IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 11, Issue 2 Ver. II (Mar.- Apr.2016), PP 45-51
www.iosrjournals.org

Evaluation of Antimicrobial Potential of *Saccharomonospora oceani* VJDS-3: A Study on optimization of Fermentation Parameters

Mani Deepa Indupalli, Vijayalakshmi Muvva*, Rajesh Kumar Munaganti
Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur-522 510, A.P., India.

Abstract: *A study has been undertaken to isolate potent rare actinobacteria from Mangrove ecosystem of Nizampatnam, Guntur district of Andhra Pradesh, India. Among the 20 different actinobacterial strains isolated, one potent strain with broad spectrum antagonistic activity was identified as Saccharomonospora oceani VJDS-3. Attempts were made to optimize the cultural parameters for enhanced antimicrobial metabolite production. Production of bioactive metabolites by the strain was high in yeast extract malt extract dextrose broth as compared to other tested media. The strain utilized maltose and tryptone as good carbon and nitrogen sources for the elaboration of bioactive metabolites. The optimum temperature and pH for bioactive metabolite production of the strain were recorded as 30^oC and 7.0 respectively. The secondary metabolites produced by the strain grown under optimal conditions exhibited high antagonistic activity against a variety of Gram positive, Gram negative bacteria and fungi. This is the first report on the optimization studies of bioactive metabolite production by Saccharomonospora oceani.*

Key words: *Mangrove ecosystem, Saccharomonospora oceani, optimization, bioactive metabolites.*

I. Introduction

Microbial resources have made an incredible contribution to the antibiotic drug discovery and development process over the last few decades [1]. In particular, actinobacteria are the most important source of bioactive natural compounds with a long track record of producing novel molecules. However drug resistant superbugs including Vancomycin Resistant Enterococci (VRE), Methicillin Resistant *Staphylococcus aureus* (MRSA), Extended Spectrum ß-lactamase (ESBLs) producing Gram negative bacteria, *Klebsilla pneumonia,* Carbapenemases (KPC) producing Gram-negatives, Imipenem resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa* became very common now-a-days. The multiple drug resistance problem demands that a renewed effort be made to seek antimicrobial agents effective against these multi drug resistant organisms.

Although the diversity of life in the terrestrial environment is extraordinary, the greatest biodiversity is in the oceans [2]. Microbial assemblages and their species distribution in marine environments are mostly determined by specific environmental conditions, which may translate into novel chemistry [3]. Marine actinobacteria are potential sources of bioactive compounds, and the work done so far has shown that these microbes are the richest source of secondary metabolites. They hold a prominent position as targets in screening programs due to their diversity and proven ability to produce novel metabolites [4]. The application of bioprospecting strategy with a combination of selective isolation, strain dereplication and screening leads to the discovery of new natural products from novel rare actinomycetes isolated from geographically diverse samples. The relevance of the rare actinomycetes in this regard can also be demonstrated by the fact that many of the successful antimicrobial agents such as rifamycins produced by *Amycolatopsis mediterranei*, erythromycin by *Saccharopolyspora erythraea* which are currently available in the market [5]. Eventually, the focus of industrial screening has also shifted to the members of other lesser-exploited prolific groups of rare actinomycetes such as *Actinomadura, Amycolatopsis, Dactylosporangium, Kibdelosporangium, Saccharomonospora* etc.

In view of the significant contribution being made by the rare actinomycetes to the novel chemistry, we have been exploring the diversity of our near marine ecosystems. In this survey a rare actinobacterium with potential antagonistic activity was isolated from Mangrove ecosystem of Nizampatnam, Guntur district, and identified as *Saccharomonospora oceani* VJDS-3. With an intention to improve the antagonistic strategy of the strain attempts were made to optimize various cultural parameters.

