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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 endospor e-formed 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

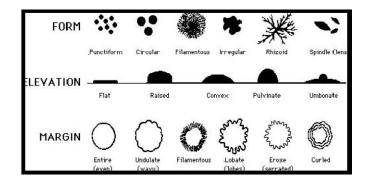
Neemuch being a developing Industrial town. . It has a longitude 23.40-24.80 East and Latitude 74.20-75.50 North is situated in North Western part of Madhya Pradesh popularly known as malva region. The approximate urban area of Neemuch is 1075 km² and its population is 1.25 lakh. From the geographical and government point of view Neemuch acquires an important position. Neemuch the whole city is spreaded over three regions namely Baghana, Chhawni 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, Rawatkheda

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. Rawatkheda and Gwaltoli are two another region of Neemuch where different types of waste by the Muncipal 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 platecalled 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.

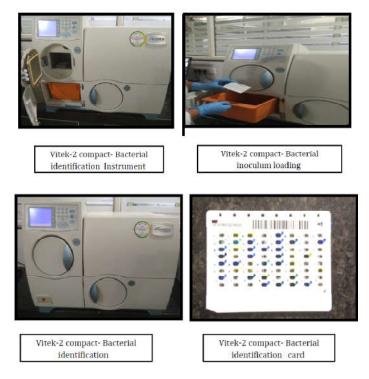
Gram Staining: The gram stain, a differential stain was developed by Dr. Hans Christian Gram in 1884 that is why named Gram staining. Gram staining (or Gram's Method) is an empirical method of staining differentiating bacterial species into two large group (Grampositive and Gram negative) based on the 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 stateoftheart technology platform for phenotypic identification methods.



RESULT AND DISCUSSION

for microbiological Eleven isolates were obtained 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.

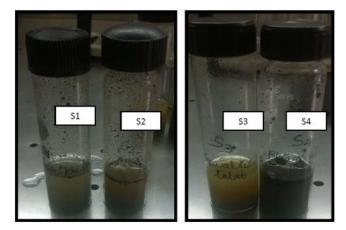
Kocuria kristinae is found widespread in nature, frequently as normal skin flora on humans and other mammals. It is usually non-pathogenic. There are very few documented cases with infections caused by *Kocuria kristinae*. It was previously classified into the genus Micrococcus, but was dissected from 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

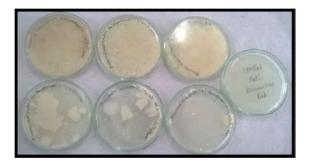




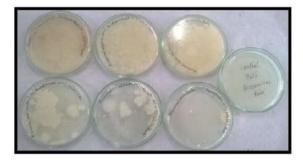
STEP 2 : Preparation of Soil Samples in Saline



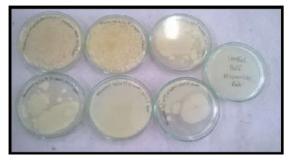
STEP 3 : Preparation of Soil dilution (Serial Dilution of soil sample)



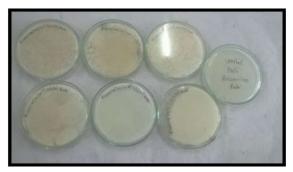
Rawatkheda Serial Dilution Plate 101-106



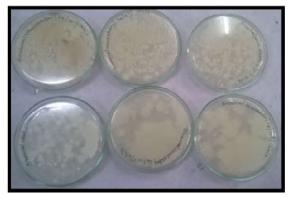
Bholiyawas Dilution Plate 10⁻¹ - 10⁻⁶



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.		10 ⁻¹	TNTC
2		10-2	TNTC
3	Bacteria	10-3	TNTC
4	Dacteria	104	84
5		10-5	22
6		10-8	13

Table 2. Sample 2Bholiyawas Soil Sample

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

Table 4. Sample 4Bhaghana

S. no	Microorganism	Dilution	Colony no/ per plate.			
1.		10-1	TNTC			
2		10-2	TNTC			
3	Bacteria	10-3	TNTC			
4		104	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.		10 ⁻¹	TNTC			
2		10-2	TNTC			
3	Bactería	10-3	TNTC			
4		104	TNTC			
5		103	10			
6		10.0	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	15 S2 B	Nutrient Agar	Circular	1-lat	Light cream	Latire	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.	Reference	Isolate	Media	Shape	Elevation	Color	Margin	Surface
no	no.	no.						
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	Circular	Flat	Orange	Lobate	Smooth

Table 11. Microscopic identification by Grams Staining

Isolate	Gram Stain	Shape
S1A	Gram Positive	Cooci 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

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2	APPA	+	14	CDEX		15	AspA		16	BCAR	-	17	AMAN	-	19	PHUS	-
	PALERAY.		100	ProA		24	BGURr		25	AGAL	+	26	PvrA	-	27	BGUR	1.
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13 20 28	LeuA AliaA		23	TyrA	+	30	dSOR	+	31	URE		132			37	(GGB)	
13 20 28 38	LeuA AlaA dRIB	+ + +	29 39		+	30 42	dSOR LAC	+	31	URE	+	32	dMAL	•	37	dGAL BACI	•
2 13 20 28 38 47 57	LeuA AliaA		29	TyrA	+ +			+ + +	-	and the second se	-	-		+ +	37 46	dGAL BACI PUL	•

