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In Vitro Anti-Oxidant and Anti-Inflammatory Potential of Marine Actinomycetes

Lavanyalatha.Y[1]. , Rashmi H.K.[2] , Uma Maheswari Devi P[1*]
Department of Applied Microbiology, Sri Padmavathi Mahila Visvavidyalayam
Tirupati , 517502, A.P.

Abstract:

Marine Actinomycetes are considered as the potent source for the production of pharmaceutically important secondary metabolites. The enormous bio chemical diversity of marine micro organisms renders as potential producers of bio active compounds. In the present work 15 strains of Actinomycetes were isolated from marine sediments collected from kottakodduru of Nellore district, Andhra Pradesh. Out of these 15 strains, the strain (KN8) showed potential activity and was used for the present study. Ethyl acetate extractions of Marine Actinomycetes derived from marine sediments were tested to investigate the level of anti oxidant potential by DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity and also for invitro Anti inflammatory activity by Red blood cells membrane stabilization. The results obtained suggest that the KN8 possess anti oxidant and anti-inflammatory metabolites and could be considered as a potential source for anti cancer drug development.

1 INTRODUCTION

Marine ecosystem is highly complex environment with extreme variations in temperature and salinity and is proved to be good habitat of diverse assemblage of microorganisms. Among the marine microbes, Actinomycetes are prominent in the pharmaceutical and medical industry due to their capacity to produce secondary metabolites with diverse biological activities. Marine actinobacteria are the most economically and pharmaceutically price less prokaryotes. The genera of actinobacteria represents Streptomyces, Actinomycetes, Corynebacterium, Frankia, Micrococcus and others[1] Literature is abound with isolation of thousands of bio active compounds which have been characterized and developed into drugs for the treatment of various diseases in humans. Although heavily studied over the last 10 years, Actinomycetes continue to prove themselves as prolific source of novel bio active metabolites [2] Actinomycetes are filamentous Gram +ve bacteria characterized by the presence of both bacterial and fungal characters. At present the researchers' are converged on the response of anti oxidant system of Bacteria, which is important in terms of biotechnology, such as Streptomyces growth in various oxidative stress condition[3].The studies with respect to antioxidant and anti inflammatory activities of marine actinobacteria are very limited and hence the present study is designated to investigate the anti inflammatory and antioxidant potential of KN8 isolated from Bay of Bengal of Andra Pradesh(A.P), India.

- *Corresponding author: Prof. P Uma Maheswari Devi, Head, Department of Applied Microbiology, Sri Padmavati Mahila Visvidyalayam, Tiruptiti, 517502.*
Email: umadevi66@yahoo.co.in,
Ph No: 9848894900

2 MATERIAL AND METHODS

2.1 Isolation and collection of Actinomycetes

Marine sediments were collected from kottakodduru of Nellore district (Andhra Pradesh) from a depth of 5m in `sterile containers. The samples were transported immediately to the laboratory and were air dried in laminar air chamber for 2hrs and then dried in hot air oven for about 25 min at 70oC]4[. 10gms of dried sediment soil was serially diluted (4) and inoculated onto starch casein agar, Nutrient glucose agar and Maltose yeast extract agar independently [5]. The media were supplemented with 5µg/ml Rifampicin and 25µg/ml Nystatin to minimize the growth of Bacteria and fungi[6].The samples were incubated at 30oC for 21 days to observe the growth on different media.

2.2 Bio chemical characteization of Marine Isolate

The enzymatic properties are useful to characterize the Actinomycetes. The isolate was inoculated on suitable medium by streaking and the plates were incubated at 30oC for 4 -7 days. Biochemical characterization like Indole Methyl Red, Voges-Proskauer, Citrate utilization and Nitarte reduction were performed and the Amylase, protease, Cellulase, Gelatinase, lipase and Urease activity of the isolate.