II. Materials And Methods

Isolation and screening of actinomycetes

Mangrove soils collected from the Nizampatnam, Guntur district were used for the isolation of novel actinomycetes. The pretreated, serially diluted samples were plated on Humic acid vitamin (HV) agar and yeast extract malt extract dextrose (YMD) agar media. The actinomycete colonies, which appeared different from one another to the naked eye (surface texture) were transferred and maintained on YMD agar (yeast extract, 0.4%;

malt extract, 1%; dextrose, 0.4%; agar, 2%; pH, 7.2) slants at 4°C [6]. A total of 20 distinct actinomycete strains were picked and screened for their potential antagonistic activity by employing primary and secondary screening techniques. One promising strain with potential activity was identified as *Saccharomonospora oceani* VJDS-3. The test organisms used in the present study were procured from ATCC, University Boulevard, Manassas, USA, MTCC, IMTECH, Chandigarh, India and NCIM, Pune and preserved at 4^{o}C.

Selection of the production medium

From the screening experiments, strain VJDS-3 showing broad spectrum antimicrobial activity against the test organisms was selected for further studies. To select the suitable growth medium, the strain was grown in 8 different culture media, such as tryptone-yeast extract broth (ISP-1), YMD broth (ISP-2), Oat-meal broth (ISP-3), Inorganic salts Starch broth (ISP-4), glycerol-asparagine broth (ISP-5), tyrosine broth (ISP-7), starch-casein salts broth and starch yeast extract broth [7]. The biomass accumulation and bioactive metabolite production by the strain were determined after 8 days of incubation. The medium in which the strain exhibited maximum bioactive metabolite production was fixed for further studies.

Growth pattern and antimicrobial activity of the strain *S.oceanii* VJDS-3

The growth pattern and antimicrobial profile of *S.oceani* was studied at regular intervals for up to 11 days. The strain was inoculated into 250 ml flasks containing 100 ml YMD broth and incubated at 30 ±2 °C for optimum yields on a rotary shaker at 120 rpm. At every 24 h interval, dry weight of the biomass of the strain and production of antimicrobial metabolites were determined. The culture filtrates were extracted with ethyl acetate and antimicrobial activity of crude extract was determined by agar well diffusion method. The production of bioactive metabolites was assessed by measuring the diameter of the inhibition zone against the test bacteria and fungi mentioned below.

Gram positive bacteria - ***Bacillus megaterium*** **(NCIM 2187).**
Gram negative bacteria - ***Xanthomonas campestris*** **(MTCC 2286),** ***Pseudomonas aeruginosa*** **(ATCC 9027)** and ***Escherichia coli*** **(ATCC 35218).**
Fungi - ***Candida albicans*** **(MTCC 183).**

Optimization studies

Bioactive metabolite production by *S.oceani* VJDS-3 was optimized by employing different cultural parameters viz., pH, temperature, carbon and nitrogen sources and minerals.

Effect of initial pH and incubation temperature on biomass and bioactive metabolite production

Influence of initial pH on growth and bioactive metabolite production of the strain was determined by adjusting the pH of production medium ranging from 4-10. The optimal pH achieved at this step was used for further study [8]. Similarly, the optimum temperature for growth and bioactive metabolite yield was measured by incubating the production medium at temperatures ranging from 20-40ºC, while maintaining all other conditions at optimum levels [9].

Effect of supplementary carbon and nitrogen sources on biomass and bioactive metabolite production

Carbon sources such as maltose, glycerol, fructose, galactose, lactose, mannitol, starch, sucrose and xylose @1% were supplemented separately into the fermentation medium in order to study the influence on growth and bioactive metabolite production. The influence of varying concentrations (0.5-4%) of the best carbon source on the growth and bioactive metabolite production was also examined. Similarly, various nitrogen sources, such as urea, glycine, glutamine, casein, asparagine, tryptone, peptone, ammonium oxalate and ammonium sulphate at 0.5% were individually supplemented into the fermentation medium [10]. Further, the impact of different levels (0.1- 1.5%) of optimized nitrogen source was studied to enhance antimicrobial metabolite production [11].

Effect of minerals on biomass and bioactive metabolite Production

Impact of minerals on the production of biomass and bioactive metabolites was determined by supplementing different minerals such as K_2HPO_4, KH_2PO_4, KCl, $MgSO_4$, $FeSO_4$, $MnCl_2$ and NaCl each at a concentration of 0.05% (w/v) in to the production medium [9].