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13	APPA		14	CDEX		15	AspA	-	16	BGAR		17	AMAN		19	PHOS	
20	LeuA		23	ProA	-	24	BGURr	-	25	AGAL	+	26	PyrA	+	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR		31	URE		32	POLYB	-	37	dGAL	-
38	dRiB		39	ILATE	+	42	LAG-	-	44	NAG		45	dMAL	+	46	BAGI	t
47	NOVO		50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	
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20	LEIA		23	ProA		24	BGURr		25	AGAL		26	PyrA		27	BGUR	
28	AlaA	+	20	TyrA	-	30	dSOR		31	UR-		32	POLYB	-	37	UGAL	
38	dRIB	+	39	ILATE		42	LAC	+	44	NAG	-	45	dMAL	+	46	BACI	
47	NOVO	+	6C	NC6.5	+	52	dMAN	+	53	dMNE		54	MBdG		56	PUL	
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13	APPA	-	14	CDEX	-	15	AspA		16	BGAR	+	17	AMAN	+	19	PHOS	-
20	LeuA	+	23	ProA	-	24	BGURr		25	AGAL	+	28	PyrA	+	27	BOUR	
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2	ANY	-	4	PIPLC		5	dXYL		8	ADH1	-	3	BGAL	+	111	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA		18	BGAR	+	17	AMAN	+	19	PHOS	-
20	LeuA		23	ProA	-	24	BGURr	-	25	AGAL	+	28	PyrA	+	27	BGUR	-
28	AicA.	-	29	TyrA	+	30	dSOR	-	31	URE	-	32	POLYB	1	37	dGAL	+
38	dRIB	(+)	39	ILATK	1	42	LAC	-	44	NAG	-	45	dMAL	+	48	BACI	+
47	NOVO	-	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	-
57	SRAF		58	0129R		59	SAL	+	60	SAC	+	62	dTRE	+	63	ADH2s	-
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20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA		29	TyrA	-	30	dSOR		31	URE		32	POLYB	+	37	eGAL	-
38	dRIB	+	39	ILATE	+	42	LAC		44	NAG	+	45	dMAL.	+	46	BACI	-
	NOVO		50	NC6.5		52	dMAN		53	dNNE	-	54	MBdG	+	56	PUL	+
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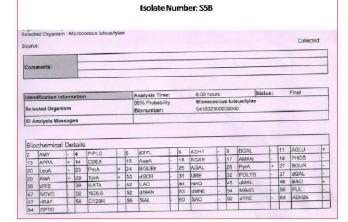
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20	LeuA.		23	ProA	-	24	BGUR	-	25	AGAL		26	PyrA	-	27	BGUR	¢.
28	AlaA	1	29	TyrA	-	30	dSOR.		31	URE	1	32	POLYB	-	37	dGAL	-
38	dRIB	+	39	ILATK	+	42	LAC	-	44	NAG	-	45	dMAL.	-	46	BACI	-
47	NOVO	1	80	NC6.5	+	52	dMAN.	+	53	dMNE		54	MBdG	-	56	PUL	+
	BRAT	1	58	01295	+	59	SAL		60	SAC		62	TRE	-	63	ADH2s	
57.	OPTO													14			

							Isolate	Nu	mb	er: S3E	5						
iele iour		6m : 1	Staph	ylococcus i	entus											Collec	led.
Cor	nments:							_	_					_			
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Sel	ected Orga	nism					97% Probe Bionumbe					0000	us lentus 631				
10/	Analysis Me	essag	985		_	_		-									_
Bid	chemica	al De	tails			-			-	_	-	-	_				
2	AMY	+	4	PIPLO	F	5	BXYE	-	8	ADH1	+	9	BGAL	+	11	AGLU	+
13	APPA	ŀ	14	CDEX	-	15	AspA	-	16	BOAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	-	23	ProA		24	BGURT	-	25	AGAL	+	26	РутА	+	27	BGUR	-
28	AlaA	-	28	TyrA	-	30	dSOR	+	31	URE	-	32	POLYB	-	37	dGAL	-
38	dRIB	+	39	LATK	+	42	LAC	-	44	NAG	+	45	dMAL.	+	46	BACI	+
47	NOVO	-	60	NC6.6	+	62	dMAN	+	63	dMNE	+	54	MBdC	+	56	PUL	-
57	dRAF	-	58	0129R	+	59	SAL	+	60	SAC	+	62	OTRE	+	63	ADH2s	-
64	OFTO	+							1			1					

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47	MOVO	-	58	0129R	-	52	SAL		53 60	dMNE SAC	+	54 62	MBdG	+	63	ADH2s	-
64	OPTO	,															
Selec	cted Organ	ism :	Каси	ria rosea			Isolat	e Ni	umi	ber: 55/	A						

lder	tification I	inform	natio	a			Analysis	Tim	e 1		8.001	ours		Statu	1981	Final	
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2	AMY		4	PIPLO	-	5	dXYL.	-	8	ADH1	ŀ	19	BGAL	-	11	AGLU	1
13	APPA	-	14	CDEX	-	15	AspA	+	16	BGAR		17	AMAN		19	PHOS	1
20	LeuA	+	23	ProA	+	24	BGURr	1	25	AGAL	-	26	PyrA	-	27	BOUR	1
28	AlaA	+	29	TytA	+	30	d3OR	-	31	URE		32	POLYB	-	37	dGAL	-
38	dRIB	12	39	ILATE	1	42	LAG	-	44	NAG	-	45	dMAL.	-	46	BACI	-
47	NOVO	-	50	NC6.5	-	52	dMAN	-	53	dMNE		54	MBdG	-	56	PUL	1
	dRAF		58	0129R	-	59	SAL	-	60	SAC	-	62	DITRE		63	ADH2s	
57																	



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 coagulasepositive staphs (*S. aureus*) and coagulase-negative (CoNS) staphs (*S. epidermidis, S. haemolyticus*, and *S. saprophyticus*).

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