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RESEARCH **P**APER

Isolation of ureolytic bacteria from different sources and their characterization

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The present study was aimed at isolation and characterization of urease producing bacterial strains for improving the strength of cement concrete. A total of 1500 colonies were isolated from different samples like cowshed, poultry farm, milk, soil and pigeon dung. These isolated cultures were purified on phenol red agar plates. Out of total, 17 bacterial cultures showed pink coloured colonies indicating the presence of urease enzyme. These isolates were characterised on the basis of Gram's staining, size, shape, colour (also known as pigmentation), texture and elevation, ability to form endospores, catalase test, hydrogen sulphide production and acid production and carbohydrate utilization test.

Key words : Urease, Calcium carbonate precipitation, Characterization, Endospore

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INTRODUCTION

Increasing fuel and electricity cost leads to increased number of animal dung based biogas plants to be installed in increasing number in our country. The costs of fixed dome biogas plants are relatively low. There are no rusting steel parts and hence, a long life of plants (20 years or more) can be expected. The construction materials such as bricks and concrete are subjected to the weathering action of several physical, chemical and biological factors (Warscheid and Braams, 2000). Because of their composition and textural characteristics, carbonate stones (limestones, dolostones and marbles) are also susceptible to weathering. Progressive dissolution of the mineral matrix as a consequence of weathering leads to an increase in porosity and as a result, a decrease in mechanical strength. (Tiano *et al.,* 1999).

Fixed dome biogas plants calls for high quality work. If cracks/ broken bucks etc are noticed at the time of installation, the whole digester masonry needs to be demolished which is most cumbersome process. Urease producing microbes can be used for sealing the cracks of biogas plants. It can also be used for biocementing at commercial scale like building repairs, crack filling and making earthquake resistant building. Very few reports are available for the characterisation of urease producing bacteria, therefore, the present study is related to the characterisation of urease producing bacteria.

RESEARCH METHODOLOGY

Isolation and purification of urease producing bacterial strain :

Ureolytic bacteria used in the present studies were isolated from various sources, like poultry dung compost, cowshed, milk sample, etc. Ureolytic bacteria were

isolated by inoculating 1ml of serially diluted soil suspension on modified urea agar (composition gl^{-1}: potassium dihydrogen phosphate: 2.0; glucose: 1.0; peptone: 0.2; sodium chloride: 5.0; urea: 20.0; phenol red: 0.012; agar: 15.0; pH: 7) plates and incubating at 25±2°C in dark (Burbank *et al.,* 2012). Colonies that turn the agar red or pink were picked and streaked for isolation onto urea agar plates.

Further, colonies were picked and further streaked for purification. The cultures were maintained at refrigerated temperature on nutrient agar as by repeated sub-culturing fortnightly.

Cultural characteristics of bacteria :

Cultural characteristicsof all the isolates were studied on the basis of Gram's staining (Smith and Hussey, 2013) and colony morphology. These are the characteristics used to describe the morphology of a bacterial colony (size, shape, colour also known as pigmentation, texture and elevation).

Biochemical characterization of urease producing bacteria :

Biochemical characterization of all bacterial isolates was done on the basis of following tests.

Catalase production :

Small amount of colony from a well-isolated 18- to 24-hour old culture was placed on the microscope slide and 1 drop of 6 per cent H_2O_2 was placed over the culture on the microscope slide. Rapid and sustained production of gas bubbles or effervescence constituted positive test (Reiner, 2010).

Carbohydrate utilization test :

Triplicate test tubes of phenol red broth containing different sugars (such as one for glucose, lactose) were prepared and Durham tube was placed in inverted position in each test tube and was used to test gas production. The tubes were inoculated and incubated for 18-24 hrs at 37°C (Reiner, 2012).

Hydrogen sulphide gas and acid production test :

A loop full of culture was used to stab the centre of the Kligler's agar medium into tube butt. The needle was withdrawn and surface of the slant was streaked with culture. Tubes were incubated at 35°C for 3-4 days and were observed for acid production on slant/butt, gas production and hydrogen sulphide production (Lehman, 2005).

