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ANTIBACTERIAL ACTIVITY OF A BACTERIOCIN-LIKE INHIBITORY SUBSTANCE PRODUCED BY BACILLUS SP. FROM HALOPHILIC ENVIRONMENT

VISHAL R. DHUNDALE[1] AND VIJAYSHREE M. HEMKE*[2]

[1]New Arts Commerce and Science College Ahmednagar, Maharashtra (India)
*[*2]Jijamata Mahavidyalay Buldhana, Maharashtra (India)*

ABSTRACT

The objective of this study was to investigate a bacteriocin like inhibitory substance (BLIS) produced by a Bacillus sp. isolated from the halophilic Lonar crater. In present study total fourteen Bacillus sp isolated, out of them twelve Bacillus species showed that the antimicrobial activity against the *E. coli* and *S. typhi* except the CW1 and CW3. The Bacillus sp. CW2 was found 24 mm zone of inhibition that considered as prominent BLIS producer against *E. coli* while the Bacillus DS3 was found 25 mm zone of inhibition that considered as prominent BLIS producer against *S. typhi*. The Bacillus Sp. GW3 and Bacillus Sp. HS4 were found moderate activity with 22 mm zone of inhibition against *P. aeruginosa* and 28 mm zone of inhibition against *P. vulgaris* respectively. Out of fourteen, nine bacillus sp were found antibacterial activity against *B. subtilis*. The Bacillus Sp EW2 and Bacillus Sp. DS7 was found moderate activity with 21 mm zone of inhibition against *B. subtilis* and 22 mm against *B. megaterium* respectively. The isolated Bacillus sp. DS3 and DS2 were found antibacterial against all tested all gram positive bacteria. In conclusion, the studies suggested that halophilic environments could be sources of bacterial strains capable of synthesizing novel metabolites with antibacterial activity. These bacteria in general represent a new and rich source of BLIS that need to be explored
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KEY WORDS: ***Bacillus, bacteriocin-like inhibitory substance, halophilic***

VIJAYSHREE M. HEMKE*
Jijamata Mahavidyalay Buldhana, Maharashtra (India)

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INTRODUCTION

Hyper alkline saline lakes, with salinity and alkalinity ranges at or near saturation are extreme environments; yet, they often harbours remarkably high microbial cell densities and are biologically very productive ecosystems[1-3]. To evolve to such conditions, bacteria - have developed various mechanisms to survive in such extreme environment. Halophilic bacteria from marine environment are also better sources of secondary metabolites that may have potential of pharmaceutical and biotechnological applications[4]. The development of resistance to multiple drugs is major issue in the medication of infectious disease caused by pathogenic microorganism. The multidrug resistance is presently targeted on exploration and therefore need to search new bioactive compound. To achieve this conditions and circumstances, there is concern to ameliorate novel class of antibiotics that have distinct mechanisms of activity worldwide. The investigations of such bacteria are of great importance due to produce medically important metabolites such as bacteriocin or bacteriocin like inhibitory substance (BLIS)[5-6]. The important features of bacteriocin, such as their natural sources, wide range of activities, and their proteinaceous nature, which implies a putative degradation in the gastrointestinal tract of man and animals, have interested researchers seeking to develop new antimicrobial[7-8]. Now a days it is seen the rapid emergence of antibiotic-resistant in pathogenic bacteria and there is an essential for discovering novel antimicrobial compounds[9-10]. The hypersaline environment including halophilic microorganisms may, as indicated, serve as source of antibacterial compounds[11]. Only a minor fraction of the halophilic and alkaliphilic microbiota is culturable and assessing the complete potential of bioactivity of the extremophilic microbial diversity would require inclusion of both cultured and uncultured organisms from marine environment[4,12-13]. The alkaline Lonar crater is a unique basaltic rock meteorite impact crater, ranking third in the world and is filled with saline water having an average pH of 9.5- 10. In Lonar, no significant studies have been conducted so far to isolate and produce useful antibiotics. Therefore, present study is intended to isolate, screen and characterize bacteriocin producing bacteria from Lonar Lake which could be further explored for a biotechnological potential.

