

ISOLATION AND CHARACTERIZATION OF BACTERIAL ISOLATES FROM AGRICULTURAL SOIL AT DURG DISTRICT

CHITRA BHATTACHARYA^{a1}, P. HARSHA^b, SADHANA GUPTA^c AND SONI ROY^d

Department of Microbiology and Biotechnology Bhilai Mahila Mahavidyalaya, Hospital Sector, Bhilai

ABSTRACT

Agriculture soil was collected from Durg district of Chhattisgarh state. This study aimed to isolation from soil and to biochemical characterization of bacterial isolates. Total of bacterial isolates were characterized as *Escherichia*, *Micrococcus*, *Staphylococcus* sp. The *Escherichia* sp. was rod shaped, gram negative acid and mucous producing bacteria and *Micrococcus*, *Staphylococcus* sp. was coccus shaped gram positive bacteria they were found in any natural ecosystem. They utilize glucose, lactose and sucrose as sole carbon source, it showed fermentation by both acid and gas production. *Escherichia* sp. was unable to hydrolysis the gelatin, citrate and starch, urease test but it showed positive results in VP, catalase, methyl red and indole production test. *Micrococcus* sp. was able to hydrolyzed starch catalase, gelatin and urease by producing enzymes but it was unable to hydrolysis the methyl red, VP, carbohydrates fermentation and indole production test. *Staphylococcus* sp. was able to hydrolyzed catalase, carbohydrates fermentation as acid production, gelatin and MR-VP test but it was unable to hydrolysis starch, indole, urease production test. In this study shows that bacterial organisms are present also in agricultural soil.

Keywords: Agricultural Soil Sample, Biochemical Characterization, *Micrococcus* sp., *Escherichia* sp., *Staphylococcus* sp.

Soil contains a variety of microorganisms included bacteria that can be found in any natural ecosystem. Microorganisms play an important role on nutritional chains that are an important part of the biological balance in the life in our planet. Where, bacteria are essential for the closing of nutrient and geochemical cycles such as the carbon, nitrogen, sulfur and phosphorous cycle. Without bacteria, soil would not be fertile and organic matter such as straw or leaves would accumulate within a short time (Kummerer, 2004). Microorganisms can be used to determine the bioavailability of a given chemical compound in soil. Specifically, measurement of plasmid containing bacteria, using either an endogenous or exogenous approach, serves as a general indicator of environmental contaminants (Arias *et al.*, 2005). In the endogenous approach, plasmids are extracted from soil bacteria isolated on agar plates followed by a visualization of the plasmids on agarose gels (Campbell *et al.*, 1995). Soils normally contain low background levels of heavy metals. However, in areas where agricultural, industrial or municipal wastes are land-applied as fertilizer, concentrations may be much higher. Excessive levels of heavy metals can be hazardous to man, animals and plants. Although most organisms

have detoxification abilities (i.e. mineralization, transformation and/or immobilization of pollutants), particularly bacteria, play a crucial role in biogeochemical cycles and in sustainable development of the biosphere (Diaz, 2004).

MATERIALS AND METHODS

Collection of soil samples:

Samples were randomly collected from the agricultural field of Durg district of Chhattisgarh state, during the post-rainy season of 2013. The top 0–15cm soils contained high organic carbon and were neutral to alkaline in pH (7.5–8.3). One whole leguminous plant, after chopping off the shoots, was carefully uprooted (along with the adhering soil; without breaking the secondary and tertiary roots), placed in a polythene bag, labeled and tied (in order to minimize the evaporation loss), and further placed in a box containing ice and separate loosely adhering soil aggregates around primary, secondary and tertiary roots, and the adhering soils were collected and stored in a refrigerator at 4°C for further studies.

