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ISOLATION AND CHARACTERIZATION OF BACTERIA WITH BIOCHEMICAL IMPORTANCE FROM SOIL SAMPLES OF RANCHI CITY, INDIA

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***Key words**: Soil bacteria, Isolation, Antibacterial activity*

Abstract–In the present study soil samples from Ranchi municipality area was used for isolation and characterization of bacteria having biochemical potential and pharmacological importance. Total twenty five soil samples were collected from fish, vegetables and fruits dump area from Ranchi town. Bacterial population was sub-cultured in Trypticase Soya Agar (TSA) Plate. Fifteen colonies were isolated, cultured and characterized by Gram staining and Biochemical tests. Five isolates were found to be gram negative while ten were gram positive. All isolates were positive in oxidase, catalase, citrate, and protease tests. Eight isolates showed coagulase negative and seven were coagulase positive. The intrinsic levels of resistance to antibiotics Chloramphenicol, Kanamycin, Streptomycin, Ampicillin, Tetracycline and Nalidixic acid were studied and resistance pattern showed, all the bacterial isolate were found to be resistant to Kanamycin, Streptomycin and Ampicillin till the concentration of 200 µg/mL. of the three isolate, showed maximum resistance 200 µg/mL to all the antibiotics tested, three isolate could grow only up to 50 µg/mL in Chloramphenicol. The two can be further differentiated from each other in their resistance to Nalidixic acid and Tetracycline. It was found that all bacterial isolates were sensitive to Tetracycline, Chloramphenicol, Gentamycin, Ciprofloxacin, Azithromycin and Ceftriaxone.

INTRODUCTION

Finding strategies against the development of antibiotic resistance is a major global challenge for the life sciences community and for public health. The past decades have seen a dramatic worldwide increase in human-pathogenic bacteria that are resistant to one or multiple antibiotics. More infections caused by resistant microorganisms fail to respond to conventional treatment, and even last-resort antibiotics have lost their power. In addition, industry pipelines for the development of novel antibiotics have run dry over the past few decades.

Microbial population in soil counts for a huge mass of organic matter on earth. Importance of microorganism in maintaining human habitat on earth is now beyond a question of discussion. These microorganisms are of very diverse that includes Bacteria, Archaea, Yeast, Fungi, Algae, and Protozoa (Braga *et al*., 2016).

Microorganisms can live in environment along with human and in extreme conditions such hot springs, miles deep in the ocean, inside rocks and in extreme cold temperature (Hongmei *et al*., 2005). Although the number of microorganisms varies in different places, it has been indicated that mass of carbon from these microorganisms could be trillions of tons (Eilers *et al*., 2012; Angelov, 2008; Brock and Madigan, 1991). Bacterial population in soil performs key roles in nutrient cycles including Nitrosomonas and Nitrobacter (nitrification), Thiobacillus (sulfur and iron oxidation), Rhizobium and Frankia (N2 fixation), *Bacillus* and *Clostridium* (carbon cycling) and *Caulobacter* and *Pseudomonas* (manganese oxidation) (Makhalanyane *et al*., 2015).

In addition, a large number of bacteria have been discovered to produce various types of chemicals that are being exploited in biotechnology industries (Smith, 1989; Bulock, 1987; Vandemme, 1948). Most of the bacteria in soil remain unidentified. Both academic and industrial scientists realized that soil bacteria are a potential source to find unique

biologically active metabolites and novel commercially important products. Bacteria have been found as a source of producing many valuable chemicals including ethanol, acetone, enzymes, perfumes, antibiotics. In the last few decades thousands of antibiotics have been discovered (Saha and Santra, 2014).

Badosa *et al*. (2004) Antibiotic resistance is a great challenge to modern medicine and demands discovery of novel antibiotics (Ball *et al*., 2004; Hancock, 2007). Microorganisms were found of producing secondary metabolites with a diverse chemical structure and antimicrobial activities (Stachelhaus *et al*., 1995; Drablos *et al*., 1999). In recent years prevalence of multiple-antibiotic resistance strains have been encountered by clinicians all over the world. These findings raise a high possibility to identify bacterial strains from soil samples that might produce antibiotics resistance pattern.