MATERIALS AND METHODS

Enrichment and isolation of microorganisms

The alkaline Lonar Lake in Buldhana district of Maharashtra is unique ecosystem and wonder in the India (latitude 19º58', Longitude 76º36'). Water and sediment sample were collected in sterile bottles and polythene bags respectively, from defined sampling site. Enrichment of water samples and sediment samples were carried out in Horikoshi I, Horikoshi II, and nutrient broth at pH 10, nutrient broth at pH 10.0 with 30 g l−1 sodium chloride. All the flasks were incubated at room temperature (RT) on a rotary shaker (100 rpm) for 48h. After enrichment, the organisms were isolated on respective media agar plates and incubated at 37°C for 24h. Well isolated and differentiated colonies from these enrichment media were transferred on the respective medium slants and cultures were maintained as stocks[14-15].

Identification of the bacterial culture

The *Bacillus* culture isolated from Lonar lake water and sediment samples were identified by conventional biochemical tests in according with Bergey's Manual of systematic bacteriology.

Detection of antagonistic activities

The antagonistic properties of *Bacillus* preparations were determined by modifying the disc diffusion method. Sterile blotting paper discs (6mm) were dipped into 48h incubated cell free culture broth and then placed on solidified Nutrient agar seeded with 3h old culture of test oraganism, which included *Escherichia coli* (MTCC 443) *Proteus vulgaris* (MTCC 426), *Salmonella typhi* (MTCC 734), *Bacillus subtilis, Bacillus megaterium, Bacillus cereus.* The plates were kept for incubation at 37°C for 24h. Zones of inhibition were measured[16].

Antibacterial assay of bacteriocin

As potential bacteriocin producers grown in respective broth at 37°C for 48h. Cell suspensions were centrifuged at 5000 rpm for 15 min. The antagonistic activity of antibacterial substance was determined by disc diffusion method[17-18].

RESULT AND DISCUSSIONS

The alkaline Lonar Lake is a unique basaltic rock meteorite impact crater. Lonar Lake is a one such soda Lake in which the indigenous microflora is present, and such microbial flora has ability thrives in alkaline condition. Total fourteen isolates obtained in the isolation exercise, cultural, morphological characteristics of all the strains were studied. The isolates were screened on the basis of biochemical characteristics as described earlier All the fourteen bacterial cultures were revealed to be Bacillus by their positive Gram reaction, spore bearing, motile, and rods in cell morphology. Then a various conventional phenotypic tests were used to identify them tentatively. In present investigation was to determine antibiotic substance producing diversity of Lonar Lake using different enrichment media with various substrates as peptone, yeast extract, glucose, and starch, Casein and egg yolk. The media composition seemed to have an effect on the recovery of different species of the *Bacilli.* There are evidence to show that the moderately halophilic bacteria have strong potable solutes, fermented foods, enzyme, polymers and degradation of toxic compounds[19-22].

Table 1
Biochemical characteristic of Bacilli isolated from Lonar Lake

		Marphological characteristic		Biochemical characteristic										Bacteriocin-like inhibitory substance against						
Sr.NO	Isolation Code	Gram	Shape of Bacteria	Colony Size	Catalase	Oxidase	Glucose	Arabinose	Mannitol	Xylose	Sucrose	Maltose	Fructose	*B. subtilis*	*B. cereus*	*P. vulgaris*	*P. aeruginosa*	*E. coli*	*S. typhi*	*B. megaterium*
1	IS4	+	SR	1.5	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+
2	IW3	+	SR	1	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+
3	EW1	+	SR	1	+	+	+	+	-	+	-	-	+	+	+	-	-	+	+	-
4	EW2	+	SR	1	+	+	+	+	-	+	-	-	+	+	+	-	-	+	+	+
5	DS3	+	SR	1.5	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
6	DS7	+	SR	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	CW1	+	SR	1	+	+	+	+	-	+	+	-	+	-	-	-	-	-	-	-
8	CW2	+	SR	1	+	+	+	+	-	+	+	-	+	-	+	-	-	+	+	-
9	CW3	+	SR	1	+	+	+	+	-	+	+	-	+	-	-	-	-	-	-	-
10	HS3	+	LR	2.5	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-
11	HS4	+	LR	3	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-
12	ES1	+	LR	1	+	+	+	+	-	+	-	-	+	-	+	-	+	+	+	-
13	DS2	+	LR	1	+	+	+	+	-	+	-	-	+	+	+	-	-	+	+	+
14	GW3	+	LR	1.5	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-