Isolation of bacterial strains:

One grams of soil from each sample were separately suspended in 10 ml of physiological saline

(0.85% of NaCl) in a flask and placed on shaker (at 100 rpm) at room temperature (37°C) for 1 h. At the end of shaking, the soil samples were serially diluted up to 5 dilutions with physiological saline. Dilutions 10^{-3} – 10^{-5} were placed on nutrient agar medium (NAM) by spread plate technique and incubated at 37°C for 48 h. The most prominent colonies were isolated and maintained on NAM slants at 4°C for further studies.

Colony Morphology: The colony morphology of isolates was examined on nutrient agar medium plates. After an incubation of 2 days at 30°C, individual colonies were characterized based on their colour, shape, appearance, colony, diameter, transparency and colour (K. R. Aneja 2003).

Gram's Staining: Colonies that were grown on nutrient agar were gram stained in accordance with standard gram staining procedure described by Todar *et al.*, 2005.

Biochemical Characterization

Carbohydrate Fermentation: The test was performed to determine capability of bacterial isolate to use as carbohydrates. Three sugars (glucose, lactose, sucrose) fermentation broth were inoculated and Durham's tube with culture incubated at 37°C temperature for 24 hours. After incubation to determine capability of bacterial isolates to acid and gas production.

Starch Hydrolysis: The test was performed to determine capability of bacterial colonies to use starch as carbon source (de Oliveira *et al.*, 2007). Starch agar medium were inoculated with culture and incubated at 37°C temperature for 24 hours. After incubation iodine solution was added in starch plate to determine capability of bacterial isolates to use starch.

Gelatin Hydrolysis: The test was performed to determine capability of bacterial colonies to produce gelatinase enzyme as use gelatin as media source. Degradation of gelatin indicates the presence of gelatinase enzyme (Aneja 2003). The actively grown cultures were inoculated in nutrient gelatin medium and incubated at 37°C and grown for 48 hours.

Catalase Test: This was carried out by taking 2.0 ml. of hydrogen peroxide on a clean glass tube. With the edge of test tube a colony of the organism was picked and allowed to be in contact with the hydrogen peroxide. Presence of bubbles indicates positive reaction while absence of bubble indicates negative reaction.

Indole Test: One percent tryptophan broth in a test tube was inoculated with bacteria colony. After incubation period of 37°C for 48 hours, then 1.0 ml of chloroform was added to the broth. The test tube was shaken gently, and then 2.1 of Kovac's reagent were added and this was also shaken gently and allowed to stand for twenty (20) minutes. The formation of red colouration at the top layer indicated positive and yellow colouration indicates negative.

Citrate Utilization Test: This was carried out by inoculating the test organism in test tube containing Simon's citrate medium and this was inoculated for 24 hours to 48 hours. The development of deep blue colour after incubation indicates a positive result.

MR-VP Test: 5.0 ml of MR-VP broth was inoculated with the test organism and incubated for 48-72 hours at 37°C after which, 1.0 ml of the broth was transferred into a small tube. Some small quantity (2.3 drops) of methyl red solution was added. A red colour on the addition of the indicator signified a positive methyl red test while yellow colour signified a negative test. To the rest of the broth in the original tube some drops (five) of 4% potassium hydroxide (KOH) were added followed by some (fifteen) drops of 5% -naphthol in ethanol within 1 hour indicated a VP positive test while no colour change indicated a VP negative test by R.C. Dubey (2002).

Urease Test: Test was performed to determine capability of bacterial colonies to produce Urease enzyme as use of urea as media source. Urea agar medium were inoculated with culture and incubated at 37°C temperature for 48-72 hours. After incubation colour was changed in pink it shows positive results to determine capability of bacterial isolates to use urea.

