International Scholars Journals

African Journal of Environmental and Waste Management ISSN 2375-1266 Vol. 8 (7), pp. 001-006, July, 2021. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Antagonistic activity of marine bacteria *Pseudoalteromonas tunicata* against microbial pathogens

Sivasubramanian K[1]*, Ravichandran S[1] and Vijayapriya M[2]

[1]Center of Advance Study in Marine biology, Annamalai University, Parangipetti-608 502, India.
[2]Department of Microbiology, Annamalai University, Annamalainagar-608002, Tamilnadu, India.

Accepted 28 March, 2021

Marine bacteria have been recognized as an important and untapped resource for novel bioactive compounds. In the present study the antagonistic activity of marine bacteria was investigated. The selected *Pseudoalteromonas tunicata* showed a very broad range of antagonistic activity against many pathogenic bacteria and fungi. The antagonistic strains were also tested for the production of exo-enzymatic activities, 83% of the selected marine bacterial strains produced proteases except *Pseudoalteromonas denitificans* and *Pseudoalteromonas piscicida.* Lipase activity was produced by *Pseudoalteromonas aliena*, *Pseudoalteromonas aurantia*, *Pseudoalteromonas tunicata* and *Pseudoalteromonas citrea* strains. The *P. tunicata* strain isolated in the present study seems to have potential antifungal and antimicrobial activity against most of the human bacterial pathogens and phytopathogenic fungi. Thus, the marine bacterial strain *P. tunicata* was having the potential of producing bioactive compounds.

Key words: Antagonistic activity, marine bacteria, human pathogens, enzymatic activities, bioactive compounds.

INTRODUCTION

Ocean remains as an unexploited and unexplored source for many drugs and pharmacologically active substances. In recent years there has been a growing awareness on the bioactive potential of marine microbes of all available marine forms, the Pseudoalteromonas merits special correlation in view of its proven biosynthetic properties. Pigmented Pseudoalteromonas species possess broad range of bioactivity associated with the secretion of extracellular compounds, several of which include pigment compounds. This realization provided greater credence to earlier studies of alteromonads, that this group of marine bacteria represents a rich source of biologically active substances. In the present study we have carried out our research on the production of bioactive substances isolated from the pigmented marine Pseudoalteromonas species and its purification properties.

MATERIALS AND METHODS

Collection and isolation The marine water samples were collected at different marine environment that is, Mandapam, Parangipettai and Cuddalore (Southeast coast of India) at varying depths of 8 to 10 m. Samples were serially diluted and 0.1 ml of each sample was spreaded on marine agar medium and incubated at 37°C for 48 h. Colonies were counted and the results were expressed as CFU/ml. The isolated bacterial strains were identified up to species level (Buchanan and Gibbons, 1974) and were stored in nutrient agar slants for further study.

Antagonistic activity

Antagonistic activity of marine bacterial isolates was tested (Geels and Schippers, 1983). Initial screening for in vitro antagonistic activity was done on nutrient agar against bacterial pathogens and on Potato Dextrose Agar plates regarding fungal strains. It was performed by placing 0.6 cm diameter mycelium disk on the agar in the center of the Petri dish from a 5 day fungal culture grown at room temperature, which was followed by inoculation of the bacterial strains at a distance of about 3 cm from the fungus. Bacterial strains

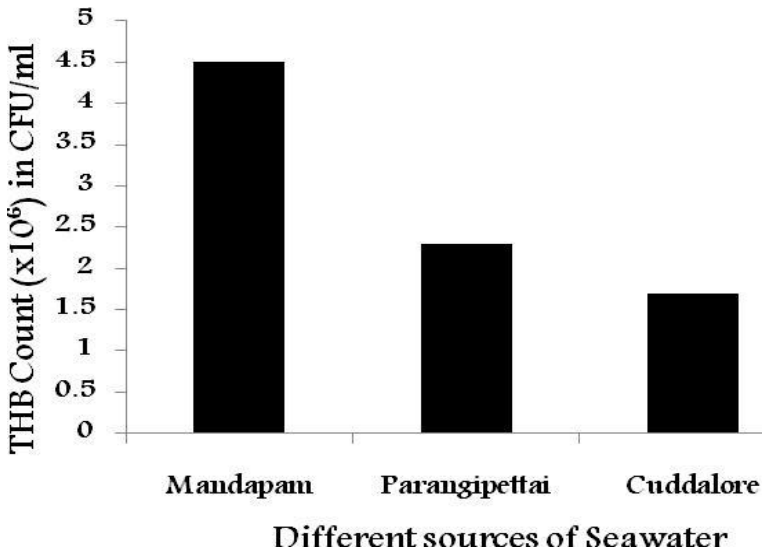

Figure 1. Percentage of identified seawater associated pigmented bacterium in different sources.

inhibiting mycelia growth after five days, growth was determined by the inhibition zone of the strains and the potential strains were selected for further characterization. The present study deals with *P. tunicata* strain selected through this procedure. Among 42 strains tested this strains was found to be more potential.