The numbers and species of microbes in soil is depended on environmental conditions like nutrient availability, soil texture, presence of moisture in soil and type of vegetation cover, and other environmental conditions (Brakstad *et al*., 2015). Among many other commercially important enzymes, proteases are another large group of industrial enzymes with significant industrial use being isolated from microorganisms (Nurullah, *et al*., 2011). Nutrient content in soil varies from area to area. A high content of nutrient is likely to be soil where organic waste is dumped. Accordingly, soil samples of organic dump site will have varieties of nutrient in higher amount. Bacteria will favorably grow in such soil samples and historically preferable sites for sample collection for isolation of novel bacteria (Louis *et al*., 2016).

MATERIALS AND METHODS

Chemicals and reagents : Trypticase soy broth (Hi-Media, India), Trypticase soy agar (Hi-Media, India), Muller-Hinton agar (Hi-Media, India), Luria Bertani broth (Hi-Media, India), Kligler's iron agar (Hi-Media, India), hydrogen peroxide, N, N, N1 N1- tetra-methyl-P-phenyldiamine-dihydrochloride (sigma), simmons citrate agar (Hi-Media, India), commercial antibiotic disc (Hi-Media, India).

Sample collection : Soil samples were collected from fish, vegetables and fruits dumped area during april-july, 2018 from Ranchi City.

Bacterial culture : Five gram of each soil sample was suspended in 25 mL of TSB. Bacterial suspension was diluted (10^{-4}) with saline water and 100 μL of bacterial suspension was spreaded on TSA plate and incubated for 24 hours. Bacterial colonies were isolated and grown in TSB. Bacteria were characterized by biochemical analysis as described below.

Gram staining : Gram staining was performed for all isolated colonies according to the standard procedure. A smear of bacterial cells was prepared on a clean glass slide by a gentle heat fixation. The heat fixed smear was flooded with crystal violet solution for one minute. Smear was washed with water followed by adding mordant Gram's iodine. The smear was decolorized with 95% ethyl alcohol and rinsed with water. Finally safranin was used as counter stains for 60-80 sec and washed with water. Cells were then examined under microscope.

Catalase test : A drop of 3% hydrogen peroxide was added to a bacterial colony on a sterile glass slide and mixed well. Production of air bubble was observed for a minute. Production of air bubble indicated catalase positive and no bubble indicated catalase negative.

Coagulase test : All isolates were tested for coagulase test using human plasma serum. Two drops of saline water was taken onto the slide and mixed with the bacterial sample. A drop of serum was added on the saline drop and mixed well. The slide was rocked gently for about 10 seconds. Positive test was indicated by clumping of bacterial cells in the plasma within 10 seconds. Failing to form bacterial cells clump indicates a negative result for coagulase test.

Oxidase test : Oxidase test was performed with 1% solution of N, N, N^1N^1-Tetra methyl-p-phenyldiamine-dihydrochloride which was socked in a piece of filter paper. A portion of the colony of the test organism was picked up with a sterile tooth pick and touched on to the paper with impregnated reagent. A dark purple color development within 5-10 second was considered positive and no change of color was interpreted as a negative result for oxidase.

Kilger's iron agar (KIA) test : All bacterial isolates were tested for KIA test to study the mode of dextrose utilization in oxidative/fermentative test. Slant of KIA media were inoculated by stabbing the butt and streaking the slant and incubated at 37 °C for 24hours. Results were recorded for changing in color of the butt or slant, H_2S or other gas

production. Production of hydrogen sulphide causes change of color of the medium to black and the gas production give rise to bubble formation

Simmons citrate test : Isolates were tested to determine the utilization of citrate as the sole source of carbon for metabolism. Tubes of citrate media were inoculated by streaking the slant with bacteria and incubated at 37 °C for 24 hours. Results were recorded for change in color of citrate media.