Statistical analysis

Statistical data are recorded on biomass and antimicrobial metabolite production by using One-way Analysis of Variance (ANOVA).

Test organisms

The Antimicrobial metabolites produced by the strain under optimized conditions were tested against bacteria including *Staphylococcus aureus* (MTCC 3160), *Bacillus megaterium* (NCIM 2187), *Xanthomonas campestris* (MTCC 2286), *Proteus vulgaris* (MTCC 7299), *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 35218) and *Streptococcus mutans* (MTCC 497) grown overnight at 37^{o}C for antibacterial assay while *Candida albicans* (ATCC 10231), *Aspergillus niger, A. flavus, Fusarium solani, F. oxysporum* (MTCC 3075) and *Penicillium citrinum* were used as test fungi by using agar plate diffusion assay [12].

Nucleotide Sequence accession number

The 16S rRNA gene (rDNA) sequence of the strain VJDS-3 has been deposited in the NCBI GenBank with the accession No **KP170478.**

III. Results And Discussion

Growth pattern and antimicrobial profile of the strain

The growth curve and antimicrobial profile of *S.oceani* VJDS-3 was studied at regular intervals for up to 11 days in batch culture. The stationary phase of *S.oceani* VJDS-3 extended from 168 h to 216 h of incubation. The secondary metabolites obtained from eight-day old culture showed high antimicrobial activity against the test microbes (Fig.1). However the seven day culture extracts of *Saccharomonospora halophila* and *Saccharomonospora paurometabolica* isolated from Algerian Sahara soils exhibited strong antimicrobial activity against Gram negative bacteria [13]. Munaganti *et al*., (2015) noted the production of antimicrobial metabolites from five day old culture of *Rhodococcus erythropolis* VL-RK_05 [14]. Naragani *et al*., (2014) reported that five day old culture extracts of *Streptomyces violaceoruber* VLK-4 evidenced the production of antimicrobial compounds [15]. Narayana *et al*., (2008) stated that *Streptomyces albidoflavus* elaborated maximum antimicrobial metabolites production after 120h [16]. The secondary metabolites obtained from four-day old culture of *Nocardia levis* MK-VL_113 isolated from laterite soils of Guntur showed high antimicrobial activity against the test microbes [17].

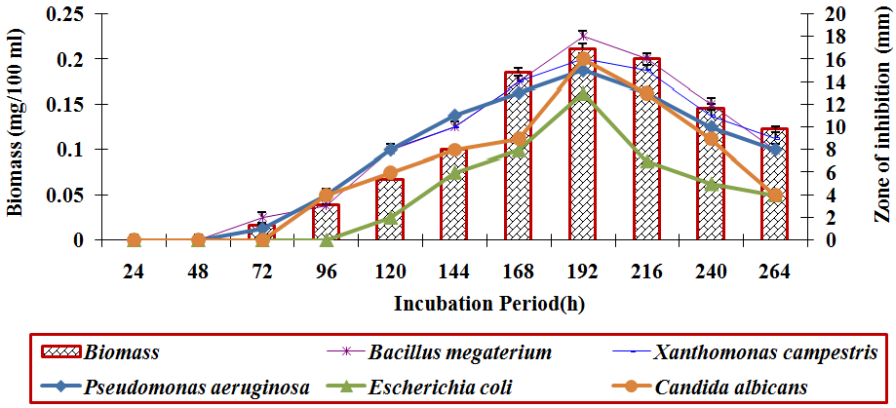

Fig.1 Effect of incubation period on biomass and bioactive metabolite production by *Saccharomonospora oceani* VJDS-3.
(Data are statistically analyzed and found significant at 5 %)

Selection of culture media suitable for biomass and bioactive metabolite production:

The influence of different media on the production of biomass and bioactive metabolites was recorded (Fig.2). Among the media tested, YMD broth supported high bioactive metabolite production followed by inorganic salts starch broth and tyrosine broth.

