



ISOLATION AND IDENTIFICATION OF BACTERIAL POPULATION FROM VARIOUS SOIL SAMPLES

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ABSTRACT

The soil is one of the main reservoirs of microbial life. Typical garden soil has millions of bacteria in each gram. The most numerous microbes in soil are bacteria. Soil bacteria include aerobes and anaerobes with a wide range of nutritional requirements, from photoautotrophs to chemoheterotrophs. As usable nutrients and suitable environmental conditions (such as light, aeration, temperature) become available, the microbial populations and their metabolic activity rapidly increase until the nutrients are depleted or physical conditions change, and then they return to lower levels.

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INTRODUCTION

The soils is one of the main reservoirs of microbial life. Typical garden soil has millions of bacteria in each gram. The most numerous microbes in soil are bacteria. Soil bacteria include aerobes and anaerobes with a wide range of nutritional requirements, from photoautotrophs to chemoheterotrophs. As usable nutrients and suitable environmental conditions (such as light, aeration, temperature) become available, the microbial populations and their metabolic activity rapidly increase until the nutrients are depleted or physical conditions change, and then they return to lower levels. Human pathogens, with the exception of endospore-forming bacteria, are uncommon in the soil. Soil microorganisms are responsible for recycling elements so they can be used over and over again. The numbers of bacteria and fungi in soil are usually estimated by the plate count method.

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The actual number of organisms is probably much higher than the estimate, however, because a plate count only detects microbes that will grow under the conditions provided (such as nutrients and temperature),

Study Area

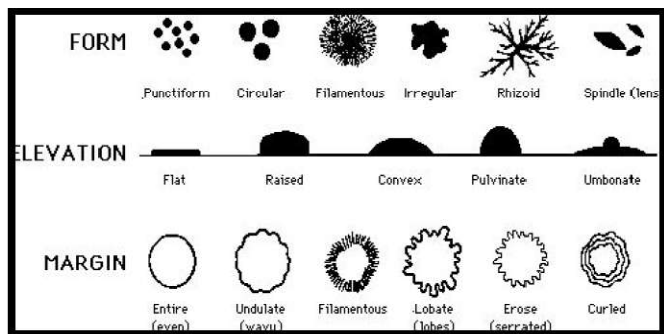
Neemuch being a developing Industrial town. It has a longitude 23.40-24.80 East and Latitude 74.20-75.50 North is situated in North Western part of Madhya Pradesh popularly known as malva region. The approximate urban area of Neemuch is 1075 km² and its population is 1.25 lakh. From the geographical and government point of view Neemuch acquires an important position. Neemuch the whole city is spreaded over three regions namely Baghana, Chhawani and City. The Alkaloid and Opium Factory, factory was founded in 1993. In 1996, it began extracting alkaloids in addition to processing opium. It is one of the largest producers of opium in the world. It is also very large producer of oil seeds. The study where it carried out is four different regions of Neemuch. These four regions mainly known as Bhaghana, Bholiyawas, Rawatkhedra

and Gwaltoli Talab. Baghana place comes under neemuch district. Its geographical situation for latitude is 24° 27' 27" North and for longitude is 74°50' 59" East. Baghana is near of Railway station of neemuch municipal treching ground of solid waste. Bholiawas are collateral with neemuch. It is near of M.P.E.B. substation. Bholiawas is a place where solid waste of Neemuch city comes for dumping by Municipal Corporation without any proper land filling and treatment. Rawatkhedha and Gwaltoli are two another region of Neemuch where different types of waste by the Muncpal Corporation thrown out without any proper and prior treatment.

MATERIALS AND METHODS

The method for isolation and identification of bacteria were based on morphological, microscopic and biochemical characteristics. And these characteristics carried out by different standard methods according different standard protocols. The methods which used for the study purpose are given below

Morphology Characterization: Bacteria grow on solid media as colonies. A colony is defined as a visible mass of microorganisms all originating from a single mother cell; therefore a colony constitutes a clone of bacteria all genetically alike. In the identification of bacteria and fungi much weight is placed on how the organism grows in or on media. This help to identify the cultural characteristics of a bacterium on agar plate called colony morphology. Although one might not necessarily see the importance of colonial morphology at first, it really can be important when identifying the bacterium. Features of the colonies may help to pinpoint the identity of the bacterium. Different species of bacteria can produce very different colonies.



Microscopic identification by gram's staining

Microorganisms were characterized on the basis of microscopic characteristics.

Gram Staining: The gram stain, a differential stain was developed by Dr. Hans Christian Gram in 1884 that is why named Gram staining. Gram staining (or Gram's Method) is an empirical method of staining differentiating bacterial species into two large group (Gram positive and Gram negative) based on the chemicals, primarily the presence of high levels of peptidoglycan, and physical properties of their cell walls.

