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Biological activities of marine Actinobacteria isolated from West Coast of Kerala and Goa: A screening and pilot scale optimization study

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Abstract

The diversity and the distribution of extra-cellular enzymes and antifungal compound producing marine actinobacteria isolated from the Arabian Sea (west coast of India), Kerala and Goa were studied. A total of 46 actinobacterial isolates were obtained from different soil samples, and all the isolates were preliminarily characterized morphologically by using colonial and microscopic properties. All the isolates were screened for the production of extra-cellular enzymes namely amylase, chitinase, protease and xylanase. The actinobacteria with potential enzyme producers were also evaluated for their antifungal activity against some fungi namely *Colletotrichum fulgatum, Fusarium semitectum* and *Trichoderma viridae* by agar disc method. The suitable conditions for maximum enzyme production were optimized with respect to pH, temperature, salinity and incubation period. The actinobacterial isolates with prominent enzyme and antifungal activities were further characterized using cultural, biochemical, chemotaxonomical and physiological properties and identified as *Streptomyces* sp. KA1-3 and *Streptomyces* sp. KA2-2.

Key words: Actinobacteria, Antifungal activity, Characterization, Enzyme activity, Optimization, West coast of India.

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INTRODUCTION

Plant pathogenic fungi cause serious problems worldwide to the growers and agricultural scientists. The current practice of the use of the agrochemicals/ chemical fungicides in agriculture has led to human health and environmental issues (Nannipieri, 1994; Pal and Gardener, 2006). The harmful and hazardous impacts of pesticides on natural ecosystems have made scientists consider seriously about alternative strategies such as powerful fungicides of natural origin, which should help to make a safer, more acceptable environment. With this background, biological control has been widely studied as an alternative method of controlling plant diseases, since the maximizing use of fungicides has caused development of pathogenic microbial resistance, problems with environmental pollution, and human and animal health risks. Microbe-based biocontrol agents, as a replacement or supplement for agrochemicals, have been addressed in many recent reports (Dhingra and Sinclair, 1995; Shimuzu *et al*., 2000; Vijayakumar *et al*., 2012a).

According to Goodfellow and Williams (1983) actinobacteria are among the most studied noteworthy biocontrol agents, which are worldwide familiar for the production of antibiotics and bioactive compounds, which act in the control of plant pathogens (Compant *et al*., 2005). Totally, 85% of the known commercial metabolites are produced by actinobacteria which includes chemically diverse compounds such as biopolymers like lignocellulose, hemicellulose, pectin, keratin and chitin, enzymes and other bioactive molecules with a wide range of biological activities, and are of considerable importance in industry (Stutzenberger and Berdine, 1992). Among the actinobacteria, especially those belonging to the genus *Streptomyces* are studied for their bioactive compound producing potentiality (Schrempf, 2001; Thirumurugan *et al*., 2018). Further, streptomycetes are of medical and industrial importance because, they synthesize over 9500 important antibiotics including streptomycin, neomycin, chloramphenicol, tetracyclines, etc., (Keiser *et al*., 2000) and numerous enzymes with notable agricultural and other industrial applications. Comparatively, marine actinobacteria are more important sources of novel bioactive compounds than terrestrial species (Newman *et al*., 1989). In addition, a wide array of enzymes and their products from various genera of actinobacteria are applied in biotechnological industries and biomedical fields and have been reported. Actinobacteria have been continuously employed for the production of proteases, cellulases, chitinases, amylases, xylanases

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and others (Prakash *et al.*, 2013). Thus, the present study deals with extra-cellular enzyme production and antifungal potentiality of actinobacteria isolated from the west coast of Goa and Kerala, India, and the morphological, cultural, physiological and chemo-taxonomical characterization of the selected isolates.

MATERIALS AND METHODS

Soil sample collection

Totally six different soil samples were collected from the sampling stations namely Alapuzha beach, Varkala beach, Fort Kochi beach and Kollam beach from Kerala, and Vagatore beach and Baga beach from Goa, Arabian Sea, West Coast of India for the isolation of marine actinobacteria. The soil samples were collected from the above mentioned sites at a depth of 10-20 cm below from soil surface and aseptically transferred in to sterile polythene bags. Soil samples were brought to the laboratory and stored at 4°C for further process.

Isolation of actinobacteria

Starch casein agar (SCA) plates were prepared with the supplementation of griseofulvin 50 µg/L to prevent the growth of bacterial and fungal contaminants. The collected soil samples were diluted up to 10^{-6} and 0.1 mL of the diluted samples was spread over the SCA plates. The plates were incubated at 28°C for 7-10 days (Vijayakumar *et al.*, 2007). After incubation, the actinobacterial colonics were observed, purified using streak plate method and maintained in SCA medium for further assay.

Morphological characterization of actinobacteria

Colony morphology of actinobacterial isolates on SCA medium was recorded with respect to the colour, aerial mycelium, size and nature of colonies, reverse side colour and pigmentation as per standard methods of Shirling and Gottlieb (1966). Microscopically, actinobacterial isolates were identified upto generic level using cover slip culture technique by the method as described by Pridham *et al*. (1958). The morphological features of spores, sporangia and aerial and substrate mycelia were observed, and the identity of actinobacteria was compared with the standard manual of Bergey's Manual of Determinative Bacteriology and confirmed.