Effect of initial pH and incubation temperature on biomass and bioactive metabolite production:

The various environmental requirements influence growth and bioactive metabolite production by actinomycetes. Maximum growth and antimicrobial metabolite production was obtained at pH 7 (Fig.3). The influence of temperature on the biomass and bioactive metabolite production of the strain is presented in fig.4. Good growth as well as anti-microbial metabolite production was obtained at 30°C. The organism appeared to be mesophilic in terms of its optimum temperature for growth. Several strains of Actinobacteria belonging to the genus *Streptomyces* including *S.galbus* [18], *S.rochei* G164 [19], *S.hygroscopicus* [20], *S.marinensis*, *S.fradiae*, *S.lavendualae*, *S.fulvissimus* showed optimum levels of antibiotic production at 30^{o}C [21].

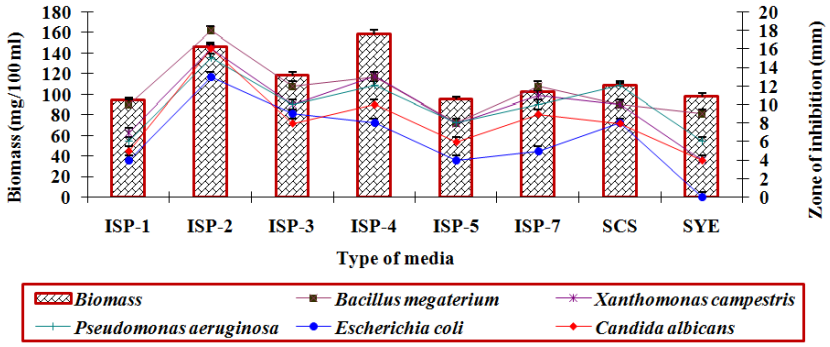

Fig. 2 Effect of different growth media on biomass and bioactive metabolite production by *Saccharomonospora oceani* VJDS-3.
(Data are statistically analyzed and found significant at 5 %)

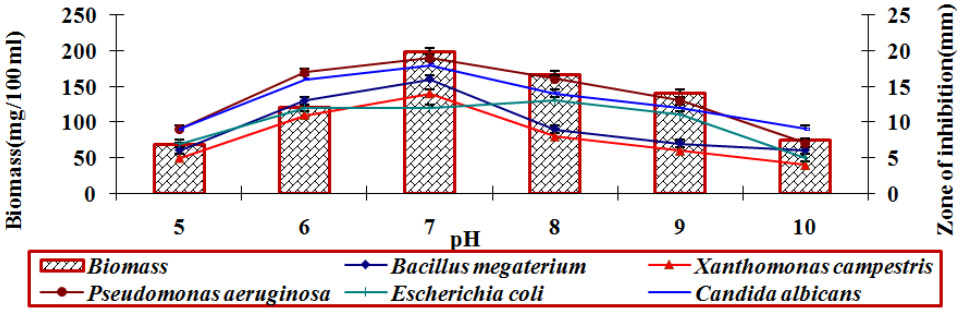

Fig. 3 Effect of pH on biomass and bioactive metabolite production by *Saccharomonospora oceani* VJDS-3.
(Data are statistically analyzed and found significant at 5 %)

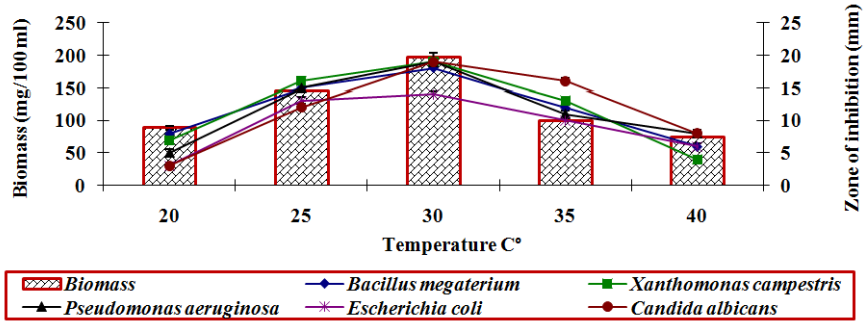

Fig. 4 Effect of temperature on biomass and bioactive metabolite production by *Saccharomonospora oceani* VJDS-3.
(Data are statistically analyzed and found significant at 5 %)

