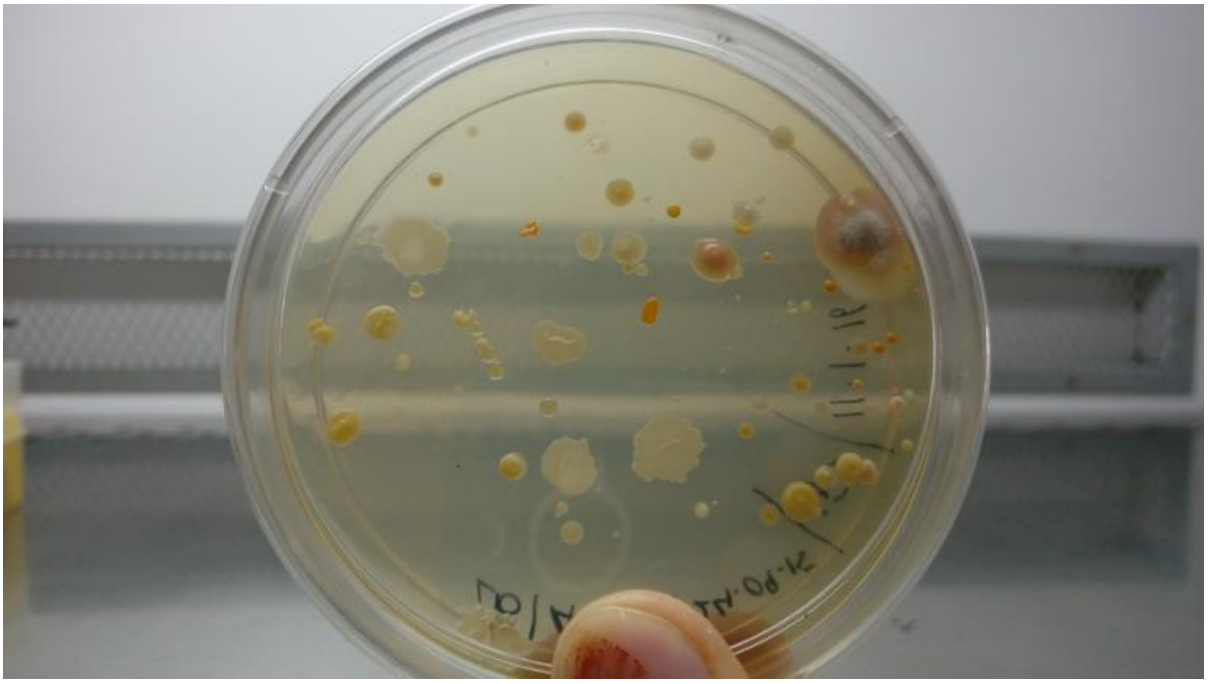

**Fig. 2 CFUs obtained after serial dilution and plating on LB agar media**

The colonies were picked and purified by repeated streaking on LB agar and designated as strains BR1, PK1, PV2, SU1, NW1 and YO1 (Fig 3a, 3b, 3c, 3d, 3e, 3f respectively). The colonies varied in size, shape, color and showed some preliminary indication of secondary metabolites like pigments and surfactant.