RESULTS

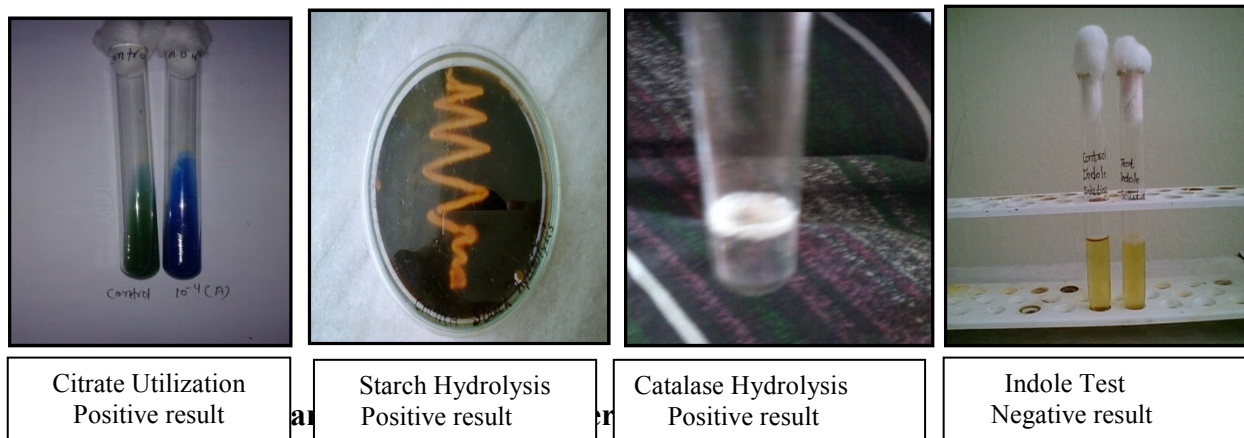


Fig.1 Biochemical characterization of Bacteria-2 (*Micrococcus* sp.)

Table 1: Morphology and biochemical characterization of the bacterial isolates

Biochemical Characters	Bac-1	Bac-2	Bac-3
Gram's reaction	+	+	-
Shape	Cocci	Cocci	Rod
Catalase test	+	+	+
Citrate utilization	-	+	-
Starch hydrolysis	-	+	-
Indole test	-	-	+
Methyl Red	+	-	+
VP test	+	-	-
Glucose fermentation	A	-	AG
Lactose fermentation	A	-	AG
Sucrose fermentation	A	-	AG
Urease activity	-	+	-
Gelatin hydrolysis	+	+	-
Probable identify	<i>Staphylococcus</i> sp.	<i>Micrococcus</i> sp.	<i>Escherichia</i> sp.

Key: (+) = Positive, (-) = Negative, sp.= Species, A= Acid, AG=Acid-Gas, Bac = Bacteria



**Methyl Red Test
Negative result**



Carbohydrate Fermentation (Glucose, Sucrose, Lactose- Negative result)