Reagent used

- Crystal violet (primary stain)
- Gram's Iodine (mordant that fixed the crystal violet to the cell wall)

- Decolorizer (e.g. ethanol)
- Saffranin (counter stain)

Biochemical Identification of the bacterial isolates: This was done with VITEK 2 compact technology. The VITEK 2 is an automated microbial identification system that provides highly accurate and reproducible results as shown in multiple independent studies. With its colorimetric reagent cards, and associated hardware and software advances, the VITEK 2 offers a stateofheart technology platform for phenotypic identification methods.



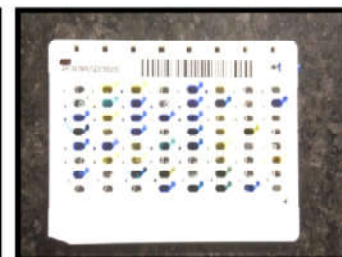
Vitek-2 compact- Bacterial identification Instrument



Vitek-2 compact- Bacterial inoculum loading



Vitek-2 compact- Bacterial identification



Vitek-2 compact- Bacterial identification card

RESULT AND DISCUSSION

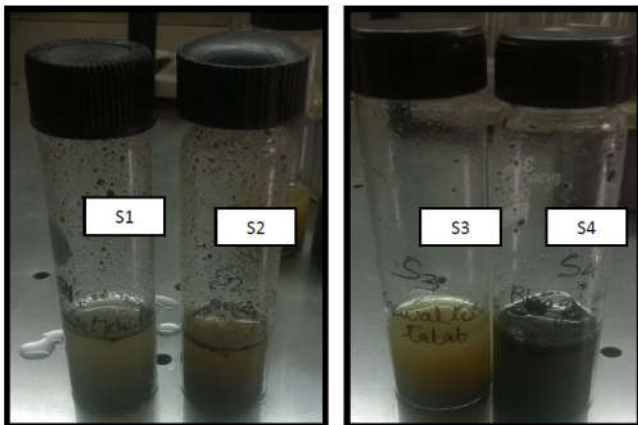
Eleven isolates were obtained for microbiological characterization from various soil samples and this was notified that all the eleven isolates are rarest in the environment with specific characteristics. The six isolates were from same genera of *Staphylococcus* (*S. haemolyticus*, (*S. lentus* - obtained two times from two different study area), *S. arlettae*, *S. aureus*, *S. sciuri*) and other was like *Kocuria kristinae*, *Kocuria rosea* and (*Bacillus altitudinis* – this also obtained two times from two different study area). Staphylococci have the ability to tolerate high salt concentration (Kloos and Lambe, 1991). Members of the genus *Staphylococcus* are catalase positive and oxidase negative. The catalase test differentiates Staphylococci from Streptococci. These genera also differ in the composition of their cell walls. Pathogenic Staphylococci such as *S. aureus* can generally be identified by their ability to produce coagulase enzyme. The coagulase negative strains of *Staphylococcus* genus (CoNS) are commensals or saprophytic but some of them can cause opportunistic infections (Murray et al., 2002). *M. luteus* has been shown to survive in oligotrophic environments for extended periods of time. Recent work by Greenblatt et al. demonstrate that *Micrococcus luteus* has survived for at least 34,000 to 170,000 years on the basis of 16S rRNA analysis, and possibly much longer.

Kocuria kristinae is found widespread in nature, frequently as normal skin flora on humans and other mammals. It is usually non-pathogenic. There are very few documented cases with infections caused by *Kocuria kristinae*. It was previously classified into the genus *Micrococcus*, but was dissected from *Micrococcus* based on phylogenetic and chemotaxonomic analysis. It has been reclassified in the new genus *Kocuria* along with *K.rosea*, *K. varians*, *K. palustris* and *K. rhizophila*. *Kocuria kristinae* is a facultative anaerobic, nonmotile, gram positive coccus occurring in irregular clusters and tetrads.

STEP 1 : Processing of the Soil samples

Four different sites of soil samples details are following

- Sample 1: RawatKheda Soil Sample
- Sample 2: Bholiyawas Soil Sample
- Sample 3: Gwal Toli talab Soil sample
- Sample 4: Bhaghana



STEP 2 : Preparation of Soil Samples in Saline



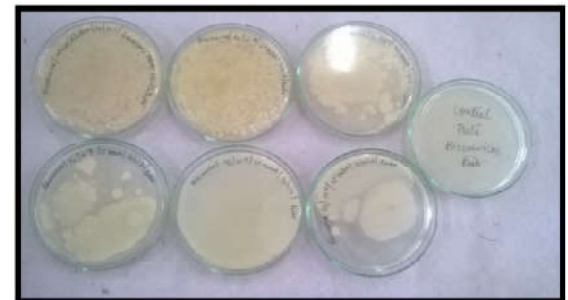
STEP 3 : Preparation of Soil dilution (Serial Dilution of soil sample)