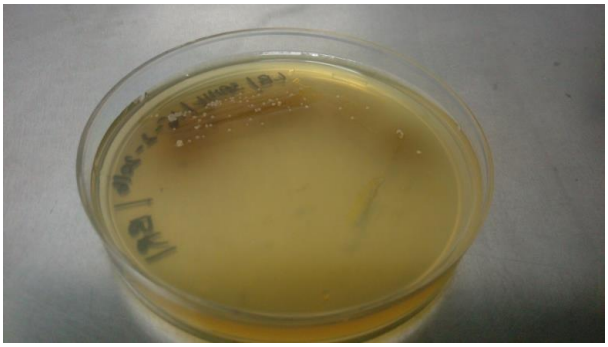

**Fig 3a: Bacterial strain BR1**

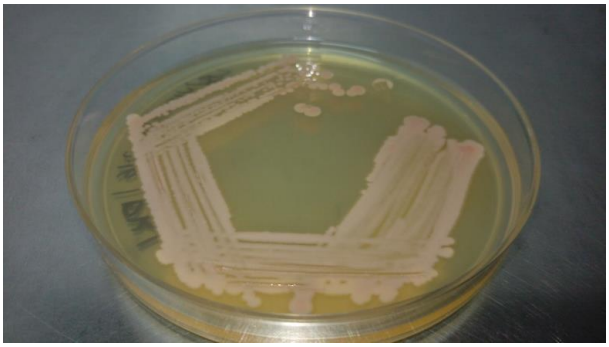

**Fig 3b: Bacterial strain PK1**

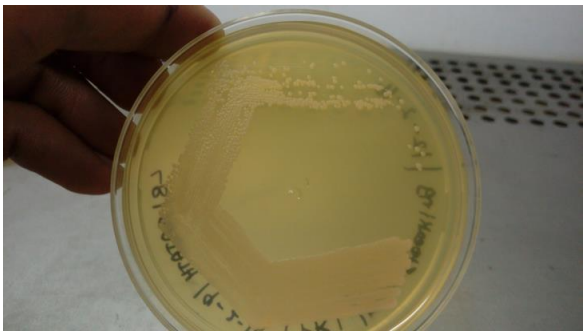

**Fig 3c: Bacterial strain PV2**

**Fig 3d: Bacterial strain SU1**

**Fig 3e: Bacterial strain NW1**

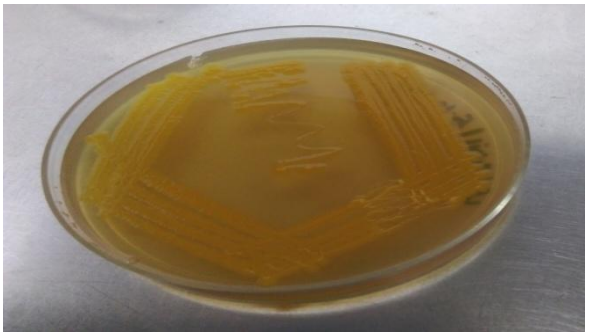

**Fig 3f: Bacterial strain YO1**

**Assay for antibacterial activity**

The isolated strains of various bacteria were then screened for any antibacterial activity. Three strains were used for these purposes E*. coli*, *S. aureus* and *B. subtilis*.

E coli is known to cause gastroenteritis, urinary tract infections, and neonatal meningitis. *B. cereus* is responsible for a minority of foodborne illnesses (2–5%), causing severe nausea, vomiting, and diarrhea. *S. aureus* can cause a range of illnesses, from minor skin infections such as from minor skin infections, such as , and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteremia, and sepsis.

Lawns of these bacteria were made on LB agar which were then inoculated by the isolated strains in the center. After incubation at 28 for 2 days the plates were observed for the clearance zone (halo around the inoculated strain). Two strains PK and YO were found to show positive results.

Strain PK showed visible clearance zone around it against *B. cereus* thus proving it's antibacterial potential against it (Fig 4).

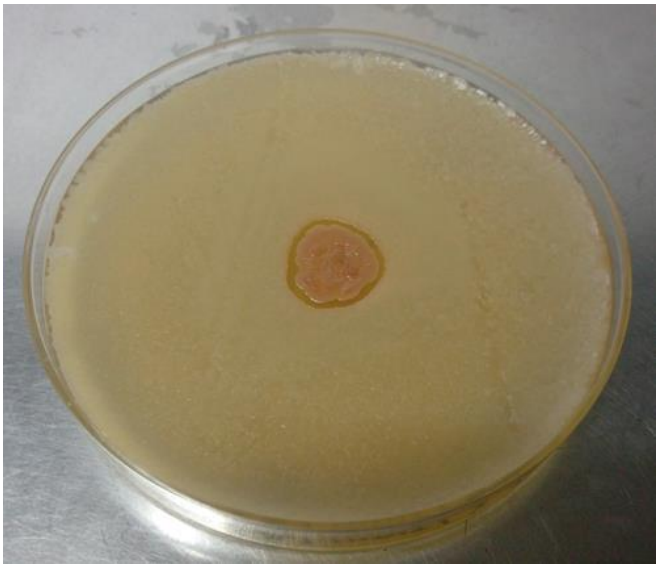

**Fig 4. Bioassay of strain PK1 against *Bacillus cereus* showing zone of inhibition**

Strain YO1 showed antibacterial activity against strain *E. coli* as proved by clearance zone around it against the mentioned strain (Fig 5)

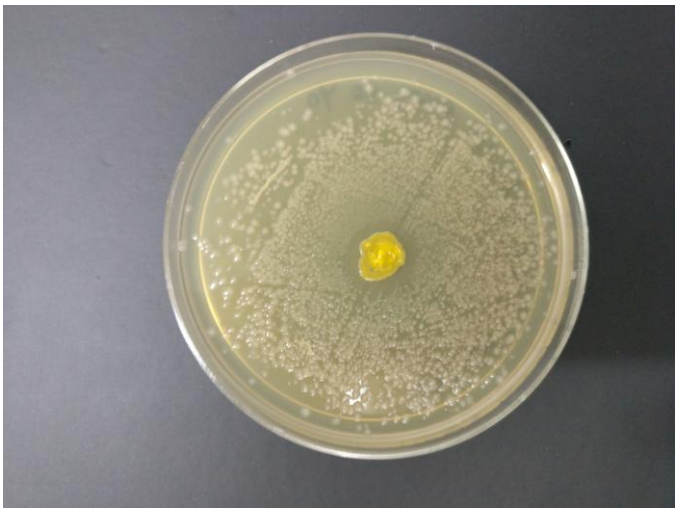

**Fig 5. Bioassay of strain YO1 against *E. coli* showing zone of inhibition**

## GENOTYPIC CHARACTERIZATION OF STRAIN PK1

The gene sequence of 16S rRNA gene of strain PK1 obtained after sanger sequencing was constructed to a stretch of length 1291 bp. Alignment based searches (BLASTN, Evalue X $1e^{-10}$) of 16S rRNA gene of *Bacillus filamentosus* SGD-14(T) was performed against Ribosomal Database (Sonalkar et al. 2015) *Bacillus filamentosus* SGD-14(T) showed closest sequence similarity to *Bacillus endophyticus* 2DT(T) (99.1 %, 1289 bp) (Reva et al. 2002) and Ornithinibacillus contaminans CCUG 53201(T) (96.07 %, 1120bp) (Kämpfer et al. 2010). Rhodococcus rhodochrous DSM43274T was considered an out-group in phylogenetic analysis (Fig 6).