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2.3 Effect of carbon on the growth of marine isolate

To study the effect of carbon on the growth of marine isolate, sugars like starch, glucose, sucrose, maltose, xylose, fructose ,cellulose, and dextrose were used as the sole source of carbon(1%) in the basal medium [7]. The marine isolate was inoculated into the flasks containing basal medium with different carbon sources. The flasks were incubated at 30±2oC for 7 days, and the growth was observed.

2.4 Influence of amino acids on growth of marine isolate

The effect of various nitrogen sources on growth of marine isolate was studied by incorporating different nitrogen sources such as Leucine, Arginine, Phenylalanine, Cysteine and Isoleucine to the basal medium and incubated at 30±2oC for 7 days, and the pattern of growth was observed.

2.5 Extraction of secondary metabolites from marine isolate

The selected isolate was inoculated into the Bennet broth to extract the bio active metabolites and incubated for about 7 days at 30oC. After incubation the culture was harvested and centrifuged at 10,000rpm for 20 min at 4oC. The supernatant was collected and extracted twice with ethyl acetate (1:1ratio).The organic phase was collected and concentrated in rotary vacuum evaporator [8].The powdered sample was collected and weight of the sample was determined.

2.6 DPPH radical scavenging assay

The scavenging activity of the isolate was analyzed based on the scavenging of stable DPPH free radical [9]. Different concentrations (50, 75, 100 and 125µg/ml) of ethyl acetate extract was added to methanolic solution of 1ml of DPPH (0.1 mM). The mixture was shaken and allowed to stand at room temperature for 30 min and the absorbance was measured at517 nm using UV VIS spectrophotometer with methanol as blank [10]. The concentration of ethyl acetate extract required to scavenge 50 % of free radicals was determined and the scavenging activity was expressed as the percentage of inhibition and was calculated by using the formula,

Scavenging activity (%) = (Ac-At)/ Ac x100.

Where Ac is Mean absorbance of DPPH control and At is Mean absorbance of the extract.

2.7 Invitro anti inflammatory activity

Human red blood cell (HRBC) membrane stabilization method Fresh healthy human blood (10ml) was collected and was mixed with equal volumes of Alsevar solution (dextrose 2%,sodium citrate 0.8%,citric acid 0.05%, sodium chloride 0.42% and distilled water 100ml) and centrifuged with isosaline [11].To 1ml of HRBC suspension equal volumes of the ethyl acetate extract of different concentrations((100,200,300 and 400ug) was added. The assay mixtures were incubated at 37oC for 30min. After incubation the samples were centrifuged at 10000 rpm, the supernatant was collected and estimated at 560 nm.

% of Haemolysis=OD of control-OD of sample/OD of control x 100

The % of protection can be hence calculated from the equation as given below.

% of Protection =100-OD of test /OD of control x100

OD of test = test sample absorbance.

OD of control = absorbance of negative control (Alsevers solution with blood in it and it contained no Aspirin or extract).

RESULTS AND DISCUSSION

At present, the researchers have started gazing towards marine organisms for novel drugs from natural products. Many structurally unique compounds with diverse biological activities have been derived from marine sources. During the present study, The actiobacterial colonies were enumerated from the sediment samples of Kottakoddur, Nellore district A.P. Amon the 15 distinct morphological strains, the isolate KN8 was used for further study.

Biochemical characterization of KN8

All the media used were proved to be the good source for the growth of KN8 isolate (Fig 1). The KN8 isolate was identified at generic level on the colony, microscopic and biochemical characteristics as shown in Table1. Based on the growth on different media, morphological, microscopic and biochemical characteristics the KN8 isolate was identified as Nocardia species strain KN8. The KN8 isolate demonstrated Amylolytic, Gelatinase, Lipase (Fig2.) and Cellulase activity (Table2.) was not observed with isolate KN8. KN8 showed maximum growth in glucose as carbon (Table3) source and Arginine as nitrogen source (Table4)

Anti oxidant activity of isolate KN8

DPPH is a stable free radical to form a stable diamagnetic molecule. Therefore DPPH has been used extensively as free radicals to evaluate reducing substances and DPPH is useful for determining the free radical scavenging activities of marine actiomycetes (Navya et al 2013). Ethyl acetate extract of Nocardia species KN8 strain showed significant DPPH radical scavenging activity at all concentrations and IC 50 was observed at 50µg/ml(Table5.). Sargassum wightii a brown alga derived marine sources showed DPPH radical scavenging with 50% at 1.01mg/ml concentration(Neelakandan

yuvarag and venkatesan arul 2014). The scavenging activity was increased with increasing concentrations of ethyl acetate extract. The present data strongly suggest the anti oxidant potential of Nocardia species strain KN8.