Culture filtrate activity

The culture filtrate activities of marine bacterial isolates were tested against bacterial pathogens and pathogenic fungi by following the well assay method. After swabbing the pathogens on the plates 0.1 ml of cell free culture broth of *P. tunicata* centrifuged at 10.000 rpm for 20 min was poured into the well and plates were incubated at 37°C for 48 h. The bacterial culture filtrate inhibiting the growth of pathogen around the well after two days was assessed by the inhibition zone around the well. Results were recorded.

Enzyme mediated activity

The selected morphologically different bacterial strains were tested for the production of protease, lipase, amylase, cellulose and gelatinase (Gerhardt et al., 1994).

Proteinase and non-proteinase compound inhibitory activity

Protein precipitation was carried out (James et al., 1996; Barja et al., 1989). The proteinase and non-proteinase compound inhibitory activity of *P. tunicata* was tested against bacterial pathogens and pathogenic fungi and the results were recorded. The extraction of the compound produced by *P. tunicata* was done by solvent extraction method.

RESULTS AND DISCUSSION

The numbers of culturable bacterial cells present in the seawater samples were estimated. The total heterotrophic bacterial load ranged from $1.7x10^6$ to $4.5x10^6$ CFU/ml of seawater sample and it was found to be the maximum of $4.5x10^6$ CFU/ml in Mandapam seawater sample, followed by Parangipettai and Cuddalore seawater samples $2.3x10^6$ CFU/ml in $1.7x10^6$ CFU/ml, respectively (Figure 1). In Mandapam seawater sample the presence of *P. tunicata* was indicated by its green yellow pigment on nutrient agar medium. A total of 42 bacterial isolates were tested for their antagonistic ability against highly virulent 11 pathogenic fungi and 12 human bacterial pathogens. A set of 12 bacterial strains were selected on the basis to growth inhibitory activity against pathogenic fungi and bacterial pathogens, and they are identified up to species level. The bacterial strain identified as *P. tunicata* showed highest activity and more percentage of antagonistic activity (45%) against fungal pathogens.

The maximum antagonistic activity of *P. tunicata* was screened against *Fusarium moniliforme* (10 mm) and *Trichoderma* sp. (10 mm) and the minimum antagonistic activity was observed against *Penicilium citrinum* (2 mm) and *P. oxalicum* (2 mm) (Figure 2) . The *P. tunicata* exhibited the highest antagonistic activity against bacterial pathogens like *Staphylococcus aureus* (2 mm) and *Lactobacillius vulgaris* (2 mm) and the lowest antagonistic activity was observed against *Salmonella typhi* (1 mm) and *Vibrio spp.* (1 mm) (Figure 3). The culture filtrate activity of *P. tunicata* the highest activity was exhibited against the bacterial pathogens *S. aureus, S. typhi, L. vulgaris* and *Vibrio cholerae* and the zone around the well was ranged from 7 to 9 mm. The maximum activity was observed against, *L. vulgaris* (9 mm) and *Vibrio spp.* (9 mm) and the minimum in