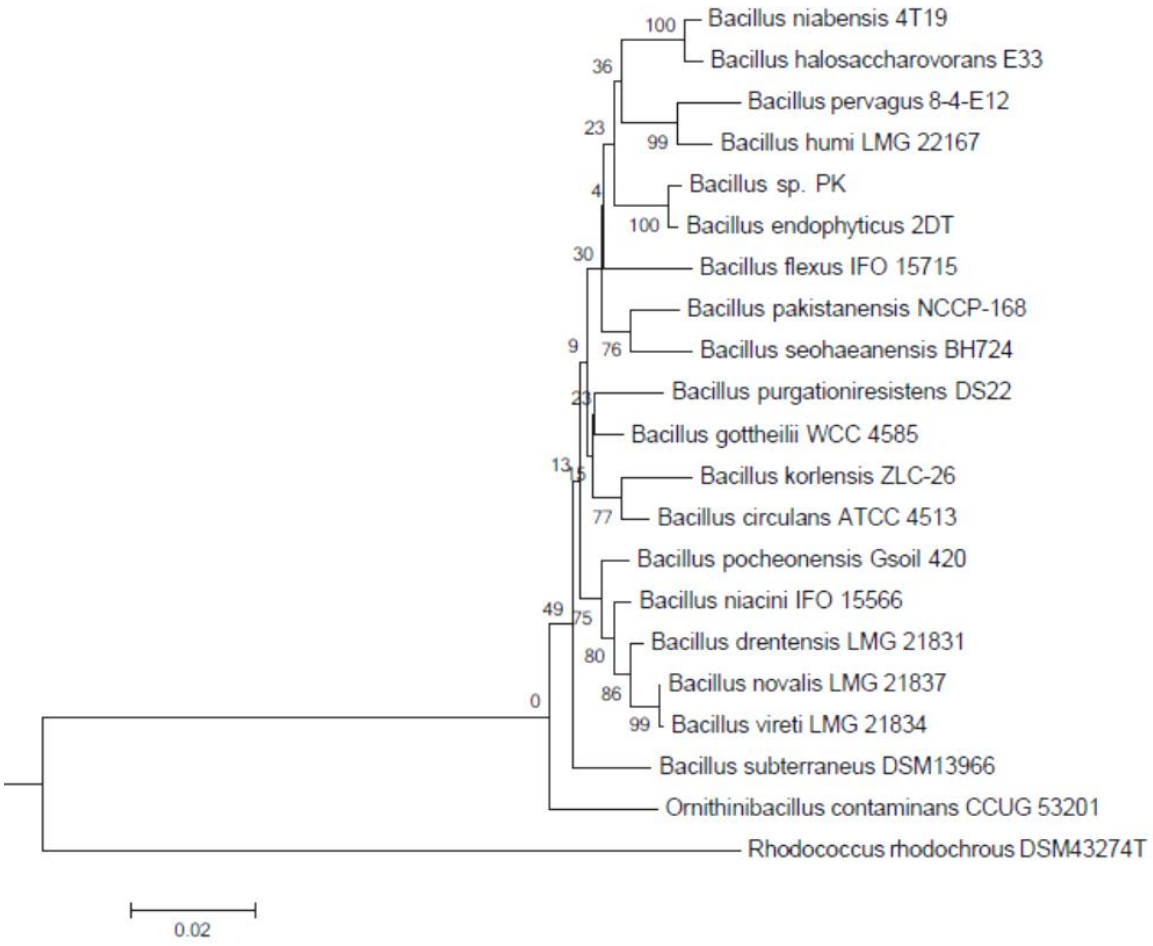


**Fig 6:** Phylogenetic tree of PK1 constructed by software MEGA version 5.2.2 by neighbor-joining method of the conserved marker 16S rRNA genes showing the evolutionary relationship of strain PK1 and its neighbors. Rhodococcus rhodochrous DSM43274T was taken as outgroup of the rooted tree. Bootstrap values fall within 100 replications. Bar 0.02, substitutions/nucleotide position.