Rawatkhedha Serial Dilution Plate 10¹-10⁶



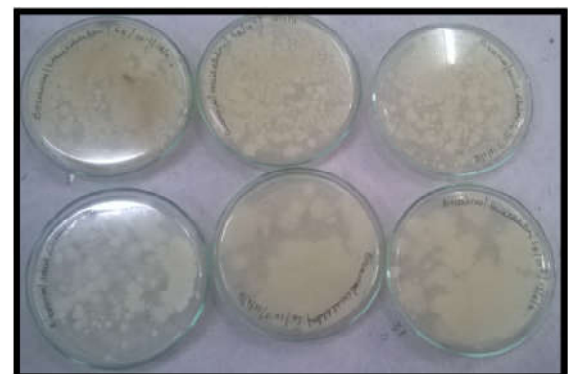
Bholiyawas Dilution Plate 10¹ - 10⁶



Toli Serial Dilution Plate 10¹-10⁶



Bhaghana Serial Dilution Plate 10¹-10⁶



Mix Sample Serial Dilution Plate 10¹-10⁶

Colony Calculation

Table 1. Sample S1Rawat Kheda Soil Sample

S. no	Microorganism	Dilution	Colony no/ per plate.
1.	Bacteria	10^{-1}	TNTC
2		10^{-2}	TNTC
3		10^{-3}	TNTC
4		10^{-4}	84
5		10^{-5}	22
6		10^{-6}	13

Table 2. Sample 2Bholiyawas Soil Sample

S. no	Microorganism	Dilution	Colony no/ per plate.
1.	Bacteria	10^{-1}	TNTC
2		10^{-2}	TNTC
3		10^{-3}	TNTC
4		10^{-4}	06
5		10^{-5}	05
6		10^{-6}	11

Table 3. Sample 3 Gwal Toli Soil Sample

S. no	Microorganism	Dilution	Colony no/ per plate.
1.	Bacteria	10^{-1}	TNTC
2		10^{-2}	TNTC
3		10^{-3}	TNTC
4		10^{-4}	19
5		10^{-5}	No growth Observed
6		10^{-6}	03

Table 4. Sample 4Bhaghana

S. no	Microorganism	Dilution	Colony no/ per plate.
1.	Bacteria	10^{-1}	TNTC
2		10^{-2}	TNTC
3		10^{-3}	TNTC
4		10^{-4}	TNTC
5		10^{-5}	12
6		10^{-6}	10

Table 5. Sample 5 Compost (Mix Culture)

S. no	Microorganism	Dilution	Colony no/ per plate.
1.	Bacteria	10^{-1}	TNTC
2		10^{-2}	TNTC
3		10^{-3}	TNTC
4		10^{-4}	TNTC
5		10^{-5}	10
6		10^{-6}	11

TNTC - Too numerous to count

Table 6. Sample 1: Morphological Characterization

S. no	Reference no.	Isolate no.	Media	Shape	Elevation	Color	Margin	Surface
1.	Sample S1	IS-S1-A	Nutrient Agar	Circular	Flat	Creamish yellow	Entire	Smooth glistening
2	Sample S1	IS-S1-B	Nutrient Agar	Punctiform	Flat	Creamish	Entire	Smooth glistening
3	Sample S1	IS-S1-C	Nutrient Agar	Punctiform	Flat	White	Entire	Smooth

Table 7. Sample 2

S. no	Reference no.	Isolate no.	Media	Shape	Elevation	Color	Margin	Surface
4.	Sample S2	IS-S2-A	Nutrient Agar	Filamentous	Flat	Yellow	Lobate	Smooth
5	Sample S2	IS-S2-B	Nutrient Agar	Circular	Flat	Light cream	Entire	Smooth
6	Sample S2	IS-S2-C	Nutrient Agar	Circular	Flat	Cream	Entire	Smooth

Table 8. Sample 3

S. no	Reference no.	Isolate no.	Media	Shape	Elevation	Color	Margin	Surface
7	Sample S3	IS-S3-A	Nutrient Agar	Circular	Flat	Yellow	Lobate	Smooth
8	Sample S3	IS-S3-B	Nutrient Agar	Irregular	Flat	Light cream	Entire	Smooth

Table 9. Sample 4

S. no	Reference no.	Isolate no.	Media	Shape	Elevation	Color	Margin	Surface
9	Sample S4	IS-S4-A	Nutrient Agar	Irregular	Flat	Yellow	Undulate	Smooth