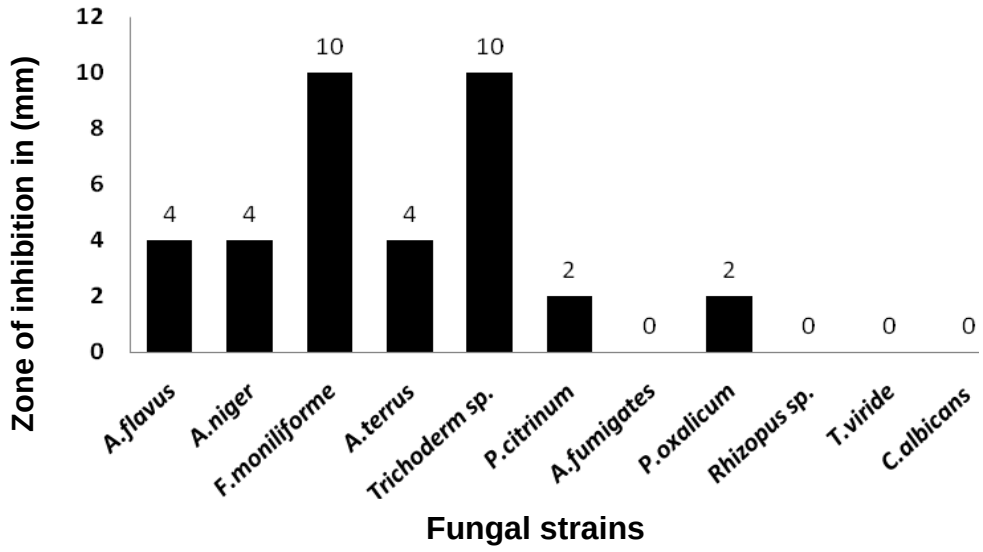

Figure 2. Antagonistic activity of *P. tunicata* against fungal pathogens.

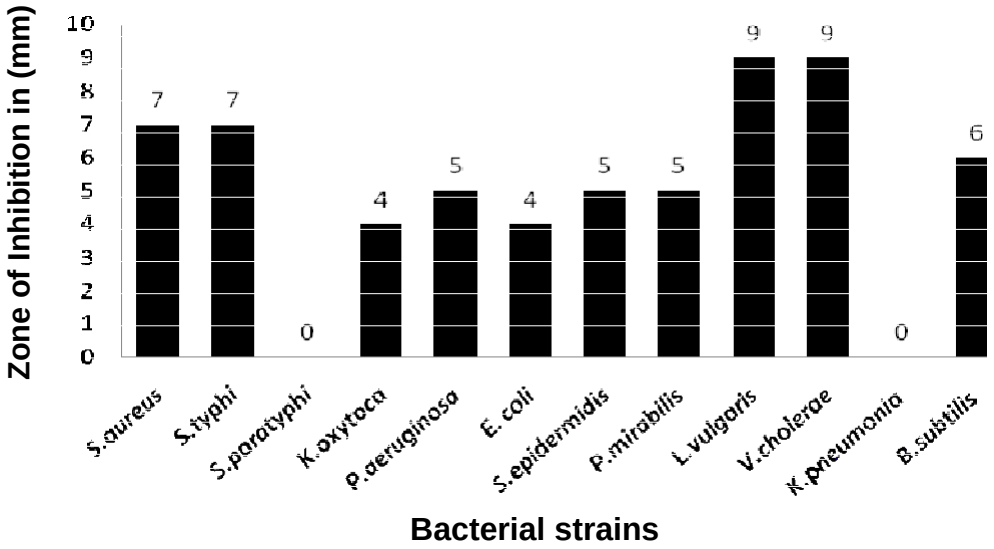

Figure 3. Antagonistic activity of *P. tunicata* against human bacterial pathogens.

Klebsiella oxytoca (4 mm) and *Proteus mirabilis* (4 mm) (Figure 4). In antifungal activity the maximum zone of inhibition was recorded in *F. moniliforme* (5 mm) and the minimum activity was observed against *Aspergillus flavus* (2 mm) and *Aspergillus niger* (2 mm) (Figure 5).

The 12 selected potential strains were tested for the production of exoenzymatic activities (Table 1). The enzymes protease, lipase and gelatinase were produced by *P. tunicata.* The enzyme proteases were produced by 83% of strains except *P. denitrificans* and *P. piscicida*. The maximum protease was produced by *P. citrea* (8 mm) and the minimum was produced by *P. nigrifaciens* (2 mm) and *P. piscicida* (2 mm). About 33% of the strains produced lipases, in which the maximum lipase activity was produced by *P. aurantia* (5 mm) and the minimum in *P. aliana* and *P. citrea* (3 mm). The amylase produced by 42% of strains in which the maximum amylase activity was produced by *P. citrea* and *P. agarivorous* (5 mm) and the minimum activity in *P. aliena* (3 mm). Cellulase was detected and maximum production in *P. aliena* and *P. aurantia* (4 mm) and *P. piscicida* (3 mm) with 25% of strains. Gelatinase were produced by the minimum