## BIOCHEMICAL CHARACTERIZATION OF STRAIN PK1

The colony morphology of strain PK1 was studied on Luria-Bertani agar (LB). Strain PK1 grew well under aerobic conditions on LB $28^0$C within 24-48 h of incubation. PK1 colonies were pink colored, entire, smooth, circular and varied in size Gram staining revealed the strain PK1 to be Gram positive. Susceptibility towards antibiotics was determined which revealed that strain PK1 was sensitive to penicillin

**Table 3 : Distinguishing phenotypic characters of *Bacillus sp* PK1 compared with closest neighbours.**

| ***CHARACTER*** | ***B. filamentosus sp SGD-14$^T$*** | ***B. endophyticus 2DT$^T$*** | ***Bacillus sp PK1$^T$*** |
|---|---|---|---|
| **Hydrolysis of :** | | | |
| Gelatin | ND | -ve | -ve |
| Starch (Amylase) | ND | -ve | -ve |
| Aesculin | -ve | ND | +ve |
| **Utilisation of :** | | | |
| Citrate | +ve | +ve | +ve |
| Tween | ND | ND | -ve |
| **Acid from :** | | | |
| Arabinose L(+) | +ve | +ve | -ve |
| Fructose D (-) | ND | ND | -ve |
| Galactose D (+) | ND | -ve | -ve |
| Glucose D (+) | +ve | +ve | +ve |
| Lactose | -ve | -ve | -ve |
| Maltose D (+) | ND | +ve | +ve |
| Sorbose L (-) | ND | ND | -ve |
| Sucrose D (+) | -ve | ND | -ve |
| Nitrate Reduction | -ve | -ve | +ve |
| H2S Production | -ve | ND | -ve |

ND- No Data

Data for *B. filamentosus sp SGD-14$^T$ (Sonalkar et al. 2015) and B. endophyticus 2DT$^T$ (Reva et al. 2002)* was taken from literature cited.

but resistant to vancomycin, tetracycline, rifampicin, ciprofloxacin, chloroamphenicol, nalidixic acid and polymixin B and kanamycin. Further characterization of strain PK1 was carried out using several biochemical and

physiological tests and the characteristics differentiating it from closely related members of the genus *Bacillus* are given in Table 3.

Based on the comparison of phenotypic, chemotaxonomic and phylogenetic analysis, with closely related members of *Bacillus* and it can be proposed that strain PK1 represents a novel strain of the genus *Bacillus* and species *filamentosus* for which the name *Bacillus filamentosus* PK1 is proposed.

## GENOTYPIC CHARACTERIZATION OF STRAIN YO 1

The gene sequence of 16S rRNA gene of strain YO1 obtained after sanger sequencing was of length 1291 bp. Alignment based searches (BLASTN, Evalue X $1e^{-10}$) of 16S rRNA gene of *Pseudomonas parafulva* NBRC 16636(T) was performed against Ribosomal Database (Uchino et al. 2002). *Pseudomonas parafulva* NBRC 16636(T) showed closest sequence similarity to *Pseudomonas fulva* NBRC 16637(T) (99.84 %, 1282 bp) (Iizuka and Komagata. 1963) and *Pseudomonas taiwanensis* BCRC 17751(T) (99.14%, 1282bp) (Wang et al. 2010). *Bacillus filamentosus* was considered an out-group in phylogenetic analysis (Fig 7).