Screening of actinobacteria for extra-cellular enzyme production

Amylase activity

Amylase producing ability of the actinobacteria was carried out as per the standard method of Ellaiah *et al.* (2002). A single line streak of the actinobacterial cultures was made on starch agar medium and incubated at 28°C for 7 days. After incubation, the plates were flooded with Lugol's iodine. Hydrolysis zones were observed and the results were recorded.

Chitinase activity

A single line streak of the cultures made on chitin mineral agar medium (Abdel- Fatah, 1995) and incubated for 7 days at 28°C. After incubation, the hydrolysis zones were observed and the results were recorded.

Protease activity

A single line streak of the cultures were made on skim milk agar medium (Ellaiah *et al*., 2002) and incubated for 7 days at 28°C. After incubation, the hydrolysis zones were observed and the results were recorded.

Xylanase activity

A single line streak of the cultures were made on nutrient agar medium supplemented with 1% xylan (Ball and McCarthy, 1989). After incubation at 28°C for 7 days, the hydrolysis zones were observed and the results were recorded. Further, the enzyme producing ability of the selected higher enzyme producing actinobacteria were enriched by the optimization of the suitable cultural conditions such as pH, temperature and NaCl concentration.

Optimization of enzyme activity

Effect of pH

The liquid enzyme assay media namely starch, chitin mineral, skim milk and nutrient media with 1% xylan were prepared for amylase, chitinase, protease and xylanase enzyme production respectively. The initial pH of the production media was adjusted to 5, 6, 7, 8 and 9 using 0.1 N HCl and NaOH, and the actinobacteria were inoculated into the appropriate media and incubated for 7 days at 28°C. Then the broth was filtered through Whatmann No.1 filter paper. The filtrate was centrifuged at 8000 rpm (Model Superspin R-V/Fm, Plastocrafts) for 20 min at 4°C. Cell free supernatant was filtered aseptically through a sterile Millipore filter (Millipore Millex - HV hydrophilic PVDF) with 0.45 µm pore size in order to remove the left actinobacterial spores and to obtain cell free metabolite suspension. The culture filtrates were centrifuged, and OD value of the supernatant was measured at 450 nm in UV spectrophotometer (Elico SL-159, UV Spectrophotometer) (Dhevendaran and Annie, 2002). One unit of enzyme was expressed as the amount of enzyme required for an increase in 1.0 absorbance unit per minute (Adhi *et al*., 1989).

Effect of temperature

The different liquid enzyme assay media were prepared and the actinobacterial culture blocks were

inoculated, and incubated at different temperature such as 20, 25, 30, 35 and 40°C for 7 days. The enzyme activities of the actinobacteria were measured as previously mentioned.

Effect of NaCl

The different enzyme assay liquid media were prepared with different NaCl concentrations (0, 1, 2, 4 and 6%) and the actinobacterial culture were inoculated and incubated for 7 days. The enzyme activities of the actinobacteria were measured as previously mentioned.

Screening of antifungal activity: Agar disc method

The actinobacterial isolates were streaked on SCA medium. After incubation at 28°C for 4-6 days, well grown actinobacterial discs (6 mm) were aseptically transferred to potato dextrose agar (PDA) plates containing lawn cultures of fungal pathogens namely *Colletotrichum fulgatum*, *Trichoderma viridae* and *Fusarium semitectum*. Plates were incubated at 28°C for 4-6 days and antifungal activity was evaluated as recommended by Dhingra *et al*. (1995). The actinobacteria with antifungal activity were selected for further confirmation of its ability to produce antifungal metabolites using poisoned food technique.

Extraction of antifungal compound

The selected actinobacterial isolates were inoculated into starch casein broth and incubated at 28°C in a shaker at 200-250 rpm for 7 days. After incubation, the metabolite suspension was prepared as described earlier by Vijayakumar *et al.* (2012a). The metabolite suspension was transferred to sterile glass bottles and stored at 4°C for further assay.

Antifungal assay

Antifungal assay of the culture filtrates of actinobacteria against test fungi were carried out by poisoned food technique (Grover and Moore, 1962). PDA medium was prepared with different concentrations (0.5, 1, 1.5 and 2%) of cell free extract of selected actinobacteria. Simultaneously, the biocontrol efficiency of actinobacteria was compared with chemical fungicide namely hexaconazole EC (0.02%). The fungal mycelial discs (6mm) were placed at the centre of the plate. A control plate without extract was inoculated with fungal mycelial discs. The plates were incubated at 28°C for 5 days. The plates were observed for the inhibition of fungal growth at 24 h interval by measuring the radial growth of fungi, and percentage of inhibition was calculated by the following formula

$$\text{Percentage of inhibition growth} = \frac{\text{Growth in control} - \text{Growth in treatment} \times 100}{\text{Growth in control}}$$

Characterization and identification of the selected isolates

Based on the promising enzyme and antifungal metabolites production, the selected actinobacterial isolates were characterized by colony morphology on different culture media such as SCA, nutrient agar, beef extract agar, yeast extract malt extract agar (ISP2), oat meal agar (ISP3), inorganic salt starch agar (ISP4), glycerol asparagine agar (ISP5), Sabouraud's dextrose agar and potatao dextrose agar. Biochemical properties of the actinobacteria were also performed and identified using standard manuals (Shirling and Gottlieb, 1966). Cell chemistry of actinobacterial isolates were also analyzed using cell wall amino acids and whole cell sugars (Lechevalier and Lechevalier, 1970; Vijayakumar *et al.*, 2012b). Based on the morphological, cultural, biochemical, physiological and chemotaxonomic properties, the prominent enzyme producers were identified. The identity of the genera was also confirmed by Bergey's Manual of Systemic Bacteriology (Williams *et al.*, 1989) Bergey's Manual of Determinative Bacteriology (Pridham and Tresner, 1974).