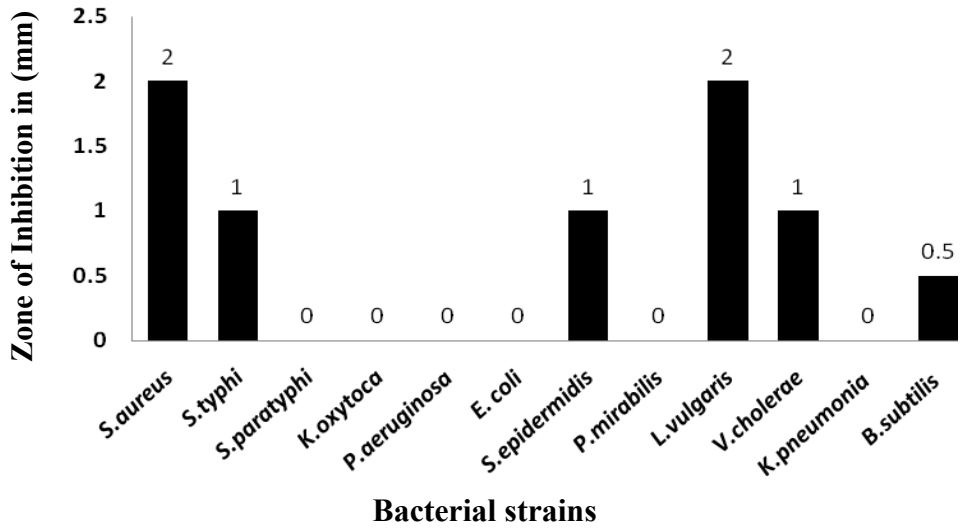

Figure 4. Culture filtrate activity of *P. tunicata* against human bacterial pathogens.

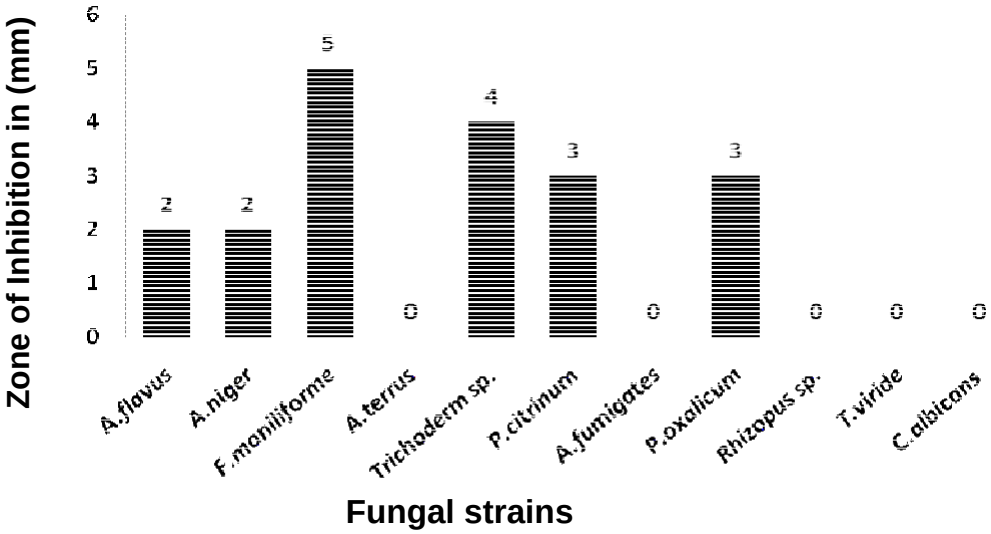

Figure 5. Culture filtrate of *P. tunicata* against fungal pathogens.

number of strains, that is, 17%, the maximum gelatinase activity was produced by *P. piscicida* (7 mm) and the minimum activity was produced by *P. tunicata* (5 mm). No cellulase and amylase activity was produced by *P. tunicata.* Partially purified non-proteinase compound of *P. tunicata* exhibited the highest biocontrol ability against bacterial pathogens *S. aureus* and *P. mirabilis* (6 mm) and the minimum non- proteinase inhibitory activity was observed against *Vibrio* sp and *S. typhi* (3 mm).

The non-proteinase compound also exhibited a biocontrol ability against pathogenic fungi *F. moniliforme* (4 mm), *A. niger* and *A. terraeus* (2 mm) and *Trichoderma* sp (3 mm).