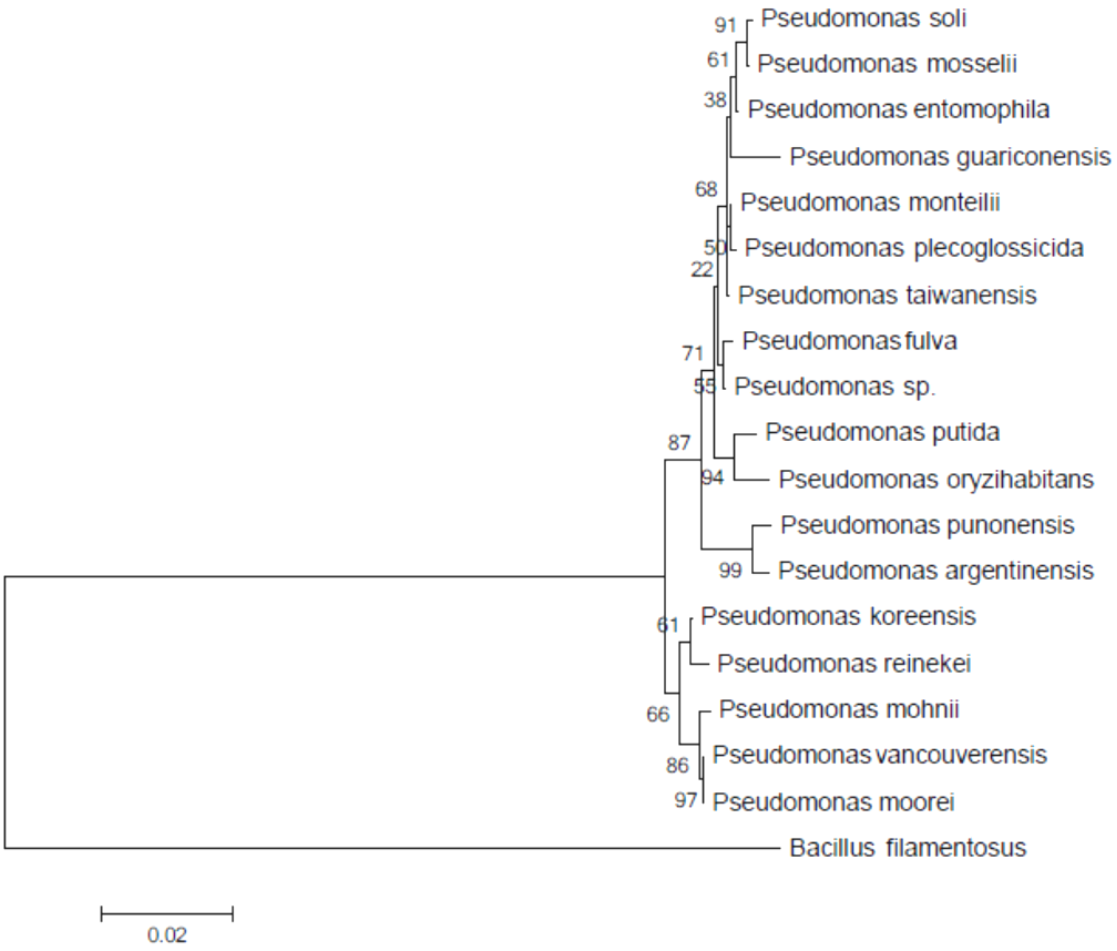


**Fig 7:** Phylogenetic tree of YO1 constructed by software MEGA version 5.2.2 by neighbor-joining method of the conserved marker 16S rRNA genes showing the evolutionary relationship of strain YO1 and its neighbors. *Bacillus filamentosus* was taken as outgroup of the rooted tree. Bootstrap values fall within 100 replications. Bar 0.02, substitutions/nucleotide position.

## BIOCHEMICAL CHARACTERIZATION OF STRAIN YO1

The colony morphology of strain YO1 was studied on Luria-Bertani agar (LB). Strain YO1 grew well under aerobic conditions on LB $28^0$C within 24-48 h of incubation. YO1 colonies were yellow colored, entire, smooth, circular and varied in size Gram staining revealed the strain YO1 to be Gram negative.

**Table 4 : Distinguishing phenotypic characters of *Peudomonas sp* YO1 compared with closest neighbours.**

| ***CHARACTER*** | ***Pseudomonas fulva* NBRC 16637(T)** | ***Pseudomonas taiwanensis* BCRC 17751(T)** | ***Peudomonas sp* YO1** |
|---|---|---|---|
| **Hydrolysis of :** | | | |
| Gelatin | ND | -ve | -ve |
| Starch (Amylase) | -ve | ND | -ve |
| Aesculin | ND | -ve | -ve |
| **Utilisation of :** | | | |
| Citrate | +ve | +ve | +ve |
| Tween | +ve | +ve | +ve |
| **Acid from :** | | | |
| Arabinose L(+) | +ve | +ve | -ve |
| Fructose D (-) | +ve | +ve | -ve |
| Galactose D (+) | -ve | ND | +ve |
| Glucose D (+) | +ve | +ve | +ve |
| Lactose | -ve | ND | -ve |
| Maltose D (+) | -ve | ND | -ve |
| Sorbose L (-) | ND | ND | -ve |
| Sucrose D (+) | -ve | ND | -ve |
| Nitrate Reduction | -ve | -ve | +ve |
| H2S Production | ND | ND | -ve |

ND- no data

Data for *Pseudomonas fulva* NBRC 16637(T) *B. filamentosus sp SGD-14$^T$ (*Iizuka and Komagata. 1963*) and B. endophyticus 2DT$^T$ (*Wang et al. 2010*)* was taken from literature cited.

Further characterization of strain YO1 was carried out using several biochemical and physiological tests and the characteristics differentiating it from closely related members of the genus *Pseudomonas* are given in Table 4.

Based on the comparison of phenotypic, chemotaxonomic and phylogenetic analysis, with closely related members of *Pesudomonas* and it can be proposed that strain YO1 represents a novel strain of the genus *Pseudomonas* and species *parafulva* for which the name *Pseudomonas parafulva* YO1 is proposed.

# INNOVATIONS SHOWN BY PROJECT

- The landfill in Mukarba Chowk has lost it's function. It should be closed immediately. The garbage lying above should be converted into manure. A new landfill should be started at an appropriate place.

- Landfill has proven to be a site of immense bacterial diversity and can be explored further for a wide range of secondary metabolites.

- A new strain of bacteria *Bacillus filamentosus* PK1 has ben isolated which shows antibacterial activity against *B. cereus* strain.

- A new strain of bacteria *Pseudomonas parafulva* YO1 has ben isolated which shows antibacterial activity against *E.coli* strain.

# CONCLUSION AND FUTURE DIRECTION

New strains of bacteria *Bacillus filamentosus* PK1 and *Pseudomonas parafulva* YO1 which produces antibacterial agents has been isolated in this project. In future the antibacterial agent can be characterized and the biosynthetic cluster of genes responsible for it’s synthesis can be identified by genome sequencing and comparative genomics.