RESULTS AND DISCUSSION

During the last few decades, actinobacteria have been rigorously studied in many unexplored habitats in various parts of the world. In the present study, a total of 46 actinobacteria were isolated from 6 different seashore soils of Kerala and Goa, West Coast of India. Among them, 29 (63%) were isolated from Kerala [Varkala Beach (9); Fort Kochi beach (8); Kovalam beach (7); Alappuzha beach (5)] and 17 (36.95%) were from Goa Vagatore beach (9); Baga beach (8) (Fig. 1). Similarly, Remya and Vijayakumar (2008) isolated a total of 173 actinobacterial colonies, of which, 64 morphologically distinct isolates were isolated from seashore soil and 24 (38%) isolates from mangrove soil of Calicut, West Coast of India. In another study, 37 soil actinobacterial strains were isolated from an agriculture field in Vengodu, Thiruvannamalai District, Tamil Nadu, India (Saravana Kumar *et al.*, 2014). Thus, the diversity of microbes especially actinobacterial diversity is not same in the environment.

The actinobacterial isolates produced white, ash, grey and brown coloured colonies with different morphological types, reverse side of the culture medium showed as cream, grey, yellow and brown colours and few actinobacteria produced brown coloured diffusible pigment on SCA medium. Microscopically, all the isolated actinobacteria were confirmed as Gram positive and non-acid fast. Based on the colonial and sporophore morphology, all the actinobacterial isolates were preliminarily identified

Table 1. Cultural characteristics of actinobacterial isolates

Name of the isolate	Isolate code	Aerial mycelium colour	Reverse side colour	Diffusible pigment	Size of the colony (mm)
Nocardia sp.	KA1-1	Grey	Cream	-	3
Saccharopolyspora sp.	KA1-2	Cream	Yellow	-	2
Streptomyces sp.	KA1-3	Grey	Cream	-	3
Streptomyces sp.	KA1-4	Pink	Pale yellow	-	4
Nocardiopsis sp.	KA1-5	Grey	Cream	-	5
Actinopolyspora sp.	KA2-1	Grey	Cream	-	3
Streptomyces sp.	KA2-2	Grey	Cream	-	2
Pseudonocardia sp.	KA2-3	Grey	Cream	-	4
Actinopolyspora sp.	KA2-4	Grey	Cream	-	6
Streptomyces sp.	KA2-5	Blue	Cream	-	3
Pseudonocardia sp.	KA2-6	Cream	Yellow	-	4
Actinopolyspora sp.	KA2-7	Pink	Cream	-	5
Streptomyces sp.	KA2-8	Cream	Cream	-	2
Nocardia sp.	KA2-9	Grey	Cream	-	3
Nocardia sp.	KA3-1	Grey	Cream	-	3
Streptomyces sp.	KA3-2	Grey	Grey	-	2
Saccharopolyspora sp.	KA3-3	Cream	Pale yellow	-	4
Streptomyces sp.	KA3-4	Grey	Cream	-	5
Nocardiopsis sp.	KA3-5	Grey	Cream	-	5
Nocardia sp.	KA3-6	Grey	Cream	-	6
Saccharopolyspora sp.	KA3-7	Grey	Cream	-	6
Nocardiopsis sp.	KA3-8	Grey	Grey	-	7
Nocardiopsis sp.	KA4-1	Grey	Cream	-	3
Actinopolyspora sp.	KA4-2	Grey	Yellow	-	4
Sacchoropolyspora sp.	KA4-3	Dull grey	Brown	-	3
Pseudonocardia sp.	KA4-4	Grey	Cream	-	5
Nocardia sp.	KA4-5	Grey	Cream	-	3
Pseudonocardia sp.	KA4-6	Cream	Pale yellow	-	3
Saccharopolyspora sp.	KA4-7	White	Pale yellow	-	4
Sreptomyces sp.	GA1-1	Grey	Cream	Brown	2
Nocardia sp.	GA1-2	White	Pale yellow	-	6
Pseudonocardia sp.	GA1-3	Pink	Brown	-	4
Streptomyces sp.	GA1-4	White	Cream	-	3
Streptomyces sp.	GA1-5	Cream	Grey	Brown	3
Pseudonocardia sp.	GA1-6	Grey	Cream	-	2
Saccharopolyspora sp.	GA1-7	White	Cream	-	5
Actinopolyspora sp.	GA1-8	White	Pale yellow	-	6
Nocardia sp.	GA1-9	Grey	Cream	-	2
Nocardiopsis sp.	GA2-1	Grey	Cream	-	6
Streptomyces sp.	GA2-2	Cream	Pale yellow	-	5
Streptomyces sp.	GA2-3	Grey	Cream	-	4
Streptomyces sp.	GA2-4	Cream	Pale yellow	-	2
Nocardia sp.	GA2-5	White	Cream	-	2
Nocardia sp.	GA2-6	Grey	Brown	-	4
Saccharopolyspora sp.	GA2-7	Cream	Brown	Brown	4
Saccharopolyspora sp.	GA2-8	White	Pale yellow	-	3

- = no pigment

to generic level. Among the 46 isolates, 13 belonged to the genus *Streptomyces*, followed by *Nocardia* (n=9), *Nocardiopsis* (n=5), *Saccharopolyspora* (n=8), *Actinopolyspora* (n=5) and *Pseudonocardia* (n=6) (Table 1).