DISCUSSION

The search for novel antibiotics has gained urgency because of increased occurrence of multi-drug resistant human pathogens. Many clinically relevant microbes have developed resistance resulting not only from the exposure to sub lethal concentrations of antibiotics in hospital environment but also in animal farms where antibiotics are used as growth enhancers (Witte, 1999). Marine bacteria have been recognized as an important and untapped resource for novel bioactive compounds. The chemical compounds of marine microorganisms are less well known than those of their terrestrial

Table 1. Production of hydrolytic activities and antifungal metabolites by bacterial strain.

Strains	Protease	Lipase	Amylase	Cellulose	Gelatinase
P. aliena	++	+	+	+	-
P. aurantia	+	+	+	+	-
P. citrea	-	+	-	-	+
P. tunicata	++	+	+	-	-
P. agarivorans	+	-	+	-	-
P. flavipulchra	+	-	-	-	-
P. luteoviolacea	++	-	-	-	-
P. denitrificans	-	-	-	-	-
P. nigri faciens	(+)	-	-	-	-
P. rubra	+	-	-	-	-
P. undina	+	-	-	-	-
P. piscicida	(+)	-	+	+	++

'++' = diameter of the growth inhibition zone > 5 mm, '+' = diameter of the growth inhibition zone 3 to 5 mm, '(+)' = diameter of the growth inhibition zone < mm, '-' = No enzyme mediated inhibitory activity observed.

counterparts. However, in the last decade several bioactive compounds have been isolated from marine bacteria and are new resources for the development of medically useful compounds (Donia and Hamann, 2003; Anand et al., 2006).

The present study is an attempt for the isolation of a potential bioactive bacterial strain from three different coastal environments. Among the 42 bacterial isolates which were isolated from seawater and screened for antagonistic against pathogenic fungi and bacterial pathogens. Marine bacteria isolated from the surface of marine algae and invertebrates has shown that a high percentage produce anti-microbial metabolites (Holmstrom and Kjelleberg, 1999). Twelve different pigmented strains were selected on the basis of growth inhibitory activity against pathogenic fungi and bacterial pathogens. The special character of *P. tunicata* was indicated by its purple yellow pigment on nutrient agar medium. Pigmented *Pseudoalteromonas* species possess broad range of bioactivity associated with the secretion of extracellular compounds, several of which include pigment compounds. The findings of SachaStelzer and SuhelenEgan (2006) also supported the present study. He also reported that the green-yellow pigmented marine bacterium *P. tunicata* produces several target-specific compounds that act against a range of common fouling organisms, including bacteria, fungi, protozoa, invertebrate larvae and algal spores. Upon testing the biocontrol ability of all these strains, it became apparent that *P. tunicata* exhibited the highest biocontrol ability in the present study. Some of the earlier study result also agreement with present investigation in Baltic sea environment on the diversity of marine bacteria associated with brown alga shows the presence of *Pseudoalteromonas sp* which have similar antagonistic property (Jonathan et al., 2009). Among the three different costal environments maximum Total Heterotrophic Bacterial (THB) count was recorded in the sample collected from mandapam station (4.5 x 10^6 CFU/ml). It may be due to more nutrients from the coral reef and mangrove environment of Gulf of Mannar than the open ocean like Cuddalore and Parangipettai. Some of the earlier work also in agreement with the present investigation. It was found that the surface or marine organisms such as seaweeds and invertebrates are more nutritious than inanimate material and sea water, and a large number of bacteria could live on it (Sponga et al., 1999).

The purpose behind the work is to check the potential of antimicrobial activity and to extract the active component from the *P. tunicata* strain. The selected antagonistic *P. tunicata* showed a very broad range of antagonistic activity against many pathogenic fungi and exhibited significant bio control activity against bacterial pathogens. Similar result was observed in *Pseudoaltromonas* isolated from the marine sponge *Haliclona simulans* (Jutta et al., 2009). The culture filtrate activity of the *P. tunicata* strain was found to be higher in bacterial pathogens compared to pathogenic fungi. The *P. tunicata* was also tested for their secretion of hydrolytic enzymes such as lipase, protease, cellulose and gelatinase and antifungal metabolites. The non-proteinase compound also exhibited biocontrol ability against pathogenic fungi and bacteria and it was extracted using ethyl acetate.

Thus, the present work on *P. tunicata* showed that it has potential antimicrobial activity. The study also revealed that marine microbes are also being useful in development of drugs against human pathogens.

ACKNOWLEDGEMENT

The authors are grateful to the Director of CAS in Marine Biology and authorities of Annamalai University for

facilities provided.

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