SR- Short Rod, LR- Long Rod, + Positive, - Negative

Lei chen *et al*.,[23] were studied on the antimicrobial and cytotoxic activities of moderately halophilic bacteria isolated from the Weihai Solar saltern. In present study twelve Bacillus species showed that the antimicrobial activity against the *E. coli* and *S. typhi* except the CW1 and CW3 were not showed activity. The Bacillus CW2 was found 24 mm zone of inhibition that considered as prominent anitibiotic substance producer against *E. coli* followed by the HS3, ES1 and DS2 were shows 23mm zone of inhibition. While the dissimilar result was observed by Motta *et al.,*[24], when the antibacterial substance produced by Bacillus sp BLS P34 was co-added with EDTA showed antibacterial activity but BLS or EDTA alone showed no inhibitory effect on the *E. coli* and *S. typhi*. The Bacillus DS3 was found 25 mm zone of inhibition that considered as prominent anitibiotic substance producer against *S. typhi* followed by the ES1 was shows 23mm zone of inhibition. Few attention have been addressed to their application as antimicrobials in clinical studies. Because attention towards the bacteriocin is important, effective and not harmful to human perspectives, it has been already investigated as an another choice for disease control[25-26]. The antibacterial substance produced by Bacillus sp. may represent an antimicrobial substance with potential application in the prevention and treatment of *E. coli* and *S. typhi.*The prominent antibacterial activity of *Oceanobacillus iheyensis* was found against *E. coli* and moderate activity against *K. pneumoniae, S. typhi, S. aureus* and poor antibacterial activity against *P. vulgaris* and *E. aerogenes* while no antibacterial activity found against *P. aeruginosa*[11]. In the present studies, five Bacillus sp. were found antibacterial activity against *P. aeruginosa.* The Bacillus Sp GW3 was found moderate activity with 22 mm zone of inhibition towards the *P. aeruginosa.* Followed that the Bacillus Sp. DS7 was found 20mm zone of inhibition while HS4 was found lowest activity. These results against *P. aeruginosa* indicate that the these antibacterial substance may be effective against *P. aeruginosa* which is responsible for the biofilm formation. Similar results were observed by Nakamura *et al*., [27] the antibacterial activity of violet pigment produced from psychrotropic bacterium RT102 Strain. These antibacterial substance produced by Bacillus sp. may represent an antimicrobial substance with potential application in the prevention of biofilm formation and treatment of *P. aeruginosa*. Shanks *et al.,*[28] was performed the isolation and identification of a bacteriocin with antibacterial and antibiofilm activity from *Citrobacter freundii*. The Bacillus Sp. HS4 was found moderate activity 28 mm zone of inhibition towards the *P. vulgaris.* Followed that the Bacillus Sp. DS7 was found 26mm zone of inhibition while DS3 was found lowest activity. Similar results were observed by Tambekar and Dhundale[5] against *E. coli*, *K. pneumoniae*, *P. vulgaris* and *E. aerogenes*.