**Urease Test
Positive result**

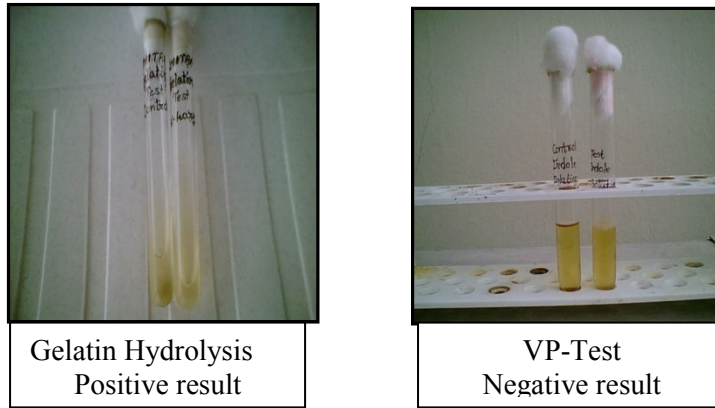
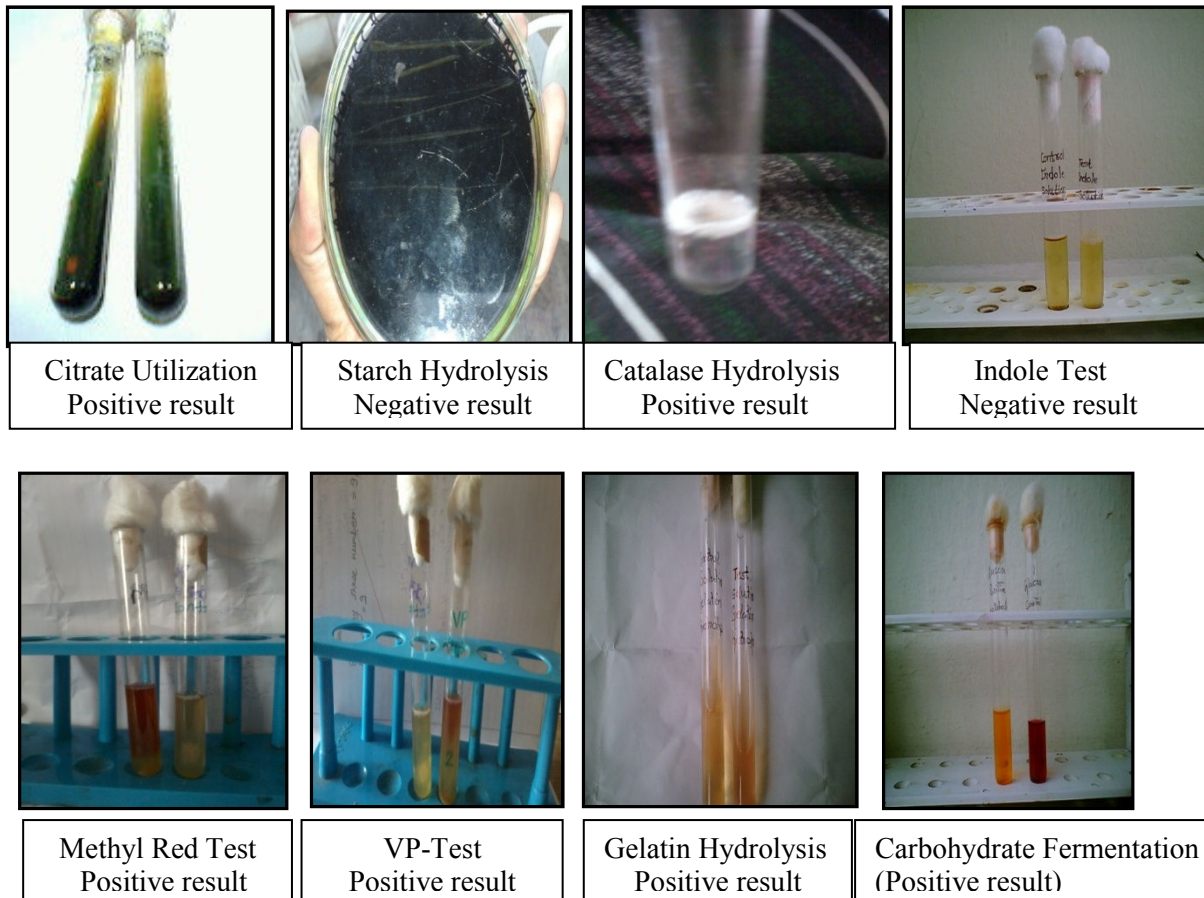


Fig.2 Biochemical characterization of Bacteria-1 (*Staphylococcus* sp.)