3.3 Anti inflammatory activity of isolate KN8.

Lysis of membrane is well documented during the process of inflammation (Govindappa etal 2011).The ethyl acetate extract showed 33% of protection at a concentration 400µg/ml as compared with standard Aspirin which showed 38% of protection at 100µg/ml (Table6). The present data indicates the presence of Nocardia species in the marine sediments of Bay of Bengal A.P, India. The ethyl acetate extract showed significant anti oxidant activity by scavenging DPPH free radical and anti-inflammatory activity through stabilization of membrane permeability.

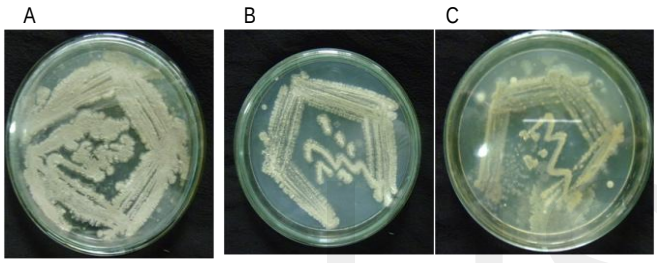

Figure1.Pure cultures of KN8 on A) Nutrient Agar B) Starch Casein agar and C) maltose Yeast Agar

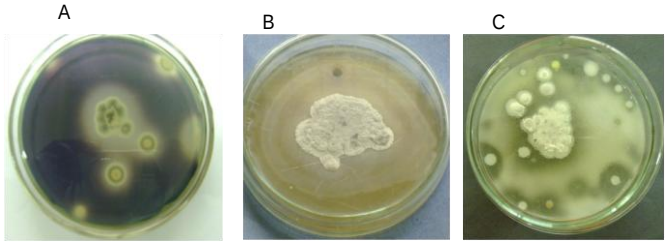

Figure2. A) Amylolytic B) Lipase and C) Gelatinase activity of KN8

Table1. Biochemical Characterization of KN8

SL NO	Biochemical test	Result
1	Indole	-ve
2	Methyl Red	+ve
3	Voges-Proskauer	-ve
4	Citrate	+ve
5	Nitrate Reduction	+ve

Table 2: Enzymatic activities of KN8

SL NO	Enzymatic activities	KN8
1	Amylase	+ve
2	Gelatinase	+ve
3	Lipase	+ve
4	Cellulase	-ve

Table3. Effect of Carbon source on the growth of Isolate KN8.

SL NO	Carbon source	KN8
1	Glucose	+++
2	Maltose	+
3	Xylose	+
4	Sucrose	+
5	Fructose	+
6	Cellulose	-
7	Dextrose	++
8	Starch	++

Table4. Effect of Nitrogen source on the growth of isolate KN8

SL NO	Nitrogen source	KN8
1	Leucine	-ve
2	Arginine	+ve
3	Phenylalanine	-ve
4	Cysteine	-ve
5	Isoleucine	-ve

Table5. Effect of ethyl acetate extract of Isolate KN8 on DPPH scavenging activity

SL NO	Concentration inμg/ml	% of scavenging activity
1	50	58
2	75	64
3	100	70
4	125	77

Table6. Effect of ethyl acetate extract of Isolate KN8 on membrane stabilization

SL NO	Concentration in μg/ml	% of protection
1	100	10
2	200	24
3	300	27
4	400	33
5	Aspirin	38

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