Protease test : The protease activity was performed using LB agar plate containing 1% skim milk. A small portion of bacterial colony was inoculated as spot agar plate and incubated for 24 hours at 37 °C. Protease producing ability of each bacterial inoculum was assessed by hydrolyzing milk protein surrounding the colony and producing a transparent clear zone. The clear zones around the colonies indicated protease activity.

Intrinsic antibiotic resistance (IAR) was studied. The eight antibiotics used for the study and the solvent used for preparing them are listed in Table. Stock solutions (10mg/mL) of the antibiotics were prepared in appropriate slovent (Table 1). They were filtered, sterilized and stored at 4 °C. Agar based medium was prepared by dispensing 50 mL in 100 mL conical flasks and autoclaved at 15lbs psi. Medium was cooled at 50-60 °C and appropriate concentration of antibiotics was added. Ampicillin, Kanamycin, Naladixic acid Streptomycin were added at concentration 25, 50, 125, 150 and 200 µg/mL where as Tetracyclin was tested at 5, 10, 15, and 25 µg/mL (Table 2). Two plates were pooled for each concentration and solidify. Bacterial isolates strains were spot inoculated on the antibiotic plates and control plates were mentioned without antibiotics. Inoculated plates were incubated for 24 hrs at 37 °C for first observation and further 24 hrs for next observation. After incubation, the growth on antibiotics plate was compared with control plates. Growth was scored as positive or negative concentration or untill which the growth occured was taken as the resistance limit.

Table 1. Antibiotics and their solvents

Antibiotics	Solvent
Ampicillin	Distilled Water
Kanamycin	Distilled Water
Nalidixic acid	Distilled Water + NaOH
Streptomycin	Distilled Water
Tetracycline	50% ethanol

RESULTS AND DISCUSSION

Some of the microbes produce enzymes and chemicals that are highly useful for human. Food waste dumps decompose to organic material providing abundant nutrient supply for microbial growth. Growth of different microorganisms in rotten food on soil depends on the type of waste food dump. Due to warm temperature in Ranchi it is likely that food dump area is an excellent environment for bacterial growth. In this report we have studied bacterial population in different food waste dumping area of Ranchi city. Soil samples were collected from fish and vegetable waste

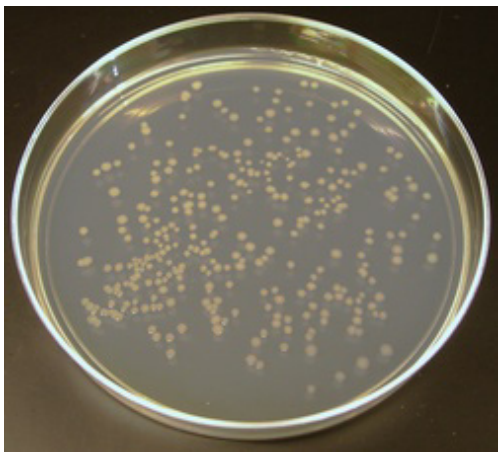

Fig. 1. Single colony of soil bacteria isolated in TSA plate

Table 2. Interinsic Antibiotics Resistance Pattern

	Kanamycin	Streptomycin	Ampicillin	Naladixic acid	tetracyclin
A1,A4,A5	200	200	200	200	100
A6,A7	150	150	150	100	25
A2,A8	150	150	150	150	100
A3,A10	200	200	NG	50	NG
A3	NG	50	200	200	15
A11,A12	NG	NG	50	NG	NG
A9,A15	NG	50	200	200	15
A13,A14	NG	50	200	200	15

dumping area of Ranchi city. Bacterial colonies were identified based on their color and morphology (Table 3, Figure 1). A total of 15 single colonies were picked up and cultured in TSB for further analysis. In order to characterize bacterial isolates, gram staining and biochemical tests were performed. It was found that 79% isolates were gram positive while 26% were gram negative (Table 1). In microscopic analysis, some bacteria were of round shape and some were rod shape (data not shown). The results obtained in this study is consistent with the previous studies in other countries (Barlaz *et al*., 1989; Song *et al*., 2015). These soil microorganisms may be an important source of producing chemicals having biochemical and pharmacological importance. Although many useful chemicals have

