International Journal of Biotechnology and Biochemistry
ISSN 0973-2691 Volume 13, Number 3 (2017) pp. 211-217

http://www.ripublication.com

Antimicrobial Evaluation of Bioactive Pigment from *Salinicoccus sp* isolated from Nellore sea coast

V. Srilekha[1], G.Krishna[2], V.Sesha Srinivas[3] and M.A.Singara Charya[2]

[1]Department of Biotechnology, Chaitanya Degree and PG College (Autonomous), Warangal-506001, Telangana, India.
[2]Department of Microbiology, Kakatiya University Warangal – 506009, India.
[3]Department of Biotechnology, SNIST, Ghatkesar, Hyderabad-501301, India.

Abstract

Presently there is a need for new antibiotics with an alternative mode of action and new chemical structures. In the present study, marine bacteria were isolated from water samples collected from the coastal areas of Nellore (mypadu, kottakoduru beach area), Andhra Pradesh, India. Among 29 different microbial colonies isolated on Zobell marine agar medium, morphologically different pigmented bacteria were characterized with yellow, orange, peach and pink colour. The isolate with bright pinkish orange pigmentation was selected for the further study. The isolate was identified as a *Salinicoccus sp* on the basis of microscopic, biochemical characteristics and 16s rRNA sequencing methods. The isolate was cultured in marine broth and the pellet extracted with methanol and acetone (5:1 ratio) for screening of antimicrobial activity against a panel of human pathogens. The crude pigment extract of marine *Salinicoccus sp* showed maximum antimicrobial activity against *Staphylococcus aureus* (24 mm) and minimum activity against *Klebsiella pneumonia* (16 mm) *Pseudomonas aeruginosa* (14 mm). Gram positive bacteria were found most sensitive to the pigment extract in antibacterial screening.

Keywords: Marine bacteria, Nellore Coast, crude extract, Antibacterial activity

INTRODUCTION

The ocean remains as an unexploited source for many drugs and pharmacologically active substances (Sivasubramanian and Vijayapriya 2011). Marine environments are vast and largely untapped source for the isolation of new microorganisms having potentiality to produce novel bioactive secondary metabolites (Baskaran *et al.,* 2011). Marine microorganisms are excellent source for antimicrobial compounds (Burgess *et al.,* 1999) are fascinating resources due to their production of novel natural products with antimicrobial activities. Microorganisms found in marine environments have attracted a great deal of attention due to the production of various natural compounds and their specialized mechanisms for adaptation to extreme environment (Solingen *et al.,* 2001).

Pigmentation is widespread phenomenon in bacteria and consists of carotenoids and many other pigments (Yehia *et al.,* 2013). The pigments produced by microorganisms viz. carotenoids, melanins, flavins, quinones, and more specifically monascins, violacein and indigo showed distinct antibacterial effect against many pathogenic bacteria (Molnár and Farkas, 2010). In this regard, a lot of attention is now being paid for synthesis of biocolors, also known as microbial pigments, by employing the microorganisms (Cho *et al.,* 2002). The purpose of this work was to isolate and screen pigmented marine bacteria and evaluate antibacterial activity of pigmented compounds against pathogenic bacteria. Hence, the present study was undertaken to isolate and investigate the antimicrobial potential of crude pigment extracts of bacteria from marine environment of Nellore coast of Andhra Pradesh, India.

MATERIALS AND METHODS

Sample collection: Sea water samples were collected, from Nellore coast in the sterilized plastic bottles and are transported to the laboratory. Samples were collected at the depth of 10-40 cm in sterilized container and transferred to the laboratory.

Isolation and Screening of Pigment Producing Bacteria

For isolation of Pigment producing micro-organisms, collected Samples were spread over surface of plates containing Zobell marine agar and incubated at 25°C temperature for 2-3 days

Extraction of Pigments

The bacterial isolates were grown in Zobell marine broth and the flasks were lodged on the shaker at a speed of 120 rpm at 25^0 C for 5 days. After fermentation, the pellet was collected by centrifugation at 10000 rpm for 10 min at 4^0c to obtain a pellet. The bacterial cell pellets were extracted using 95% (v/v) methanol and 99% (v/v) acetone in the ratio of 5: 1 until the pellet was colourless, i.e., complete pigment extraction was achieved.

Antimicrobial activity of crude pigment extract

The crude pigment extract obtained from methanol was tested for the activity against the test pathogens (*Staphylococcus. aureus, Bacillus cereus, Esherichia coli, Klebsiella pneumonia, Proteus vulgaris and Pseudomonas aeroginosa*). Screening of antimicrobial activity of crude pigment extract was determined by agar well diffusion method. About 100 µl of standardized inoculum (0.5 Mac Farland) of each test bacterium was inoculated on Petri dishes containing Nutrient agar medium under aseptic conditions. Standard cork borer of 6 mm in diameter was used to make uniform wells into which 60 µl of 10mg/ml of crude pigment extract dissolved in Methanol was added. Methanol alone was used as negative control. The plates were then incubated at 37°C for 24 hours. After incubation for 24 hours at 37^0c the zone of inhibition was measured with the help of standard scale (HiMedia).