Effect of supplementary carbon and nitrogen sources on biomass and bioactive metabolite production

Effects of different carbon and nitrogen sources were evaluated for their impact on growth and antimicrobial metabolite production (Figs.5 and 6). Among the various carbon sources tested, maltose was the best one for bioactive metabolite production followed by glycerol and fructose. Glycerol followed by maltose supported the growth of the strain. As maltose was identified as the most preferred carbon source for bioactive metabolite production by the strain, different levels of maltose (0.5-4%) were tested to determine optimal concentration for bioactive metabolite production (Fig.7). One percent maltose supplemented in the medium promoted the bioactive metabolite production.

Francois and Stephane (2001) suggested that growth and antibiotic production were found to be governed by nitrogen sources and the utilization of nitrogen sources for the production of bioactive metabolites seems to be different among actinomycete strains [22]. With reference to the nitrogen sources maximum antimicrobial activity was obtained in culture filtrates supplemented with tryptone followed by glutamine

(organic nitrogen source), where as biomass was high with casein followed by asparagine (Fig.6). Tryptone (0.5%) supported good growth as well as metabolite production by the strain (Fig.8).

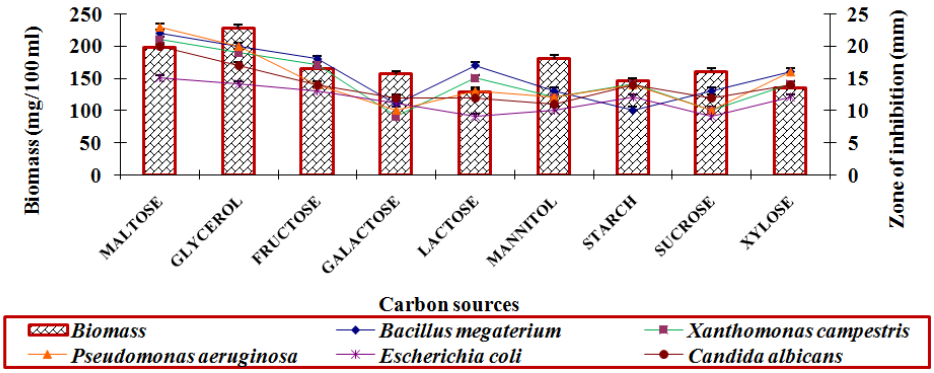

Fig. 5 Effect of different carbon sources on growth and bioactive metabolite production by *Saccharomonospora oceani* VJDS-3.
(Data are statistically analyzed and found significant at 5 %)

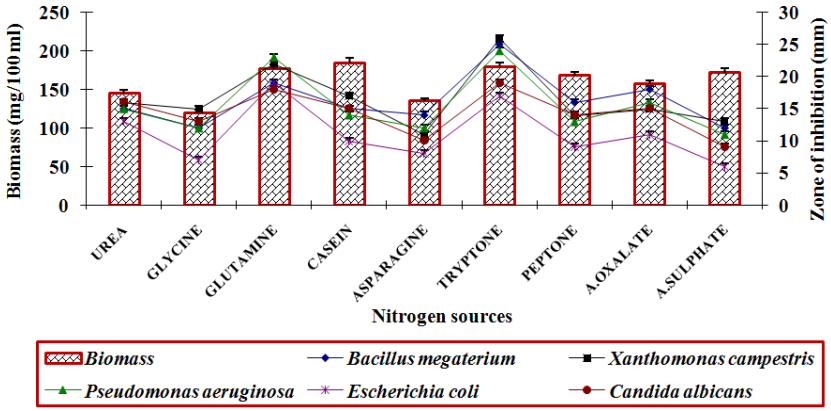

Fig. 6 Effect of different Nitrogen sources on growth and bioactive metabolite production by *Saccharomonospora oceani* VJDS-3.
(Data are statistically analyzed and found significant at 5 %)