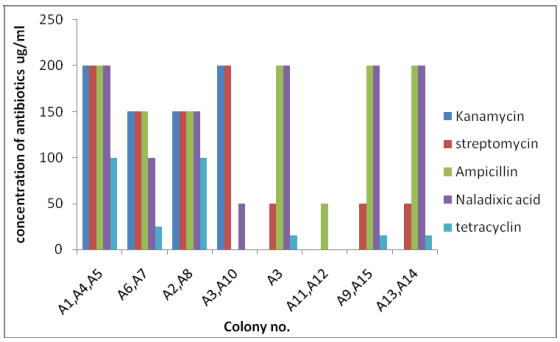

Fig. 2. Interinsic Antibiotics Resistance Pattern

Table 3. Morphological study on TSA plate and gram staining

Number of soil bacterial isolate	Color of colony	Characterisation of colony	Gram staining
A1	Greenish	Irregular, large, transparent	+
A2	Greenish	Irregular, large, transparent	+
A3	Geenish	Irregular, large, transparent	+
A4	Greenish	Irregular, large, transparent	+
A5	Copper-copper	Round shaped, small, transparent	+
A6	Copper-copper	Round shaped, small, transparent	+
A7	Greenish	Irregular, transparent, large	+
A8	Greenish	Irregular, large, transparent	+
A9	Greenish	Irregular, large, transparent	-
A10	Greenish	Irregular, large, transparent	-
A11	Greenish	Irregular, large, transparent	+
A12	Greenish	Irregular, large, transparent	-
A13	Greenish	Irregular, large, transparent	-
A14	Greenish	Irregular, large, transparent	+
A15	Greenish	Irregular, large, transparent	-

Table 4. Biochemical analysis of bacterial isolates from soil samples.

Isolate No	Biochemical test					
	Oxidase	Catalase	Coagulase	S. Citrate	KIA (Butt/Slant)	Protease
A1	+	+	-	+	acid/acid	+
A2	+	+	-	+	alkaline/alkaline	+
A3	+	+	-	+	acid/acid	+
A4	+	+	-	+	acid/alkaline (H_2S)	+
A5	+	+	-	+	acid/acid	+
A6	+	+	-	+	alkaline/alkaline (H_2S)	+
A7	+	+	-	+	acid/acid	+
A8	+	+	-	+	acid/acid	+
A9	+	+	+	+	alkaline/alkaline	+
A10	+	+	+	+	alkaline/alkaline	+
A11	+	+	+	+	alkaline/alkaline	+
A12	+	+	+	+	alkaline/alkaline	+
A13	+	+	+	+	alkaline/alkaline	+
A14	+	+	+	+	alkaline/alkaline	+
A15	+	+	+	+	acid/acid	+

+ = positive result, - = negative result, Y = yellow (acid), P = pink (alkaline), Black = H_2S, N/A = not applicable.

been discovered as microbial metabolites, there might be many more products yet to be discovered from soil microorganisms (Alexander, 1961). In our preliminary study, we screened bacterial isolates from waste dump sites for the presence of enzymes such as protease, oxidase, catalase, coagulase (Table 4). All soil bacterial isolates studied for their synthesis of proteins using casein (milk protein) as substrates.

CONCLUSION

In this study, soil bacteria from Ranchi city were found to be both gram positive and gram negative. Bacterial isolates were found to produce secondary metabolites of antibacterial principles and enzymes of commercial importance. This preliminary study may lead to discovery of antibiotics and other bioactive compounds.

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