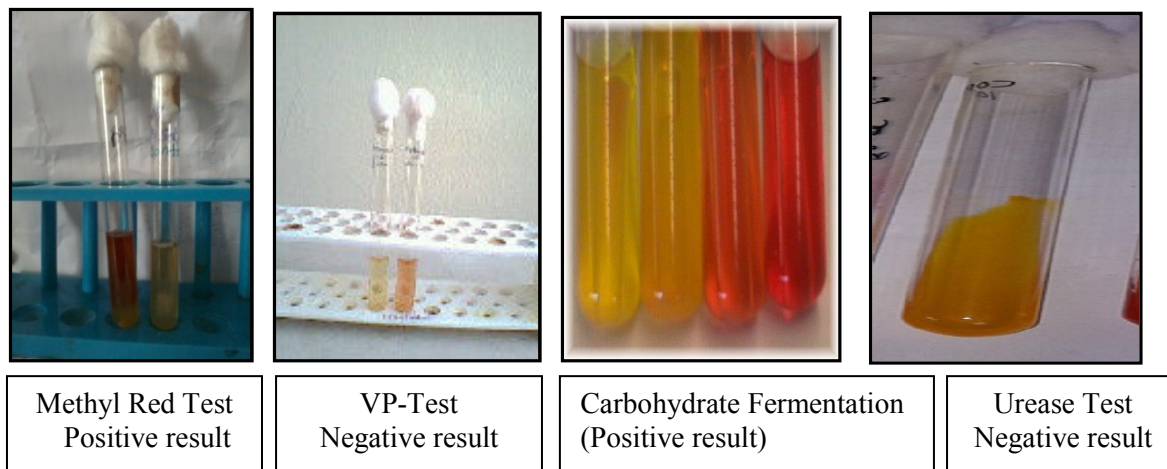
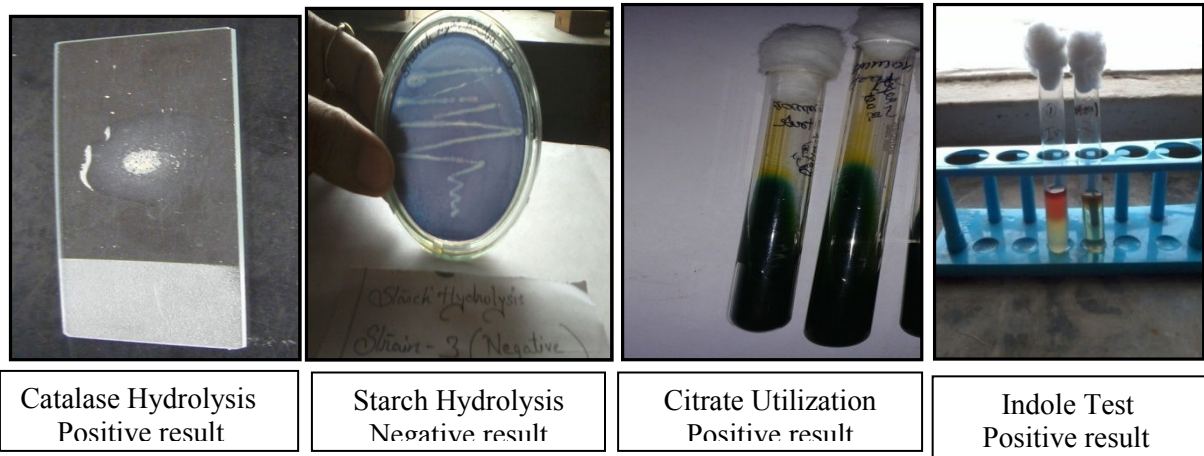


Fig.3 Biochemical characterization of Bacteria-3 (*Escherichia sp.*)

DISCUSSION AND CONCLUSION

Soil sample was collected from agricultural sites as done earlier by Emtiazi, *et al.*, 2005. Further microorganism was isolated by serial dilution method and agar plating method as done previously by (Udeani *et al.*, 2009). In farm there is constant change in microorganism of agricultural soil as the colour and texture changes. Cultures were purified by streaking techniques and the purity was cross checked by Grams staining procedure.

In the present study almost all the isolates of *E.coli* fermented glucose, lactose, sucrose with the production of both by Ali *et al.*, (1998) also studied the biochemical characteristics of the different strains of *E. coli* isolated from different sources. They

reported a little or no difference in these biochemical characters and stated that such similarity among the isolates might be due to presence of some common genetic materials. The results of Catalase, MR and indole test of the *E. coli* isolates were positive but VP test, starch and gelatin test was negative which are in agreement with the reports of Buxton and Fraser (1977).