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# CERTIFICATES OF PARTICIPATION IN WORKSHOP

Certificate of Participation

This certificate is awarded to

Sahil Yadav

for attending

**Workshop on Microbial Genomics and Metagenomics**

Held on September 22, 2016

At Department of Zoology, University of Delhi, Delhi

Dr. Amit Ghosh
Chairperson, IUMS

Prof. Neeta Sehgal
Head, Department of Zoology

Prof. Rup Lal
Organizing Secretary, IUMS

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## Workshop on Microbial Genomics and Metagenomics

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Dr. Amit Ghosh
Chairperson, IUMS

Prof. Neeta Sehgal
Head, Department of Zoology

Prof. Rup Lal
Organizing Secretary, IUMS

**DISSERTATION THESIS REPORT**

**ON**

***"Finding friends in foes: Screening local weeds for potential hyperaccumulation activity "***

**Submitted in partial fulfilment for the award of degree of**
**Master of Science in Biosciences**
**(2015-2017)**

By


**Tandrali Baruah**

**Under the guidance of :**

**Dr Vartika Mathur (External Guide)**
Department of Zoology,
Sri Venkateswara College , South Campus, New Delhi
**And**

**Dr Nadim Ahmad (Internal Guide)**
Department of Biosciences, Faculty of Natural Science ,
Jamia Milia Islamia , New Delhi -110025

# Study of Selected Tree Species and Their Associated Micro-Flora from Polluted and Less Polluted Sites in Delhi

A DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE AWARD OF DEGREE OF

MASTER OF SCIENCE

IN

ENVIRONMENTAL SCIENCE


Submitted By:

RAHUL

16/MES/014

*Under the supervision of*

Prof. Anuradha Mishra

Professor, Department of Applied Chemistry

School of Vocational Studies & Applied Sciences

Gautam Buddha University

*Under co-supervision of*

Dr. Vartika Mathur

Assistant Professor,

Department of Zoology

Sri Venkateswara College

University of Delhi




DEPARTMENT OF ENVIRONMENTAL SCIENCE

SCHOOL OF VOCATIONAL STUDIES AND APPLIED

SCIENCES

GAUTAM BUDDHA UNIVERSITY

GREATER NOIDA (U.P.)


May, 2018

# INTEGRATING CONCEPTS IN MICROBIAL DIVERSITY, BIOINFORMATICS AND PHYSIOLOGY

A Training Project Under Sri Venkateswara Programme for Research and Innovative Academics (SRI-VIPRA)

**BY:**
**Anushka Saxena**
**Third Year**
**Bachelor of Science**
**Zoology (Hons.)**

**Mentor**
Dr.Richa Misra

# DECLARATION

This is to certify that **ANUSHKA SAXENA**, student of **SRI VENKATESWARA COLLEGE** has successfully completed her training titled " **, INTEGRATING CONCEPTS IN MICROBIAL DIVERSITY**, **BIOINFORMATICS AND PHYSIOLOGY**" organised under Sri Venkateswara Programme for Research and Innovative Academics **(SRI-VIPRA**) under my guidance and supervision**.**

**Dr. RICHA MISRA**

**Assistant Professor**

**Department of Zoology**

**Sri Venkateswara College**

**(20.07.2019)**

# INDEX

SRI VENKATESWARA PROGRAMME FOR RESEARCH AND INNOVATIVE ACADEMICS

Summer Training Programme - 2019

# Exploring Microbiome: Integrating Concepts in Microbial Diversity, Bioinformatics and Physiology

Project Mentor
Dr. Richa Misra
(Department of Zoology)

Submitted by: Shiva Satija
Course: BSc. Life Sciences, III year

# Acknowledgement

I would like to show my gratitude to our project mentor Dr. Richa Misra, whose constant encouragement motivated me throughout, I am extremely thankful to ma'am for sharing her pearls of knowledge and wisdom with us during the course of this training.

I would also like to acknowledge the help provided at the laboratory of Dr.Yogendra singh, Department of Zoology, University of Delhi, who allowed us to use resources and instrumentation facility and the PhD scholars of his lab, Mohita ma'am, Vishal sir and Arushi ma'am for helping us to perform experiments in the laboratory and Anoop sir for training us to carry out metagenomic analysis.

I am extremely thankful to lab staff Partha sir and Nandu sir who permitted us to use all required instruments and essential resources from the lab.

I am immensely grateful to my team mates, Anushka and Priyanshi for their constant help and suggestions throughout the training.

# SRI VENKATESWARA COLLEGE

## SRI VENKATESWARA PROGRAMME

## FOR RESEARCH AND INNOVATIVE ACADEMICS

**Summer Training Programme - 2019**

**TOPIC- EXPLORING MICROBIOME: INTEGRATING CONCEPT IN MICROBIAL DIVERSITY, BIOINFORMATICS AND PHYSIOLOGY**

**PROJECT MENTOR: Dr. RICHA MISRA**

**Submitted by: PRIYANSHI SHARMA**
B.Sc. (H) Biological Sciences
(Second year)

# DECLARATION

This is to certify that **PRIYANSHI SHARMA**, student of **SRI VENKATESWARA COLLEGE** has successfully completed her training titled " **, INTEGRATING CONCEPTS IN MICROBIAL DIVERSITY**, **BIOINFORMATICS AND PHYSIOLOGY**" organized under Sri Venkateswara Programme for Research and Innovative Academics **(SRI-VIPRA**) under my guidance and supervision.