RESULTS AND DISCUSSION

The marine environment, which covers three quarters of the surface of the planet, is estimated to be home to more than 80 % of life and yet it remains largely unexplored. An attempt has been made to isolate the bacteria from this unexplored marine environment in order to find novel species. Since the marine environmental conditions are extremely different from the terrestrial counterparts, marine bacteria are expected to produce bioactive compounds with great potential that represent a valuable source for the development of novel therapeutic agents. The marine water samples were collected from sea shore of Nellore district, Andhra Pradesh, South India. Samples were collected in sterilized plastic containers at the depth of 10-40 cm. The Following incubation, different pigment producing colonies viz; Orange, peach, yellow, pink were selected and propagated on the same medium until pure cultures were obtained. Plates exhibiting discrete pigmented colonies were selected. Totally 29 distinct coloured bacteria were isolated from sea water samples collected from different areas along the sea coast of Nellore (Mypadu and Kottakoduru beach area). The organism under study was Pinkish orange pigmented marine bacterium **Fig 1**. The isolate was identified to be *Salinicoccus* sp based on morphological, biochemical and 16s rRNA sequencing **Table 1.**The 16S rRNA gene, consisting of 1542 bases, is highly conserved among microorganisms and is therefore an excellent tool for studying phylogenetic relationships (Sacchi *et al.,* 2002).The isolate was cultivated in zobell marine broth medium for 5 days. At the end of incubation period the fermentation medium was centrifuged at 10,000 rpm for 15 min at 4°C, to separate cell mass from fermentation medium. The cell pellets were collected and were extracted with methanol and acetone, then separated from the cells by centrifugation at 10,000 rpm for 15 min at 4°C. Different solvents were used to extract pinkish orange pigment from the wet cell pellets. Among different solvents used for pigment extraction

methanol: acetone mixture 5:1 (v/v) was found to be superior in comparison to other solvents. The pigment from selected isolate was extracted by solvent extraction (acetone and methanol). The coloured supernatant was collected and the process was repeated until the pellet turned white. The crude pigment extract from *Salinicoccus sp*. was subjected to antimicrobial activity by agar well diffusion technique against standard pathogens such as *Bacillus subtilis, Escherichia. coli, Staphylococcus aureus, Klebsiella pneumonia, Proteus vulgaris.* The crude metabolites have shown excellent antimicrobial activity against *Staphylococcus aureus*, *Proteus vulgaris*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The crude pigment extract, showed potent activity *in vitro* against Gram negative, Gram positive bacteria. The crude pigment extract of marine *Salinicoccus* sp. showed more inhibitory activity against Gram positive bacteria than Gram negative bacteria as shown in **Table 2**. The difference in susceptibility can be attributed to differences in cell wall composition. The reason was referred to the difference in the structures of the cell walls (Singh *et al.,* 2007). The component of lipopolysaccharide can also makes the cell wall impermeable to lipophilic solutes (Pandey *et al.,* 2004), hence gives more protection in Gram negative bacteria. The cell wall of Gram-positive bacteria has a thick layerof peptidoglycan and should be more susceptible because not an effective permeability barrier (Pandey *et al.* 2004,).

Results of this study indicate that the potential of the coastal region as a source of marine bacteria to produce antibacterial compounds that can be useful for many pharmaceutical applications and must be explored. The crude pigment extract of *Salinicoccus*.sp exhibited reasonably strong antibacterial activity against a series of Gram positive and Gram negative bacteria.

Fig.1. Plate showing the colonies of *Salinicoccus.sp*

Table 1 .Morphological, Physiological and Biochemical properties of marine isolate

Characteristic	
Colony Morphology	Orange pigmented,opaque,flat circular
Cell shape	Coccus
Motility	-ve
Gram staining	+ve
Starch hydrolysis	+ve
Indole	-ve
Methyl Red	+ve
Voges proskaeur	-ve
Citrate utilisation	-ve
Catalase	+ve
Urease test	+ve
Nitrate Reduction test	+ve

Table 2: Antimicrobial activity of crude bioactive compound of marine *Salinicoccus*.sp

S.NO	Test Organism	Diameter of Zone of inhibition in mm		
		Pigment extract	NC	PC
1	*Staphylococcus.aureus*	24	02	32
2	*Bacillus.cereus*	21	02	30
3	*Proteus.vulgaris*	20	02	31
4	*Klebsiella.pneumoniae*	16	02	32
5	*Escherichia.coli*	19	02	30
6	*Pseudomonas aeruginosa*	14	02	30

