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Studies on Antimicrobial Activities of Bioactive Substances Produced by Methylotrophic *Pseudomonas* Species from Lonar Lake

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ABSTRACT

The alkaline Lonar Lake is a unique ecosystem formed by meteor impact, situated in the Buldhana District, Maharashtra, India. The Lonar Lake harbours diversified microbial flora which can detoxify and degrade most harmful pollutant such as methanol. The Methylotroph which degrade harmful methanol can produced bioactive substances having antimicrobial potentials. The two methylotrophic *Pseudomonas* species were isolated and characterized by cultural, morphological, biochemical tests and by 16S rRNA gene sequencing and identified as *P. aerogenosa* (DHT2) *and P. hibiscola* (DHT 11). All these selected *Pseudomonas* species exhibited antimicrobial activities against pathogenic bacteria and data showed that and *P. aerogenosa* were stronger antimicrobial than to *P. hibiscola.* The Present study provides primary evidence that isolated *Pseudomonas* species were promising sources for production of antimicrobial bioactive substances and represent a new and rich source of secondary metabolites that need to be explored in medical microbiology.

Keywords: Lonar Lake, *Pseudomonas* species, Antimicrobial bioactive substances).

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INTRODUCTION

The alkaline Lonar Lake is a unique basaltic rocks formed by meteorite impact around 55,000 years ago, ranking third in the world. The uniqueness of the Lake water is its salinity and high alkalinity (pH of 9.5-10) and harbours diversified microbial flora which can detoxify and degrade most harmful pollutant such as methanol and are rich source of active biomolecules and secondary metabolites[1,2,3]. The study also showed that these alkaliphilic bacteria produce substances with antibacterial and antifungal activities[4,5,6]. The *Pseudomonas* species produce a large number of antibiotics, classified as ribosomal or non-ribosomal antibiotics which play a major role in competition with other micro-organisms[7]. Many antibacterial agents are available in the market now-a-days but they are less effective because of the indiscriminate use of these antimicrobial drugs in treatment of infectious diseases leading to development of resistance in bacteria. Hence, there is a need to develop new antimicrobial compound with diverse chemical structure and novel mechanism of action for new and re-emerging infectious diseases. Therefore, searches are increasingly carried out to develop better drug against microbial infections[8]. The development of resistance to multiple drugs is the major problem in the treatment of infectious diseases caused by pathogenic micro-organisms and therefore there is need to search new bioactive compound to achieve this conditions and circumstances. Halophillic bacteria from Lonar Lake marine environment are also better source of secondary metabolites in pharmaceutical and biotechnological application[9] halophillic bacteria which detoxify methane like pollutant and also can produce a potential bioactive antimicrobial substance.

MATERIALS AND METHOD

Enrichment and isolation of microorganisms:

Matt, water and sediment samples were collected from selected sites of Lonar Lake, during season 2013 and inoculated on methanol (2%) containing minimal salt medium. All the flasks were incubated at room temperature on rotary shaker (100 rpm) for 72h. After enrichment, the bacteria were subcultured on nutrient Agar media and incubated at 37^0C for 24h. Well isolated and differentiated colonies were transferred on slants and maintained as stocks. Methanol degradation potential was evaluated separately.

Identification of antimicrobial compound producing *Pseudomonas* species:

Identification of Isolates was performed by cultural, morphological, biochemical and 16S rRNA gene sequencing from NCCS, Pune (India) and Agharkar Research Institute, Pune.

Extraction of antimicrobial substances:

Extraction of antimicrobial substances from *Pseudomonas* species were done by chilled acetone treatment and precipitated was collected and treated with methanol and Chloroform to remove the impurity, dried it and collected in to the sterile glass bottles and store at 4^0C for further processing.

Antimicrobial activity of *Pseudomonas* species:

The standard cultures of pathogenic bacteria (*Escherichia coli* MTCC443, *Staphylococcus aureus* MTCC96, *Salmonella typhi* MTCC734, *Proteus vulgaris* MTCC426, *Pseudomonas aeruginosa* MTCC424, *Klebsiella pneumonia* MTCC109) were procured from IMTECH, Chandigarh, India. A loopful of culture was inoculated in 10 mL of sterile nutrient broth and incubated at 37^0 C for 3 h for lawn preparation. Sterile blotting paper discs (10 mm) were soaked in the supernatant of the *Pseudomonas* species culture medium which was collected after centrifugation. These prepared discs were dried and used for testing the antibacterial activity. The disc diffusion method was used to determine antimicrobial activity. For antimicrobial properties, 0.1mL bacterial suspension of 10^5CFU mL^{-1} was uniformly spread on Nutrient agar plate to form lawn culture. The discs were applied to the surface of the Nutrient agar plates seeded with 3 h culture of test bacterium and were incubated for 24 h at 37^0C. After incubation, zones of inhibitions were measured in mm.

RESULTS AND DISCUSSION

To survive in the environment and compete with other micro-organisms for resources, many bacteria produce antimicrobial compounds to inhibit the human and animal pathogens[10]. Search for new antimicrobial compound is very essential, as day by day resistance of the bacterial pathogens get increased therefore isolated methylotrophic *Pseudomonas* species the from extremophilic environment of alkaline Lonar Lake produces antimicrobial compounds. In the present study, total twelve samples; four each, sediments, matt and water were collected from different sites of alkaline Lonar Lake. The isolation of methylotrophic *Pseudomonas* species was done on methanol (2%) containing minimal salt medium. *Pseudomonas* species were identified on the basis of cultural, morphological, biochemical characteristics and 16S rRNA gene sequencing from NCCS, Pune and Agharkar Research Institute, Pune. Cultural, morphological and biochemical characteristics of the isolated *Pseudomonas* species were showed in Table 1. The 16S rRNA data showed that DHT2 is *Pseudomonas aeruginosa* strain mmp1 and DHT11 is

Pseudomonas hibiscicola ATCC 19867(T) (Table 2). These isolated *Pseudomonas* species were screened for the production of antimicrobial substances.