**Dr. RICHA MISRA**

Assistant Professor
Department of Zoology
Sri Venkateswara College

## ACKNOWLEDGMENT

Primarily I would like to thank God for being able to complete this training successfully. Then I would like to thank my project mentor Dr. Richa Misra, whose help, stimulating suggestions and encouragement, helped me to throughout especially in writing this report.

I would also like to acknowledge with much appreciation the help provided at the laboratory of
Dr. Yogendra Singh, Department of Zoology, University of Delhi, who allowed us to use resources and instrumental facility and Ph.D. students of his lab, Anoop Sir for training us in metagenomic analysis and Mohita ma'am , Vishal Sir and Arushi ma'am for giving their precious time to help us perform practical in laboratory.

I would like to show my gratitude of thanks to college lab staff Partha Sir and Nandu Sir for their help throughout the training part.

Special thanks go to my team mates, Anushka and Shiva who help me to assemble the parts and gave suggestions.

# CONTENT:

# Analysis of Endolichenic Communities associated with fruticose lichen "*Evernia prunastri*"

**Dissertation project work submitted in partial fulfilment for the award of degree in Master of Science in Microbiology**

By

**Manisha Gyawali**
Enrollment No- 18-05367
Batch 2018-2020

**Jamia Millia Islamia, Central University**
**New Delhi-110025**

**Under the supervision of**

**Dr. Vartika Mathur (External supervisor)**
Department of Zoology
Sri Venkateswera college, University of Delhi

**And**

**Dr. Syed Akhtar (Internal Supervisor)**
Department of Biosciences
Jamia Millia Islamia, New Delhi

**TEACHER'S ACTIVITY REPORT 2020-2021**

**FACULTY:** Science **DEPARTMENT/ COMMITTEE :** Zoology **IQAC ACTIVITY No:** SVC/2020-21/ZOO/PJ/1

**NAME OF THE ACTIVITY: student centric Project- entitled "ESTABLISHMENT OF PATIENT-DERIVED XENOGRAFTS OF RETINOBLASTOMA & CHOROIDAL MELANOMA ON THE AVIAN CHORIOALLANTOIC MEMBRANE"**

| DATE | FACULTY | DEPARTMENT/COMMITTEE | COORDINATOR NAME |
|---|---|---|---|
| from 11.02.2021 to 02.08.2021. | Science | Department of Zoology | **Dr. P.Jayaraj** |
| **TIME** | **VENUE** | **NUMBER OF PARTICIPANTS** | **NATURE: Outdoor/Indoor** |
| | Sri Venkateswara college and All Institute of Medical Science , New Delhi | 3 | Indoor |
| **SUPPORT/ASSISTANCE:** | Sri Venkateswara College university of Delhi | | |

BRIEF INFORMATION ABOUT THE ACTIVITY : **CRITERIA : 2,5**

| | |
|---|---|
| TOPIC/SUBJECT OF THE ACTIVITY | Hands on student centric Project- entitled "ESTABLISHMENT OF PATIENT-DERIVED XENOGRAFTS OF RETINOBLASTOMA & CHOROIDAL MELANOMA ON THE AVIAN CHORIOALLANTOIC MEMBRANE" |
| OBJECTIVES | Hands on research in the field of cancer biology and chick embryo development |
| METHODOLOGY | Students were taken to AIIMS, New Delhi for a hands on research on tumor tissue analysis and chick embryo development |
| OUTCOMES | 2 students from B.Sc (H) Zoology and one from B.Sc Life Sciences got an opportunity to work in the above mentioned project |

**PROOFS & DOCUMENTS ATTACHED (Tick mark the proofs attached):**

| | | | | |
|---|---|---|---|---|
| Notice & Letters✓ | **Student list of participation ✓** | **Activity report✓** | **Photos✓** | Feedback form |
| **Feedback analysis** | News clip with details | Certificate | Any other | |

| | | |
|---|---|---|
| IQAC Document No: | Criterion No: | Metric No: |
| Departmental file no | IQAC file No; | |