PC=Positive control (Ampicillin and Streptomycin); NC=Negative Control (Methanol and Acetone)

CONCLUSION

The present study was undertaken to isolate potent bacteria producing antibacterial pigments from marine environment of Nellore sea coast. In our study, it was found

that the pigmented marine bacterium was active against the series of test bacteria. Thus, the results of the present research reveal that the marine bacteria from coastal region are a potent source of novel antibacterial compounds and this marine environment warrants particular attention, for its remarkable microorganism's diversity and metabolic products. This study suggests that marine-derived bacteria are worthy of further exploration as novel drug candidates. This study also highlighted the role of other sources apart from soil community in screening of potential candidates that might be helpful for the discovery of new antibiotics. It is expected that the current attempt for the isolation, characterization and the study on marine bacteria of Nellore coast will be useful for the identification of new antibiotics that are effective against challenging pathogens.

ACKNOWLEDGEMENT

This study was supported by University Grants Commission-South Eastern Regional Office, Hyderabad. The financial assistance received from University Grants Commission-South Eastern Regional Office, Hyderabad (MRP-6073/15) was greatly acknowledged. The authors are grateful to the secretary and correspondent Dr.Ch.V.Purushotham Reddy, Chaitanya Degree and Postgraduate College, Hanamkonda, Telangana, India for the extensive support and providing facilities required to carry out this work. The authors wish to deeply thank the Principal, Dr.K.Veeravenkataiah, Chaitanya Degree and Postgraduate College, Hanamkonda, Telangana, India for the constant support and encouragement for completion of this work and for his valuable suggestions.

REFERENCES

[1] Sivasubramanian, K. and Vijayapriya, M., (2011). Antagonistic activity of marine bacteria Pseudoalteromonas tunicata against microbial pathogens. African Journal of Microbiology Research, 5(5): 562-567.

[2] Baskaran, R., Vijayakumar, R. and Mohan, P.M., (2011). Enrichment method for the isolation of bioactive actinomycetes from mangrove sediments of Andaman Islands, India. Malaysian J Microbiol, 7(1):1-7.

[3] Burgess, J.G., Jordan, E.M., Bregu, M., Mearns-Spragg, A. and Boyd, K.G., (1999). Microbial antagonism: a neglected avenue of natural products research. Journal of Biotechnology, 70(1):27-32.

[4] Solingen, P., Meijer, D., Kleij, W.A., Barnett, C., Bolle, R., Power, S.D. and Jones, B.E., 2001. Cloning and expression of an endocellulase gene from a novel streptomycete isolated from an East African soda

lake. Extremophiles, 5(5):333-341.

[5] Yehia, H. M., Abudabos, A. M., Elkhadragy, M. F., Olayan, E. M. A., Khalaf-Allah, A. R. M., & El-Shimi, N. M. (2013). Cloning, sequencing and expressing of the carotenoid biosynthesis genes of ß-carotene from epiphytic bacteria Erwinia uredovora in non carotenogenic bacteria E. coli. Life Science Journal, 10(3): 1904-1917.

[6] Molnár, K. and Farkas, E., (2010). Current results on biological activities of lichen secondary metabolites: a review. Zeitschrift für Naturforschung C, 65(3-4):157-173.

[7] Cho, Y.J., Park, J.P., Hwang, H.J., Kim, S.W., Choi, J.W. and Yun, J.W., (2002). Production of red pigment by submerged culture of Paecilomyces sinclairii. Letters in applied microbiology, 35(3): 195-202.

[8] Sacchi, C.T., Whitney, A.M., Reeves, M.W., Mayer, L.W. and Popovic, T., (2002). Sequence diversity of Neisseria meningitidis 16S rRNA genes and use of 16S rRNA gene sequencing as a molecular subtyping tool. Journal of clinical microbiology, 40(12):4520-4527.

[9] Singh, D.P., Kumar, R., Malik, V. and Tyagi, P., (2007). Synthesis and characterization of complexes of Co (II), Ni (II), Cu (II), Zn (II), and Cd (II) with macrocycle 3, 4, 11, 12-tetraoxo-1, 2, 5, 6, 9, 10, 13, 14-octaaza-cyclohexadeca-6, 8, 14, 16-tetraene and their biological screening. Transition Metal Chemistry, 32(8), pp.1051-1055.

[10] Pandey, B, Ghimire P, Agrawal V.P., (2004). Studies on the antibacterial activity of the actinomycetes isolated from the Khumbu Region of Nepal. A paper presented in the International Conference and Great Himalayas: Climate, Health, Ecology, Management and Conservation, Kathmandu, Januar. pp: 12-15.