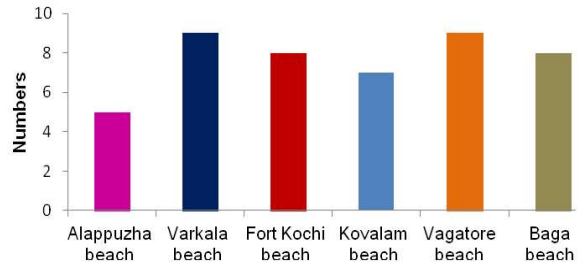

Fig. 1. Actinobacterial population in different marine soils of Kerala and Goa

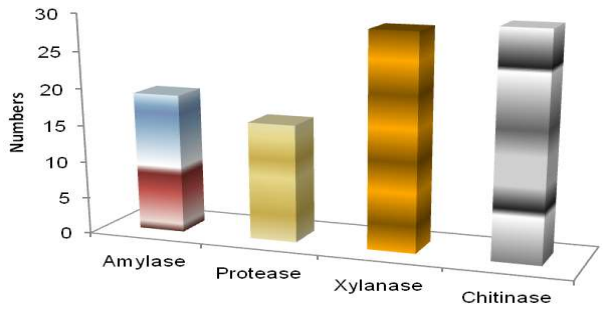

Fig. 2. Enzyme activity of actinobacteria

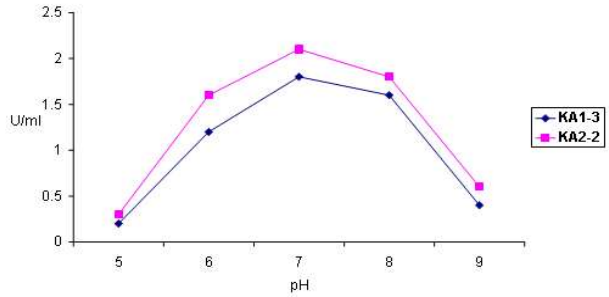

Fig. 3. Effect of pH on amylase activity

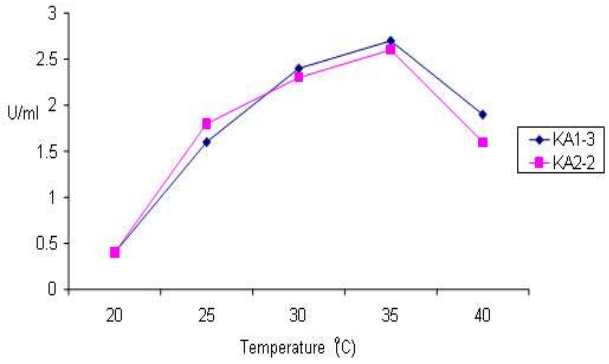

Fig. 4. Effect of temperature on amylase activity

Actinobacteria are an important source of enzymes which are involved in the degradation of cellulose, pulps, agrowastes, textile-cellulosic wastes, industrial and municipal wastes (Bhat, 2000). In the present study, among 46 isolates, 19 (41.3%), 30 (65.2%), 16 (34.8%) and 29 (63%) isolates produced amylase, chitinase, protease and xylanase, respectively (Fig. 2). Among the enzyme producers, 2 isolates namely *Streptomyces* sp. KA1-3 and *Streptomyces* sp. KA2-2 had notable enzyme activities. Hence, these two strong enzyme producers were selected for further assay and characterization. The extra-cellular enzyme activities of actinobacteria were reported by several workers including amylase activity (Yang and Wang, 1999; Ellaiah *et al*., 2002; Abou-Elela *et al*., 2009; Vaijayanthi and Vijayakumar, 2015; Vijayakumar *et al*., 2015), chitinolytic activity (Abdel-Fatch, 1995; Tahtamouni *et al*., 2006; Vaijayanthi and Vijayakumar, 2016), protease activity (Nikolova *et al*., 2005; Vinothini *et al*., 2008) and xylanase activity (Kim *et al*., 2000; Rawashdeh *et al*., 2005).