Table 1: Cultural, morphological and biochemical characteristic of Methylotrophs from Lonar Lake.

Bacterial Character	**DHT 2 *P. aeruginosa***	**DHT 11 *P. hibiscola***	**Biochemical Character**	**DHT 2 *P. aeruginosa***	**DHT 11 *P. hibiscola***
Gram character	-	-	Catalase	-	-
Shape of Bacteria	SR	SR	Oxidase	+	+
Size of Bacteria (length (um))	1-5	1-5	Indol	-	-
Size of Bacteria (Width (um))	0.5-1	0.5-1	MR	-	-
Arrangement of Cell	S	S	VP	+	+
Spore bearing	NS	NS	Citrate Utilization	+	+
Capsule	NC	NC	Urea Hydrolysis	+	+
Motility	+	+	Nitrate reduction	-	+
Growth at Temperature			Glucose	+	+
37°C	+	+	Arabinose	+	-
45°C	+	+	Mannitol	-	+
50°C	+	+	Xylose	+	+
55°C	-	-	Lactose	-	-
Growth at pH			Trehalose	-	-
pH 7	+	+	Sucrose	-	-
pH 8	+	+	Cellobiose	-	-
pH 9	+	+	Galactose	+	+
pH 10	+	+	Maltose	-	-
pH 11	+	+	Fructose	-	-
pH 12	+	+	Salicin	-	-
Growth at NaCl			Sorbitol	-	-
4% NaCl	+	+	Raffinose	-	-
5% NaCl	+	+	Starch	-	-
6% NaCl	+	+	Lipid	-	-
7% NaCl	+	+	Casein	-	-

SR=Single Rod; S = Single; NS= Non Spore; NC= Non Capsulated; + = Positive; - = Negative

Antimicrobial activity of isolated *Pseudomonas* species: In the present study these two *Pseudomonas* species from Lonar Lake showed antimicrobial activity against the *S. aureus, Sal. typhi, Prot. vulgaris, Kleb. pneumonia, Ps. aeroginosa* and *E. coli*, Previously, Borgave *et al*[4]., studied alkaliphilic bacteria isolated from Lonar Lake exhibited inhibitory effect against clinical isolates of pathogenic bacteria and phyto-pathogenic fungi. Shinde *et al.*[7], studied on antimicrobial activity of alkaliphilic *Bacillus subtilis* isolated from Lonar Lake and it is

phospholipids in nature. Tambekar and Dhundale[9], (2013) isolated *O. iheyensis* produce broad spectrum of antimicrobial agents from Lonar Lake which can be exploited for biotechnological potential and improve as promising sources for new antibacterial compound. In the present study two *Pseudomonas* species were isolated. Out of these *Ps.aeruginosa* strain mmp1 (DHT2) showed prominent antimicrobial activity as compare to *Ps. hibiscicola* ATCC 19867(T) against *Staph. aureus, Sal. typhi, Pro. vulgaris, Kleb. pneumonia*, and no activity against *P. aeroginosa* and *E. coli* (Figure 1 and Figure 2).

Figure 1: Antibacterial activities of methylotrophs.

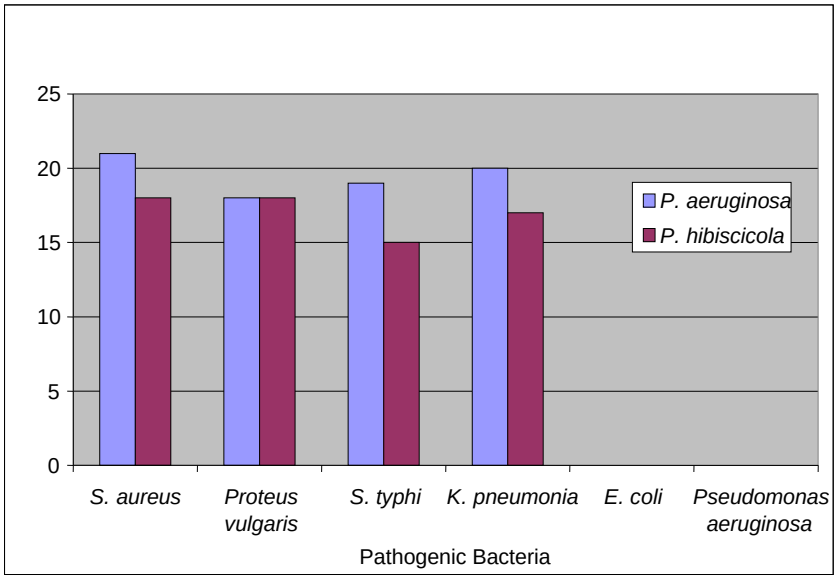

Figure 2: Antimicrobial Activities of Bioactive Substances from Methy lotrophs.

CONCLUSION

The isolated Methylotrophs produces potential antimicrobial bioactive substance having strong antibacterial activities against pathogenic bacteria. The Present study provides primary evidence that isolated *Pseudomonas species* were promising sources for production of antimicrobial bioactive substances and represent a new and rich source of secondary metabolites that need to be explored in medical microbiology.

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