RESEARCH FINDINGS AND ANALYSIS

Total of 13 samples were used to isolate urease producing bacteria (Table 1). These samples were taken from different locations of Punjab, Himachal Pradesh and Ludhiana. Approximately, 1500 bacterial colonies were purified from these samples. However, only 17 isolates were urease positive and were able to change the media colour from yellow to pink. Maximum colonies

Table 1: Isolation of urease producing bacteria

Sr. No.	Sample source	No. of colonies/plate	No. of pink colonies
1.	Soil from cowshed (Jaipur, Rajasthan)	50	Nil
2.	Soil from cowshed (Dehra, Distt. Kangra, Himachal Pradesh)	100	2
3.	Soil from cowshed (Ludhiana)	60	1
4.	Soil (lentil farm, PAU Ludhiana)	40	1
5.	Soil (construction site, PAU Ludhiana)	150	1
6.	Soil sample (COAET)	60	1
7.	Soil from Poultry farm (GADVASU Ludhiana)	150	2
8.	Poultry droppings (GADVASU)	90	1
9.	Pigeon dung (Near COAET)	More than 300	1
10.	Milk (Unpasteurised) (Ludhiana)	200	1
11.	Milk (Pasteurised) (Ludhiana)	40	1
12.	Air (Biogas Laboratory, PAU, Ludhiana)	50	1
13.	Garden soil (Near Library, PAU Ludhiana)	80	4

*Average of triplicate data Medium used: Urea agar; Temperature 25± 2°C; pH: 7.0; Incubation period, 3-7 days

were observed from Pigeon dung sample followed by soil sample from Poultry droppings (GADVASU).

Kigigha *et al.* (2012) selected three different nitrogenous waste dump-sites and a non-nitrogenous site (as control) in Port Harcourt Metropolis, Rivers State of Nigeria for isolation of bacteria. The nitrogenous waste dump-sites locations included: a urinary spot, poultry dung dump-site and a fertilizer storage site indicating suitability of urea containing soil samples for isolation and purification of urease producing bacteria.

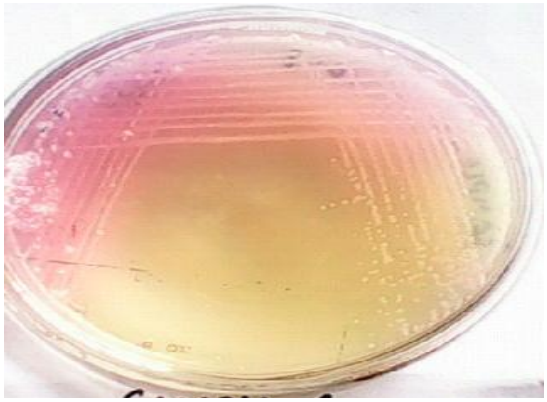

Fig. 1: Pink coloured isolate producing pink colour on urea agar

MU17 isolate formed transparent colony on nutrient agar. MU1 and MU13 formed yellow colonies. White coloured colonies were formed in MU4, MU6, MU8, MU9, MU11, MU12 and MU15. Isolates were white creamy in case of MU2, MU3, MU5, MU7, MU10, MU14 and MU16. Out of 17 isolates, only MU2 was coccobacillus, 3 (MU4, MU6 and MU7) were cocci and rest 13 were rods. Four isolates (MU1, MU4, MU9 and MU11) were able to form endospore. Four of the isolates namely MU1, MU2, MU9 and MU15 were smooth and rest were rough in nature.

Burbank *et al.* (2012) identified 10 isolates while reporting urease activity of ureolytic bacteria isolated from six soils. Out of ten isolates identified on the basis of growth conditions, motility, colony and cell morphology, nine were rods with small and cream coloured colony, two were non- motile and rest eight were motile.

Gram's reaction: Blue colour cells indicate Gram (+) bacteria.

Pink colour cells indicates Gram (-) bacteria.

Out of 17 isolates, 13 were Gram positive and 4 namely MU2, MU4, MU7, MU8 were Gram negative.

(+) catalase test: bubble formation and hence presence of catalase.