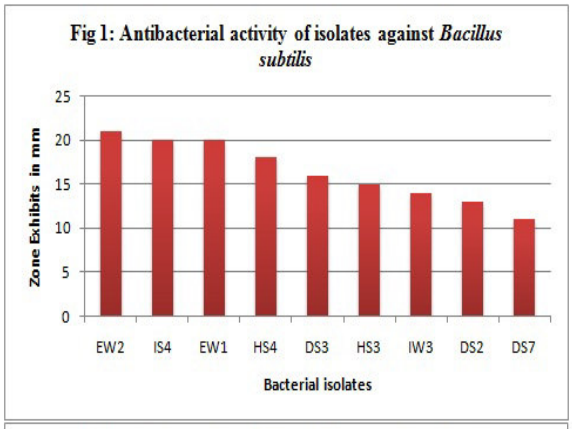

Fig 1: Antibacterial activity of isolates against *Bacillus subtilis*

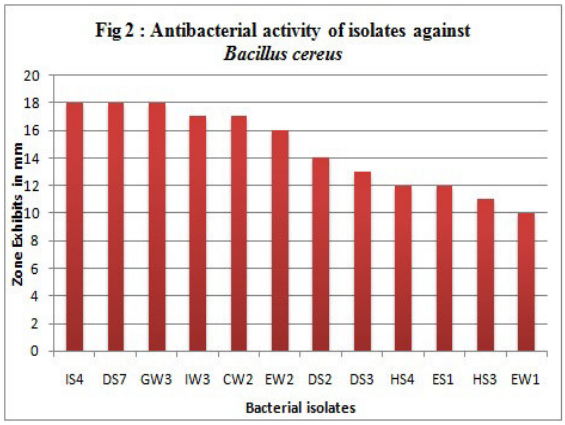

Fig 2 : Antibacterial activity of isolates against *Bacillus cereus*

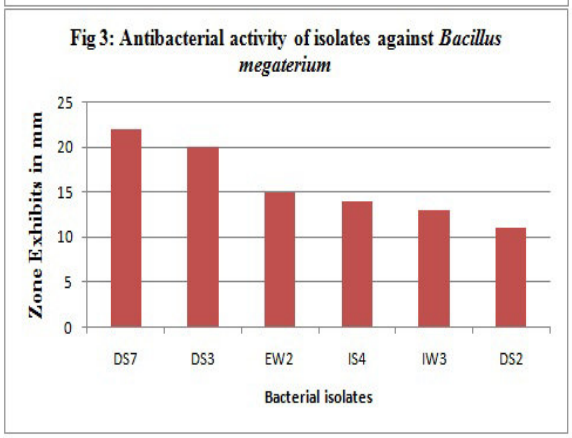

Fig 3: Antibacterial activity of isolates against *Bacillus megaterium*

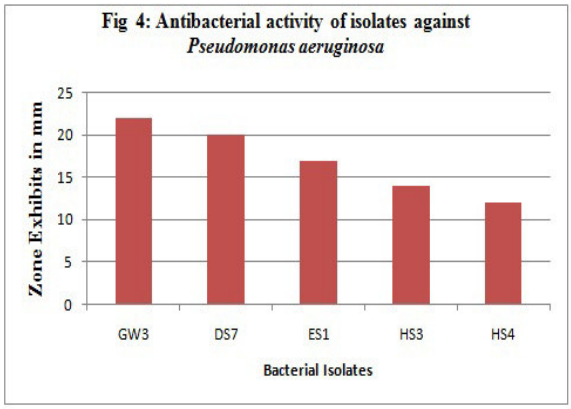

Fig 4: Antibacterial activity of isolates against *Pseudomonas aeruginosa*

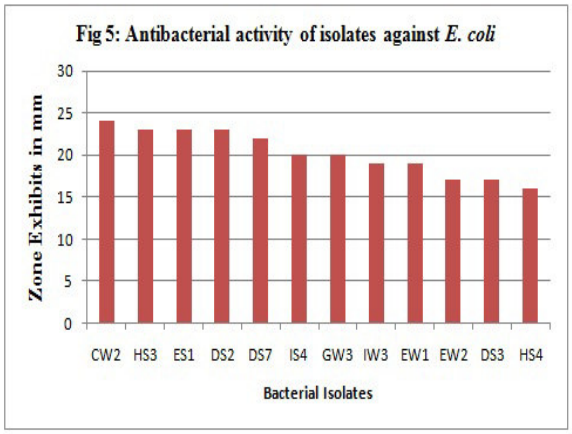

Fig 5: Antibacterial activity of isolates against *E. coli*

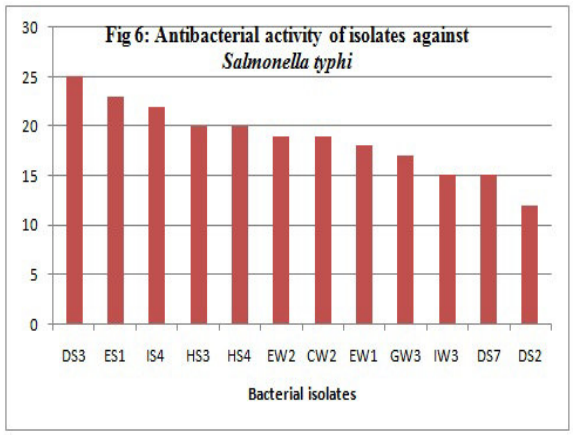

Fig 6: Antibacterial activity of isolates against *Salmonella typhi*

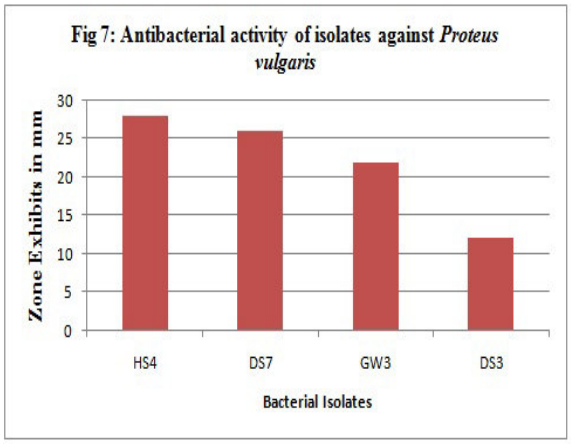

Fig 7: Antibacterial activity of isolates against *Proteus vulgaris*

The present knowledge about the inhibitory range of bacteriocins from Gram-positive bacteria is that they are narrow to inhibit the other Gram-positive bacteria. The range of sensitive strains can vary significantly, from relatively narrow as in the case of lactococcins which have been found to kill only Lactococcus, to extraordinarily broad[29]. In the present study, the isolated Bacillus sp were produced antibacterial substance against *B. subtilis, B. cereus* and *B. megaterium.* Out of fourteen, nine bacillus sp were found antibacterial activity against *B. subtilis*. The Bacillus Sp EW2 was found moderate activity with 21 mm zone of inhibition towards the *B. subtilis.* The Bacillus Sp IS4 and EW1 were found 20mm zone of inhibition while DS7 was found lowest activity. Similar results were observed by Nakamura et al.,[27] the antibacterial activity of violet pigment showed antibacterial activity against gram-positive bacteria such as *S. aureus, B. licheniformis, B. subtilis, B. megaterium.* The Bacillus sp. DS7 was exihibit 22 mm and Bacillus sp. DS3 (20mm) the prominent zone of inhibition against *B. megaterium*. The isolated Bacillus sp. DS3 and DS2 were found antibacterial against all tested gram positive bacteria such as *Bacillus subtilis, B. megaterium* and *B. cereus*, but the degree of activity were found variable towards the different