Micrococcus sp. are of gram positive cocci it isolate from soil sample all the biochemical characteristics gives starch, catalase, citrate, urease and gelatin gives positive results but MR, VP, carbohydrate fermentation test and was negative and *Staphylococcus sp.* also gram positive cocci which

gives catalase, MR, VP, gelatin positive and carbohydrate fermentation produce acid but not gas, it do not produce starch, indole citrate and urease results which are reports of Musliu Abdulkadir *et. al.*,2012 and Faiz Ahmed Raza *et. al.*,2013.

ACKNOWLEDGEMENT

I would like to thank the Bhilai Mahila Mahavidyalaya, Bhilai, C.G. I would like to thank Dr. Zehra Hasan Principal and Dr. Bhawana Pandey, Head, Department of Biotechnology & Microbiology, BMM, Bhilai, and my parents, my brother all they advice to gave me throughout the course of the project.

REFERENCES

- Ali M.Y., Rahman M.T., Islam M.A., Choudhury K.A. and Rahman M.A.; 1998. Characteristics of *E. coli* isolates of human and animal origin. *Progressive Agriculture* 9 (1-2): 221-224.
- Aneja K. R.; 2003. Experiments in Microbiology Plant Pathology and Biotechnology, 4th edition, *New Age International Publishers*, New Delhi, India.
- Arias M. E., Gonzalez-Perez J. A., Gonzalez-Vila F.J. and Ball A.S.; 2005. Soil health-a new challenge for microbiologists and chemists. *Int Microbiol* 8: 13 – 21.
- Buxton A. and Fraser G.; 1977. Animal Microbiology. *Escherichia coli*, *Blackwell Scientific Publications*, Oxford, London, Edinburg, Melbourne. Vol. 1. pp: 92-102.
- Campbell J. I. A., Albrechtsen M. and Sorensen J.; 1995. Large pseudomonas phages isolated from barley rhizosphere. *FEMS Microbiol Ecol* 18: 63 – 74.
- De Oliveria, N., de Oliveria Andrade L. A. J. S. and Chagas J.A.F.; 2007. *Rhizobia* amylase production using various starchy substances as C substrates, *Braz. J. Microbiol.* 38 208-218.
- Diaz E.; 2004. Bacterial degradation of aromatic pollutants:a paradigm of metabolic versatility. *Int Microbiol* 7: 173 – 180.
- Dubey R.C.; 2002. Practical Microbiology, first edition, *S. Chand publication* New Delhi.
- Emtiazi G., Shakarami H., Nahvi I. and Mirdamadian S.H.; 2005. African J. Biotechnol. 4(2): 172-176.
- Faiz Ahmed, Raza and Muhammad Faisal; 2013. Growth promotion of maize by desiccation tolerant *Micrococcus luteus*-chp37 isolated from Cholistan desert, Pakistan, *Australian Journal Crop Science* 7(11):1693-1698 ISSN: 1835-2707, ISSN 2079-2077.
- Kummerer K.; 2004. Resistance in the environment. *J. Antimicrob Chemoth* 45: 311 – 320.
- Musliu A. and Salawudeen Waliyu; 2012. Screening and Isolation of the Soil Bacteria for Ability to Produce Antibiotics, *European Journal of Applied Sciences* 4 (5): 211-215.
- Todar K., Ubukata M., and Hamada M.; 2005. Microbiology of Human Perspective. *Mc Graw Hill Publisher*, London.
- Udeani C.K.T., Obroh A.A., Okwuosa N.C., Achukwu U.P. and Azubike N.; 2009. Isolation and characterization of bacterial isolates. *African J. Biotechnol*, 8 (22):6301-6303.