Enzyme activity is often influenced by the components of the medium and cultural conditions such as pH, temperature, salinity, incubation period, nitrogen and carbon source, aeration, agitation and suitable media which often vary from organism to organism. In the present study, production of all the four extra-cellular enzymes was observed maximum at pH 7 for both the isolates when compared to other pH conditions tested. Maximum amylase (2.2 U/mL) production was found with *Streptomyces* sp. KA2-2, whereas *Streptomyces* sp. KAI-3 produced 1.7 U/mL of amylase. Maximum chitinase (1.9 U/mL), protease (2.7 U/mL) and xylanase (2.6 U/mL) were produced by *Streptomyces* sp. KA1-3, when compared to the enzymes chitinase (1.8 U/mL), protease (2.4 U/mL) and xylanase (1.8 U/mL) produced by *Streptomyces* sp. KA2-2 (Fig. 3; 6; 9; 12). Regarding the temperature requirement, among the various temperature tested (20, 25, 30, 35 and 40°C), enzyme production was increased with increase in the incubation temperature. Maximum enzyme activity was recorded at 35°C for both the isolates, whereas the enzyme production gradually decreased at temperatures more than 35°C. At 35°C, maximum amylase (2.7 U/mL), chitinase (2.4 U/mL) and xylanase (2.8U/mL) were produced by *Streptomyces* sp. KA1-3, whereas maximum protease (2.8U/mL) production was recorded with *Streptomyces* sp. KA2-2 (Fig. 4; 7; 10; 13). The maximum enzyme activity was found in the production media with 2% NaCl for both the isolates. Among the four enzymes tested, higher chitinase (2.8 U/mL) production was found with *Streptomyces* sp. KA2-2 in the media with 2% NaCl, while *Streptomyces* sp. KA1-3 produced 2.6 U/mL of chitinase in the media with same NaCl concentration. In amylase production, the isolate *Streptomyces* sp. KA1-