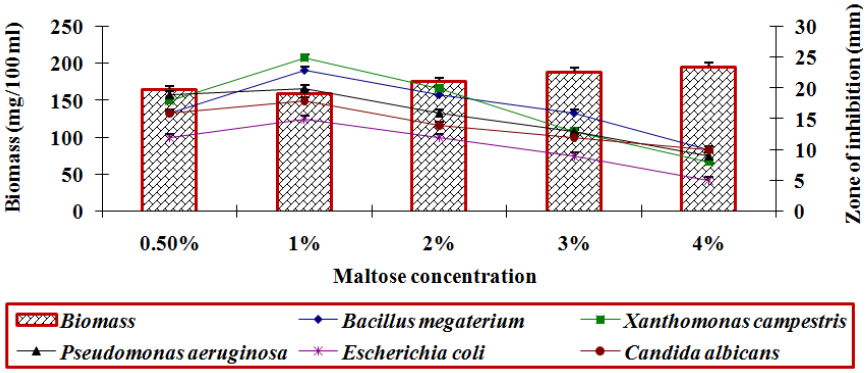

Fig. 7 Effect of Maltose concentration on growth and bioactive metabolite production by *Saccharomonospora oceani* VJDS-3.
(Data are statistically analyzed and found significant at 5 %)

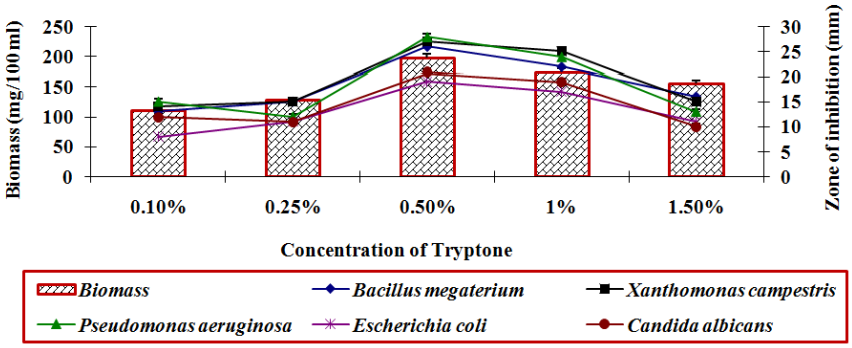

Fig. 8 Effect of different concentrations of Tryptone on growth and bioactive metabolite production by *Saccharomonospora oceani* VJDS-3.
(Data are statistically analyzed and found significant at 5 %)

Effect of minerals on biomass and bioactive metabolite production:

The influence of minerals on biomass and bioactive metabolite production by the strain is represented in fig.9. K_2HPO_4 enhanced the production of biomass and antimicrobial metabolites while the production of bioactive metabolites was very low with KCl. Maximal production of neomycin was reported in *Streptomyces fradiae* with mineral supplements of K_2HPO_4 [10].

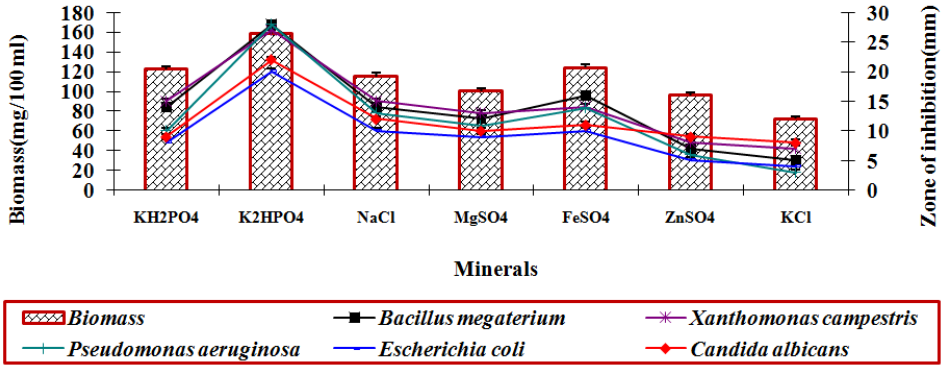

Fig.9 Effect of different minerals on biomass and bioactive metabolite production by *Saccharomonospora oceani* VJDS-3.
(Data are statistically analyzed and found to be significant at 5 %)