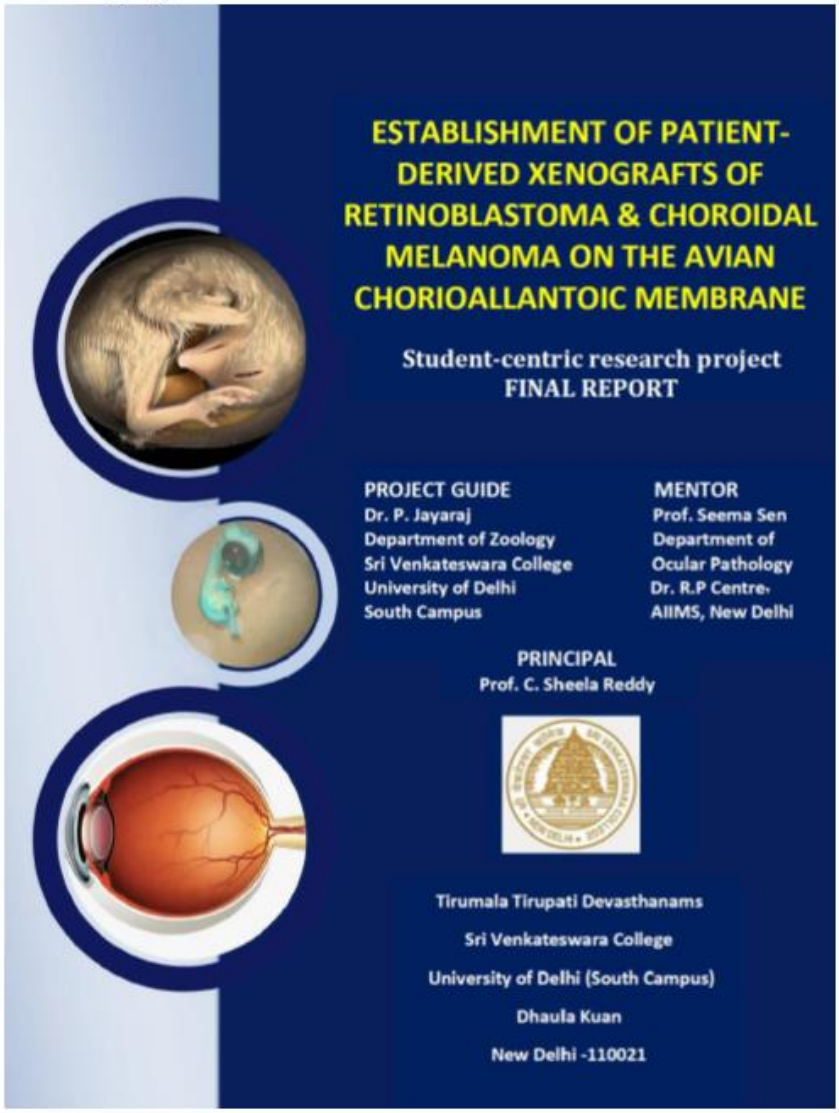
| NAME OF TEACHER & SIGNATURE | NAME OF HEAD/ COMMITTEE INCHARGE & SIGNATURE | IQAC COORDINATOR (SEAL & SIGNATURE) |
|---|---|---|
| **Dr. P.Jayaraj** | **Dr. P.Jayaraj** | |

For Reference

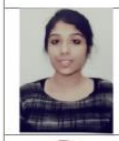
| | | | | |
|---|---|---|---|---|
| Criterion I | Curricular Aspects (planning & Implementation) | | Criterion V | Student Support & Progression |
| Criterion II | Teaching Learning & Evaluation | | Criterion VI | Governance |
| Criterion III | Research, Innovations & Extension | | Criterion VII | Institutional Values & Best Practices |
| Criterion IV | Learning Resources and Infrastructure | | | |

# Proofs

## Cover page:



**List of Enrolled students**

| Picture | Name of the Student | Class | Roll No. | Signature |
|---|---|---|---|---|
|  | Palak | B.Sc. (H) Zoology II Yr. | 2019026 | |
|  | Muskaan Gupta | B.Sc. (H) Zoology II Yr | 2019035 | |
|  | Shefali Dahiya | B.Sc. Life Science III Yr. | 1118098 | |

**Tirumala Tirupati Devasthanams**
**Sri Venkateswara College**
**University Of Delhi (South Campus)**
**Dhaula Kuan**
**New Delhi -110021**

**PROJECT COMPLETION REPORT APPROVAL CERTIFICATE**

This is to certify that the following students were involved in the student centric Project- entitled "**ESTABLISHMENT OF PATIENT-DERIVED XENOGRAFTS OF RETINOBLASTOMA & CHOROIDAL MELANOMA ON THE AVIAN CHORIOALLANTOIC MEMBRANE**" and have carried out original research work at Department of Zoology, Sri Venkateswara College, Delhi University and at Department of ocular pathology, Dr. R.P. Centre AIIMS, New Delhi from 11.02.2021 to 02.08.2021. The work and the report are original and is submitted as final Report to Sri Venkateswara College, University of Delhi.

**Project Guide:**

**Dr. P. Jayaraj**
Assistant Prof.
Department of Zoology
Sri Venkateswara College
University of Delhi

**Mentor:**

**Prof. Seema Sen**
Department of
ocular pathology
Dr. R.P. Centre,
AIIMS, New Delhi

**Principal**
**Prof. C. Sheela Reddy**
Sri Venkateswara College
University of Delhi

Students performing CAM assya to study invasive properties of cancer cells

# "Sequential induced responses in tomato (*Lycopersicum esculentum)*"

Thesis submitted for partial fulfilment of

**Degree of Master in Biochemistry**

2015-2017


Submitted by:

**Komal**

Roll No.: 15MBC0010

Enrolment No-15-1739

Under the supervision of

**Prof. Luqman Ahmad Khan**

Department of Biosciences

Jamia Millia Islamia

New Delhi-110025

Co Supervisor

**Dr. Vartika Mathur**

Department of Zoology

Sri Venkateswara College

University of Delhi

New Delhi -21