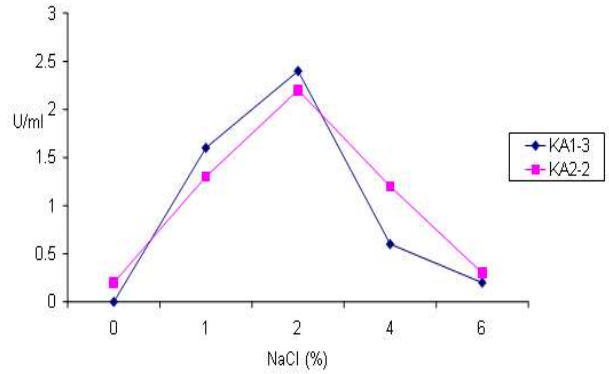

Fig. 5. Effect of NaCl on amylase activity

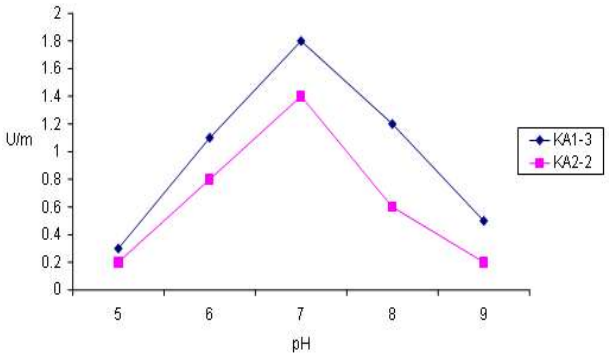

Fig. 6. Effect of pH on chitinase activity

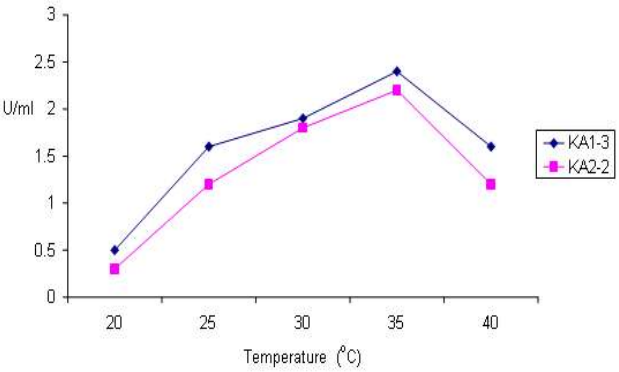

Fig. 7. Effect of temperature on chitinase activity

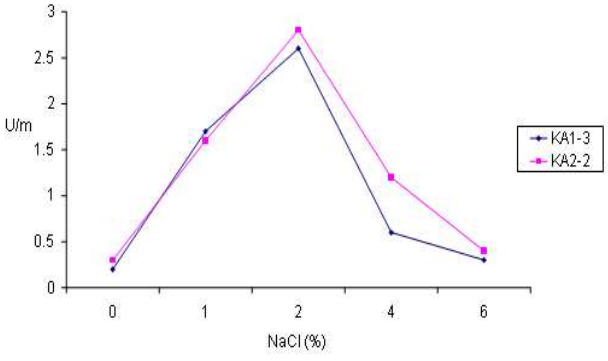

Fig. 8. Effect of NaCl on chitinase activity

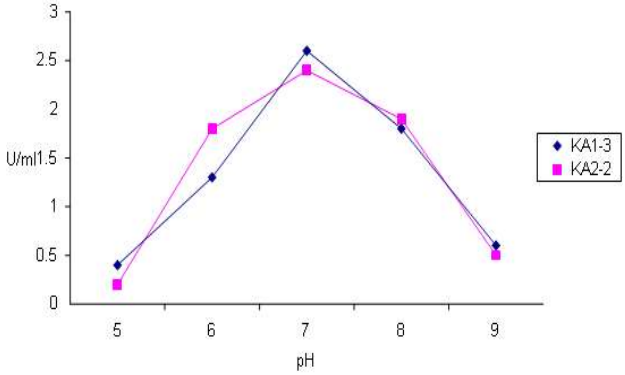

Fig. 9. Effect of pH on protease activity

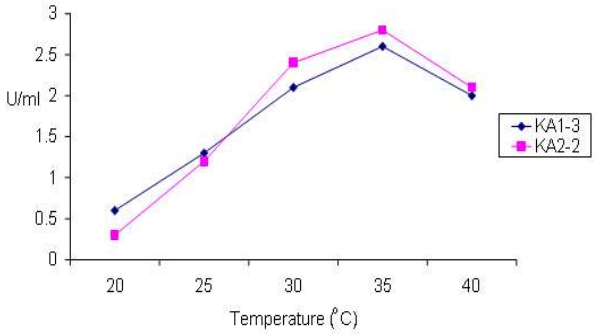

Fig. 10. Effect of temperature on protease activity

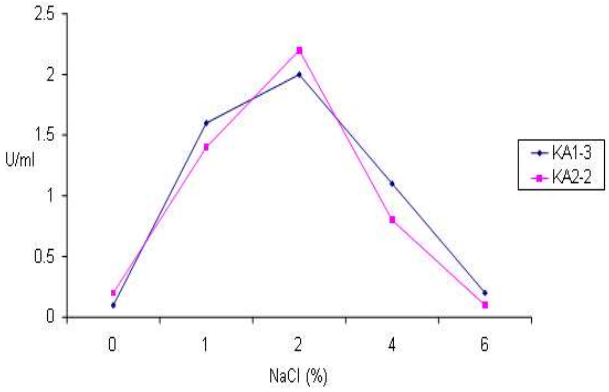

Fig. 11. Effect of NaCl on protease activity

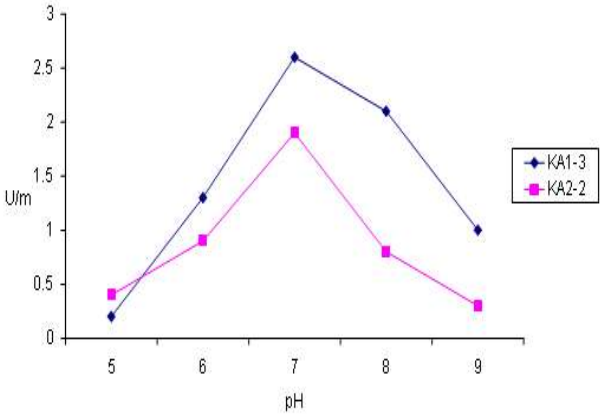

Fig. 12. Effect of pH on xylanase activity

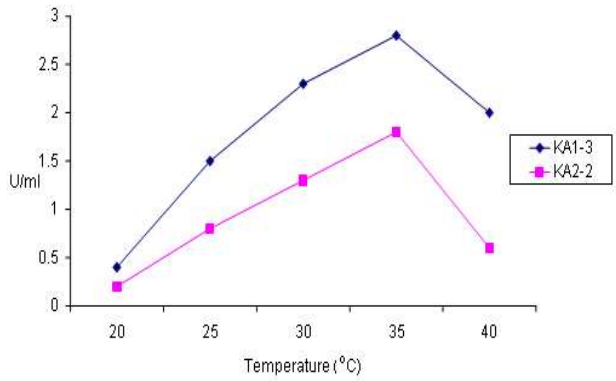

Fig. 13. Effect of temperature on xylanase activity

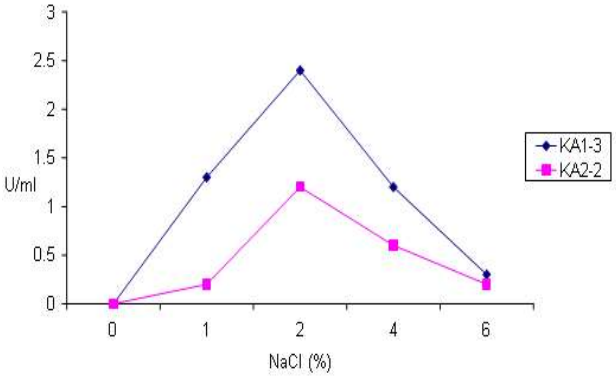

Fig. 14. Effect of NaCl on xylanase activity

3 produced 2.5 U/mL, but *Streptomyces* sp. KA2-2 produced 2.3 U/mL of amylase. The protease was (2.3 U/mL) produced higher by *Streptomyces* sp. KA2-2 at 2% NaCl, but *Streptomyces* sp. KA1-3 produced protease as minimum of 1.9 U/mL. Xylanase activity was higher (2.4 U/mL) in *Streptomyces* sp. KA1-3, whereas minimum (1.3 U/mL) xylanase activity was reported in *Streptomyces* sp. KA2-2 (Fig. 5; 8; 11; 14). Similarly, Vijayakumar *et al.* (2015) studied the production and optimization of extra-cellular enzymes from actinobacteria. Thus, these reports emphasized that the cultural conditions influenced the production of bioactive compounds by the actinobacteria.

Marine actinobacteria are of considerable value as antibiotic producers and other therapeutically useful compounds with diverse biological activities (Bernan *et al.*, 1997, Sharma and Pant, 2001). In the present study, *Streptomyces* sp. KA1-3 and *Streptomyces* sp. KA2-2 showed noticeable antifungal activity against the test fungi namely *Colletotrichum fulgatum*, *Trichoderma viridae* and *Fusarium semitectum*. The cell free culture filtrates of the antifungal compound producing actinobacteria (*Streptomyces* sp. KA1-3 and *Streptomyces* sp. KA2-2) greatly inhibited (92-100%) the test fungi namely *C. fulgatum, T. viridae* and *F. semitectum* at 2% concentration. The other concentrations of the actinobacterial culture filtrates inhibited the fungal growth at moderate to minimum (17-91%) level.