IV. Conclusion

In the present study, *Saccharomonospora oceani* VJDS-3 isolated from Mangrove ecosystems exhibited high antimicrobial activity when cultured in ISP-2 broth amended with Maltose (1%), Tryptone (0.5%) and K_2HPO_4 (0.05%) with pH 7.0 and incubated at 30°C for 192 h. Among the bacteria tested, *Pseudomonas aeruginosa* was highly sensitive followed by *Xanthomonas campestris* while *Candida albicans* exhibited high sensitivity followed by *Fusarium oxysporum* in case of fungi (Fig.10, 11). Consequently, further studies on purification, characterization and identification of bioactive metabolites of *Saccharomonospora oceani* VJDS-3 are in progress. It is the first report on the optimization of cultural parameters for improved bioactive metabolite production by *Saccharomonospora oceani* VJDS-3.

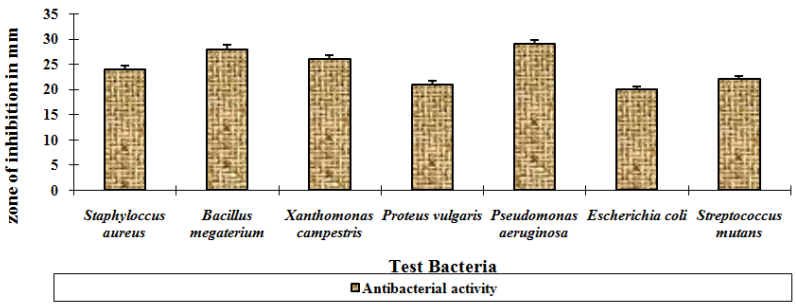

Fig.10 Antibacterial activity of *Saccharomonospora oceani* VJDS-3 cultured under optimized conditions
(Data are statistically analyzed and found significant at 5 %)

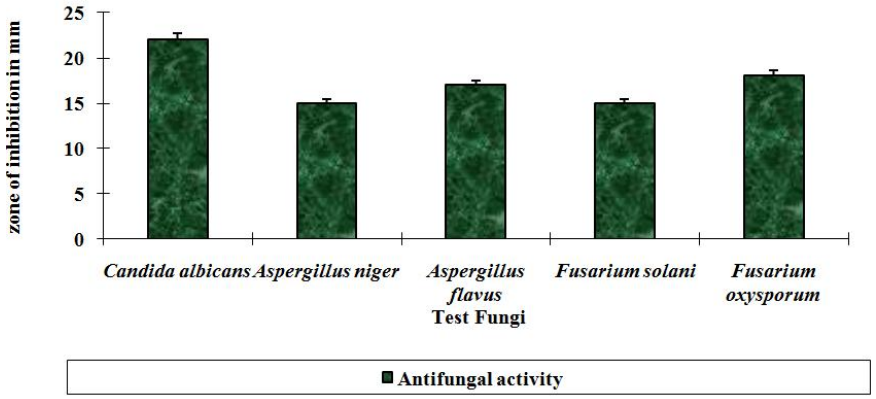

Fig.11 Anti fungal activity of *Saccharomonospora oceani* VJDS-3 under optimized conditions (Data are statistically analyzed and found significant at 5 %)

V. Acknowledgements

The authors are thankful to UGC-BSR-SAP for providing the financial support to carry out the research work and also to the Department of Botany and Microbiology for providing the laboratory facilities.