Table 10. Sample 5

S. no	Reference no.	Isolate no.	Media	Shape	Elevation	Color	Margin	Surface
10	Sample S5	IS-S5-A	Nutrient Agar	Circular	Flat	Yellow	Lobate	Smooth
11	Sample S5	IS-S5-B	Nutrient Agar	Circular	Flat	Orange	Lobate	Smooth

Table 11. Microscopic identification by Grams Staining

Isolate	Gram Stain	Shape
S1A	Gram Positive	Cocci in cluster
S1B	Gram Positive	Bacilli
S1C	Gram Positive	Cocci in pair
S2A	Gram Positive	Bacilli
S2B	Gram Positive	Bacilli
S2C	Gram Positive	Bacilli
S3A	Gram Positive	Bacilli
S3B	Gram Positive	Bacilli
S4A	Gram Positive	Bacilli
S5A	Gram Positive	Cocci in pairs
S5B	Gram Positive	Cocci in cluster

Isolate Number: S4A

Selected Organism : Unidentified Organism
Source: _____ Collected: _____

Comments: _____

Identification Information		Analysis Time:	Status:
Selected Organism		6.00 hours	Final
ID Analysis Messages		Unidentified Organism 03201003443431	

Biochemical Details																	
2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	-	9	BGAL	-	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	18	PHOS	-
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	(+)	26	PyrA	-	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	-	32	POLYB	-	37	BGAL	-
38	βRIB	-	39	ILATk	-	42	LAC	-	44	NAG	-	45	βMAL	-	46	RACI	-
47	NOVO	-	50	NC6.5	-	52	dMAN	-	53	dMNE	-	54	MBaG	-	56	PUL	-
57	dRAF	-	58	O129R	-	59	SAL	-	60	SAC	-	62	dTRE	-	63	ADH2s	-
64	OPTO	-			-			-			-			-			-

Isolate Number: S5A

Selected Organism : Kocuria rosea
Source: _____ Collected: _____

Comments: _____

Identification Information		Analysis Time:	Status:
Selected Organism		8.00 hours	Final
ID Analysis Messages		97% Probability Kocuria rosea 00003010000000	

Biochemical Details																	
2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	-	9	BGAL	-	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	18	PHOS	-
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	-	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	-	32	POLYB	-	37	BGAL	-
38	βRIB	-	39	ILATk	-	42	LAC	-	44	NAG	-	45	βMAL	-	46	RACI	-
47	NOVO	-	50	NC6.5	-	52	dMAN	-	53	dMNE	-	54	MBaG	-	56	PUL	-
57	dRAF	-	58	O129R	-	59	SAL	-	60	SAC	-	62	dTRE	-	63	ADH2s	-
64	OPTO	-			-			-			-			-			-

Isolate Number: S5B

Selected Organism : Micrococcus luteus/lylae
Source: _____ Collected: _____

Comments: _____

Identification Information		Analysis Time:	Status:
Selected Organism		8.00 hours	Final
ID Analysis Messages		96% Probability Micrococcus luteus/lylae 04103230000000	

Biochemical Details																	
2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	-	9	BGAL	-	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	18	PHOS	-
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	-	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	-	32	POLYB	-	37	BGAL	-
38	βRIB	-	39	ILATk	-	42	LAC	-	44	NAG	-	45	βMAL	-	46	RACI	-
47	NOVO	-	50	NC6.5	-	52	dMAN	-	53	dMNE	-	54	MBaG	-	56	PUL	-
57	dRAF	-	58	O129R	-	59	SAL	-	60	SAC	-	62	dTRE	-	63	ADH2s	-
64	OPTO	-			-			-			-			-			-

Conclusion

There are several aspects of the isolation and identification of bacteria. The bacteria play both positive and negative role due to presence of their in which habitat they survive. Many species of bacteria are useful from the environment and their other beneficiary point of view but sometimes they are silent killer which cause serious diseases in human body as well as in animal.

From the beginning evolutionary journey of bacteria rather now a day's scientists have much sophisticated and exact tools and techniques to emphasis more hidden peculiarities about the bacteria. It is now in the human hand how he deals with this scenario to take more advantages to go ahead with advance stage in favour of whole flora and fauna of the world.

Further Scope: *Staphylococcus* species are normal flora widespread over the body surface. They are also important pathogens. Many species of *Staphylococcus* have the ability to form biofilms which can then colonize structures such as medical catheters, stents, heart valves, prostheses, shunts, and valves. The clinically significant species are generally separated into coagulase-positive staphs (*S. aureus*) and coagulase-negative (CoNS) staphs (*S. epidermidis*, *S. haemolyticus*, and *S. saprophyticus*).

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