bacillus sp. The twelve isolated bacillus sp were found antibacterial activity including IS4, DS7 and GW3 were exihibit the optimum antibacterial activity against the *B. cereus* while the Bacillus sp EW1 display lowest activity. Similar result was found to Motta *et al.,*[24] bacteriocin like substance (BLS) produced by a novel Bacillus sp. strain P34 isolated from the Amazon basin. The effect of the BLS was tested against *Listeria monocytogenes*, showing a bactericidal effect, while dissimilar results were found against *Bacillus cereus*. The isolation, identification and biochemical characterization of bacteriocins produced by Bacillus sp., and exploration of their important use in the control of pathogenic and spoilage microorganisms. The genus *Bacillus* comprises a variety of industrially important species and has a history of safe use in both food and pharmaceutical industry[30-35]. The genus *Bacillus* encompasses a number of bacteriocinogenic species, such as *B. subtilis* which produces subtilin[36] and subtilosin[37], *B. coagulans* which produces coagulin[38], and *B. megaterium* which produces megacin[39].

CONCLUSION

In conclusion, antibacterial activity of Bacteriocin like inhibitory substance isolated from Lonar Lake bacterial culture. The comparative results, between various bacterial cultures, suggested that EW2, IS4, DS7, GW3,CW2, DS3 and HS4 were revealed important to the antibacterial performance with different tested bacterial cultures. In future the genomic data will be determined for the presence of genes encoding possible antibiotic activity.

CONFLICT OF INTEREST

Conflict of interest declared none.

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Reviewers of this article

Dr.Kapil Kample

Associate professor
Department of microbiology
Amravathi university
Amravati, Maharashtra 444602

Prof.Dr.K.Suriaprabha

Asst. Editor , International Journal of Pharma and Bio sciences.

Asso.Prof.Dr. R. Usha, MSc, M.Phil, Ph.D.

Associate Professor, Department of Microbiology,
Karpagam University, Eachanari (PO),
Coimbatore - 641 021, Tamil Nadu, India.

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