References

[1]. Sanchez S, Demain AL. Metabolic regulation of fermentation processes. Enzyme Microbial Technol, 2002, 31(7):895-906.
[2]. Donia M, Hamann MT. Marine natural products and their potential applications as anti-infective agents. Lancet Infect Dis, 2003, 3(6):338-48.
[3]. Genilloud, O., Gonzalez, I., Salazer, O., Martin, J., Tormo, J.R. and Vicente, F. Current Approaches to Exploit Actinomycetes as a Source of Novel Natural Products. J Ind Microbiol Biotechnol, 2011, 38:375-389.
[4]. Ellaiah P, Ramana T, Raju K, Sujatha P, Sankar A. Investigations on marine actinomycetes from bay of Bengal near Kakinada coast of Andhra Pradesh. Asian J Microbiol Biotechnol Environ Sci, 2004, 6:53-56.
[5]. Lazzarini A, Cavaletti L, Toppo G, Marinelli F. Rare genera of actinomycetes as potential producers of new antibiotics. Antonie van Leeuwenhoek, 2001, 79:399-405.
[6]. Usha Kiranmayi M, Sudhakar P, Sreenivasulu K, Vijayalakshmi M. Optimization of Culturing Conditions for Improved Production of Bioactive Metabolites by Pseudonocardia sp. VUK-10. Mycobiol, 2011, 39:174-181.
[7]. Krishna N, Rajesh kumar M, Usha Kiranmayi M, Vijayalakshmi M. Optimization of Culture Conditions for Enhanced Antimicrobial Activity of Rhodococcus erythropolis VLK-12 Isolated from South Coast of Andhra Pradesh, India. Br. Microbiol Res J 2014;4(1): 63-79.
[8]. Oskay M. Effects of some environmental conditions on biomass and antimicrobial metabolite production by Streptomyces sp. KGG32. Int J Agric Biol, 2011, 13:317-24.
[9]. Ripa FA, Nikkon F, Zaman S, Khondkar P. Optimal conditions for antimicrobial metabolites production from a new Streptomyces sp. RUPA-08PR isolated from Bangladeshi soil. Mycobiol, 2009, 37:211-4.
[10]. Majumdar MK, Majumdar SK. Effect of minerals on neomycin production by Streptomyces fradiae. Appl Environ Microbiol, 1967, 13:190-3.
[11]. Kathiresan K, Balagurunathan R, Selvam MM. Fungicidal activity of marine actinomycetes against phytopathogenic fungi. Indian J Biotechnol, 2005, 4:271-6.
[12]. Cappuccino JG, Sherman N. Microbiology laboratory manual. New Delhi: Pearson Education Inc, 2004 p: 282-283.
[13]. Boudjelal F, Zitouni A, Mathieu F, Lebrihi A, Sabaou S. Taxonomy and antimicrobial activities of two novel halophilic Saccharomonospora strains isolated in Algerian Sahara soils. Ann Microbiol, 2011, 61:299-305.
[14]. Munaganti RK, Naragani K, Muvva V. Antimicrobial Profile of Rhodococcus Erythropolis VL-RK_05 Isolated from Mango Orchards. Int J Pharm Sci Res, 2015, 6(4):1805-12.
[15]. Naragani K, Rajesh Kumar M, Vijayalakshmi M. Antimicrobial Potential of Streptomyces violaceoruber VLK-4 Isolated from South Coast of Andhra Pradesh, India. Int J Pharm Sci Rev Res, 2014, 25:125-129.
[16]. Narayana KJP, Vijayalakshmi M. Optimization of Antimicrobial metabolites production by Streptomyces albidoflavus. Res J Pharmacol, 2008, 2(1): 4-7.
[17]. Kavitha A, Vijayalakshmi M. Cultural parameters affecting the production of bioactive metabolites by Nocardia levis MK-VL-113. J Appl Sci Res, 2009, 5:2138-2147.
[18]. Paul, A.K. and Banerjee, A.K. Determination of optimum conditions for antibiotic production by Streptomyces galbus. Folia Microbiol, 1983, 28:397-405.
[19]. Chattopadhyay D, Sen SK. Optimization of cultural conditions for antifungal antibiotic accumulation by Streptomyces rochei G164. Hindustan Antibiot Bull, 1997, 39: 64-71.
[20]. Bhattacharyya BK, Pal SC, Sen SK. Antibiotic production by Streptomyces hygroscopicus D1.5: cultural effect. Revista de Microbiologia, 1998, 29:49-52.
[21]. Rizk M, Tahany AM, Hanaa M. Factors affecting growth and antifungal activity of some Streptomyces species against Candida albicans. Int j food agric environ, 2007, 5:446-449.
[22]. Francois V, Stephane A. Nitrogen source governs the patterns of growth and pristinamycin production in Streptomyces pristinaespiralis. Microbiol, 2001, 147:2447-2459.