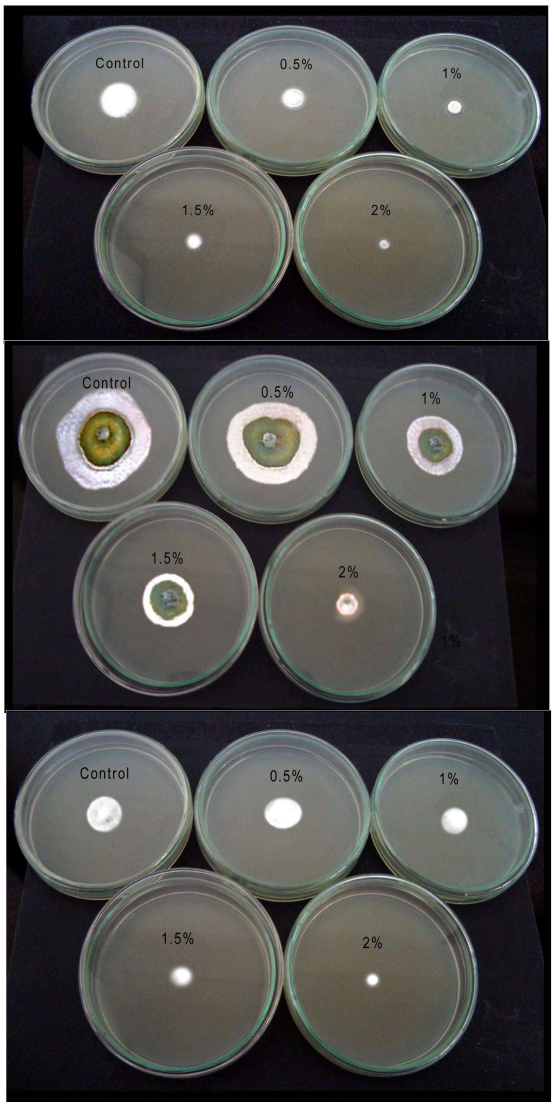

Fig. 15. Antifungal activity of actinobacteria against phytopathogenic fungi (a) KA1-3 Vs *C. falcatum* (b) KA1-3 Vs *T. viridae* (c) KA2-2 Vs *F. semitectum*

Streptomyces sp. KA1-3 inhibited 100% growth of both *C. fulgatum* and *F. semitectum,* and 95% inhibition against *T. viridae* at 2% of extract. In contrast to this, *Streptomyces* sp. KA2-2 had 100% inhibitory effect on both *F. semitectum* and *T. viridae,* whereas 92% inhibitory effect was found on *C. fulgatum* (Table 2; Fig. 15a-c). Similarly, Singh *et al.* (2016) screened a total of 80 isolates and the isolate ACITM-1 showed good antifungal activity against *Macrophomina phaseolina, F. oxysporum, C. truncatum* and *Rhizoctonia solani.* The metabolite suspensions of the potential actinobacteria such as *Streptomyces* sp. KA4-8, *Nocardiopsis* sp. KE-1, *Saccaharopolyspora* sp. KA3-3, *Streptomyces* sp. GA1-1 and *Streptomyces* sp. GA1-5 greatly inhibited the growth of fungal pathogens namely *C. falcatum, Thielaviopsis paradoxa* and *F. semitectum* even at low concentration

Table 2. Percentage inhibition of antagonistic actinobacteria against fungal pathogens after 4 days

Nature of culture filtrate	Conc. (%)	Growth rate (mm)	Inhibition (%)
Colletotrichum fulgatum			
KA1-3	Control	24	NA
	0.5	20	17
	1	14	41
	1.5	6	75
	2	0	100
KA2-2	0.5	19	21
	1	14	42
	1.5	7	71
	2	2	92
Hexaconazole	0.02%	10	60
Trichoderma viridae			
KA1-3	Control	42	NA
	0.5	22	48
	1	14	67
	1.5	6	85
	2	2	95
KA2-2	0.5	30	29
	1	14	67
	1.5	5	88
	2	0	100
Hexaconazole	0.02%	8	85
Fusarium semitectum			
KA1-3	Control	22	NA
	0.5	8	64
	1	6	73
	1.5	2	91
	2	0	100
KA2-2	0.5	10	55
	1	7	68
	1.5	3	86
	2	0	100
Hexaconazole	0.02%	4	80
NA = Not applicable			

(0.5%) (Vijayakumar *et al.,* 2012c). Thus the present and previous studies revealed the antifungal activity of actinobacteria as a biocontrol agents and suggested their activities against all the pathogens and consistency established through optimization studies.