Table 2 : Cultural characterisation of urease positive bacteria

Sr. No.	Isolate No.	Shape of colony	Size of colony (mm)	Texture	Colour	Elevation	Shape of bacteria	Endospore formation	Gram's reaction	Hydrogen sulphide and acid production	Carbohydrate test	
1.	MU1	Round	1	Smooth	Yellow	Flat	Rod shaped	+	+	-	Glucose	Lactose
2.	MU2	Round	0.1	Smooth	Creamy white	Flat	Coccobacillus	-	-	-	-	+
3.	MU3	Round	0.5	Rough	Creamy white	Flat	Rods	-	+	+	+	-
4.	MU4	Round	0.5	Rough	White	Raised	Cocci	+	-	+	+	-
5.	MU5	Round	0.5	Rough	Creamy white	Raised	Rods	-	+	-	+	-
6.	MU6	Round	1	Rough	White	Flat	Cocci	-	+	+	-	+
7.	MU7	Round	0.5	Rough	Creamy white	Flat	Cocci	-	-	-	-	-
8.	MU8	Round	0.1	Rough	White	Flat	Rods	-	-	-	+	-
9.	MU9	Round	1	Smooth	White	Raised	Rods	+	+	-	+	-
10.	MU10	Round	0.5	Rough	Creamy white	Raised	Rods	-	+	-	-	-
11.	MU11	Irregular	1	Rough	White	Raised	Rods	+	+	-	+	-
12.	MU12	Irregular	1	Rough	White	Flat	Rods	-	+	+	+	-
13.	MU13	Round	0.5	Rough	Yellow	Raised	Rods, chains	-	+	+	+	-
14.	MU14	Round	0.5	Rough	Creamy white	Raised	Rods	-	+	-	+	-
15.	MU15	Round	0.5	Smooth	White	Raised	Rods	-	+	-	+	+
16.	MU16	Round	0.5	Rough	Creamy white	Flat	Rods	-	+	-	+	-
17.	MU17	Round	1	Rough	Transparent	Flat	Rods	-	+	-	+	-

*Average of triplicate data Culture medium: Nutrient agar; temperature 25°C; incubation period: 3 days

(-) catalase test: no bubble formation and lack of catalase.

Most of the isolates were positive for catalase test except one isolate *i.e.* MU9 was catalase negative. No isolate was able to produce H_2S gas but five of them namely MU3, MU4, MU6, MU12 and MU13 produced acid that changed the colour of the slant.

(+) indicates organism ferments the given carbohydrate and produce organic acids there by reducing the pH of the medium into acidic and changes the colour of the medium to yellow.

(-) indicates organism cannot utilize the carbohydrate but continues to grow in the medium using other energy sources and broth retains the red colour.

Out of 17 isolates 12 (MU2, MU3, MU4, MU7, MU8, MU11, MU12, MU13, M14, MU15, MU16, MU17) were positive for glucose and MU1, MU5, MU6, MU9, MU10 were negative for glucose. Four isolates namely MU1, MU5, MU10, MU15 were positive for lactose and 1 isolate MU15 was positive for both sugars.

Kigigha *et al*. (2012) also characterised and identifiedvarious isolates on the basis of colony morphological and Gram-stain reactions which were used for the preliminary identification of the isolates into the basic bacterial forms. Further identification was carried out using various biochemical tests which include: sugar utilization; H_2S, gas and acid production from Kligler's Iron Agar (KIA); Coagulase test (tube/slide), urease activity, catalase production and the utilization of Manitol sugar in Manitol Salt Agar (MSA). The isolates of greatest preponderance were the *Enterobateriaceae*, followed by the *Micrococcacae*. The *Enterobateriaceae* (*Proteus, Serratia, Flavobacterium/Xanthomonas, Escherichia coli, Klebsiella*/*Enterobacter and Pseudomonas* spp.). The *Micrococcacae* (*viz.*, *Staphylococcus* and the *Streptococcus* spp.) and *Bacillus* and other Gram-positive rods were frequently isolated in all the samples.

Based on the continuous research carried out around the globe, various modifications have been made from time to time to overcome the deficiencies of cement concrete. The ongoing research in the field of concrete technology has lead to the development of special concrete considering the speed of construction, the strength of concrete the durability of concrete and the environmental friendliness with industrial material.

Therefore, from above discussion, it may be suggested that the isolated urease producing bacterial cultures can be utilised for bioconcrete production for enhancing strength of civil structures.

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