During the last few decades agriculturists used chemical agents particularly as fertilizer as well as pesticide/ insecticide to overcome the growth retardation and fungal diseases of plants. Many studies reported the side effects of these chemical fertilizers and pesticides on both soil and human health. It is very important to compare the pest killing capability, duration and cost of killer agents. More importantly it is obvious that the antagonistic

Table 3. Cultural characteristics of selected actinobacteria

Properties	KA1-3	KA2-2
Morphological characteristics		
Sporophore morphology	Spirally twisted	Spirally hooked
Colour of aerial mycelium	Grey	Grey
Colour of substrate mycelium	Cream	Cream
Spore mass	Grey	Grey
Gram staining	Gram positive	Gram positive
Acid fast staining	Non acid fast	Non acid fast
Cultural characteristics		
Starch casein medium		
Growth	+++	+++
Aerial mycelium	Grey	Grey
Substrate mycelium / Pigmentation	Cream	Cream/ Brown
Nutrient agar		
Growth	++	++
Aerial mycelium	Grey	Grey
Substrate mycelium / Pigmentation	Cream	Cream
Sabouraud's dextrose agar		
Growth	++	++
Aerial mycelium	Cream	Cream
Substrate mycelium / Pigmentation	Yellow	Brown/ Brown
Beef extract agar		
Growth	++	++
Aerial mycelium	Dark grey	Dark grey
Substrate mycelium / Pigmentation	Yellow	Cream
Yeast extract malt extract agar (ISP-2)		
Growth	+++	+++
Aerial mycelium	White	White
Substrate mycelium / Pigmentation	Brown	White/ Brown
Oat meal agar (ISP-3)		
Growth	++	++
Aerial mycelium	White	Grey
Substrate mycelium / Pigmentation	Yellow	Yellow/ Orange
Inorganic salt starch agar (ISP-4)		
Growth	+++	+++
Aerial mycelium	Cream	Grey
Substrate mycelium / Pigmentation	Yellow	Cream Light brown
Glycerol Asparagine agar (ISP 5)		
Growth	+++	+++
Aerial mycelium	White	White
Substrate mycelium / Pigmentation	Brown	Brown/ Brown
Potato dextrose agar		
Growth	+++	+++
Aerial mycelium	Cream	Cream
Substrate mycelium / Pigmentation	Yellow	Yellow/ Brown

Table 4. Biochemical and physiological characteristics of selected actinobacteria

Properties	KA1-3	KA2-2
Biochemical characteristics		
H_2S production	-	+
Nitrate	+	+
Urease	+	+
Catalase	+	+
Oxidase	+	+
Melanin	-	-
Starch	+	+
Gelatin	+	+
Casein	-	-
Triple sugar iron	Acid/Alk	Acid/Alk
Di-amino pimelic acid	L-DAP	L-DAP
Cell wall sugars	-	G/L/M
Physiological properties		
Effect of pH		
5	-	+
6	++	++
7	+++	+++
8	+++	+++
9	++	++
Effect of temperature (° C)		
20	+	+
25	++	++
30	+++	+++
35	++	++
40	+	+
Effect of NaCl (g/l)		
0	-	-
1	+	+
2	+++	+++
4	++	++
6	+	-
Effect of carbon source		
Glucose	+++	++
Lactose	++	++
Maltose	++	++
Starch	+++	+++
Effect of nitrogen source		
Potassium nitrate	++	++
Ammonium nitrate	++	+++
Yeast extract	+++	+++
Soy extract	+	+

organisms can survive for a prolonged period once introduced into the soil, and sustain its role in the biological reactions. The chemical fungicide, hexaconazole (Mass) was used at 0.02% concentration against three phytopathogenic fungi. The fungicide effectively inhibited the growth of all the four fungal pathogens tested up to 60-85% (Table 2). However, the application of chemical fungicides has several disadvantages including the development of resistance to fungicides in certain isolates of plant pathogens (Ma and Michailides, 2005), whereas the biological control methods minimise the deleterious side effects caused by synthetic fungicides (Cook and Baker, 1983).

The selected enzyme and antifungal compound producers like KA1-3 and KA2-2 were cultured on different media. The isolates showed difference in colour of colonies, size of colony and production of diffusible pigments. The isolates developed cream coloured colonies on most of the media tested, followed by grey and yellow coloured colonies. Reverse side of the culture media changed as cream, yellow and brown in colour. The strains produced brown and orange coloured pigments on most of the media tested (Table 3). Both of the isolates showed positive result for urease, catalase, gelatin hydrolysis, starch hydrolysis, casein hydrolysis, oxidase and nitrate reduction test. Both the isolates showed acid butt and alkaline slant for triple sugar iron agar test (Table 4). Chemotaxonomically, both the isolates possessed L-diaminopemilic acid in their cell wall, and the isolates also had glucose, lactose and mannose as characteristic sugars in their cell (Table 4).

Physiologically, both of the isolates showed optimum growth at pH 7, temperature at 30°C and NaCl at 2% salt concentrations, and culture media with starch and yeast extract as the carbon and nitrogen source respectively (Table 4). Based on the morphological, cultural, biochemical, physiological and chemotaxonomic properties, the prominent bioactive compounds producers was identified to be *Streptomyces* sp. KA1-3 and *Streptomyces* sp. KA2-2. The identity of the species was also confirmed by Bergey's Manual of Systemic Bacteriology (Williams *et al.*, 1989) Bergey's Manual of Determinative Bacteriology (Pridham and Tresner, 1974). In conclusion, the present study was an attempt to identify and pick-out versatile strains of actinobacteria from the regions of the West Coastal area, India that display enzyme activity and antifungal activity against a variety of phyto-pathogens intrinsically. Further, optimization of bioactive compound production necessitates the complete knowledge on optimal fermentation conditions for the strain used. The success of studies would also depend upon the development of appropriate

fermentation conditions and downstream processing technologies as to bring out commercially important new classes of antibiotics and enzymes.

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