T. Prem Anandet al /J. Pharm. Sci. & Res. Vol.5(6), 2013, 137 - 139

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

# Competitive Dominance of Potential Bacteria from Marine Organisms

**[1]T. Prem Anand*, [1]C. Chellaram and [2]Felicia Shanthini**

*[1]Department of Biomedical Engineering, Vel Tech Multi Tech Engineering College, Chennai-600062. Tamilnadu. India.*
*[2]Department of Marine Studies and Coastal Resource Management, Madras Christian College, Chennai, Tamilnadu, India*

**Abstract**
The function of secondary metabolites in nature is a controversy raging for decades. Interactions between microorganisms are well known phenomena and substrate competition and antagonism are believed to be important in a selection of microflora in a given ecological niche. Although antagonism due to antibiotic production can be easily demonstrated in rich culture media under laboratory conditions but in low-nutrient environments it is not subject to experimental approach. Studies done with natural substrates may facilitate a better understanding of the problem. In the present study the ecological role of antibiotic production by epiphytic bacterial strains was studied. The principle objective is to find out the strains which have the competitive edge over other strains. Antibiotic producer and non-producer strain isolated from different species of seaweeds, biofilm and Opisthobranch surface were used for this study. Experiments were carried out to assess the competitive dominance ofbacterial strains with four types of mixed cultures in unsupplemented seawater. Various producer strainswere found to inhibit the growth of non producer strains drastically. Competitions among producer strains were also noted and some producers competitivelydominated over others.
**Key words:** Antagonistic bacteria, seaweeds, mollusk and antagonistic bacteria

## INTRODUCTION

Competition occurs when two populations are striving for the same resource. Often it focuses on a nutrient present in limited concentrations but it may also occur for other resources including light and space [1]. Antagonism occurs when one population produces a substance inhibitory to another population. The first population gains a competitive edge as a result of its ability to inhibit the growth of competitive populations. The production of antibiotics can give the antibiotic producing population an advantage over a sensitive strain when competing for the same nutrient resources. But the function of antibiotic substances in natural ecosystems is one of the most controversial topics in the field of microbial ecology [2-4]. While some authors argue that antibiotics are simply waste products excreted by microorganisms others propose that antibiotic production is even a "purposeful behaviour" of certain micro organisms [5-7].

Interactions between microorganisms are well known phenomena and substrate competition and antagonism are believed to be important in a selection of microflora in a given ecological niche [8]. Bacterium – bacterium antagonistic interactions involving antibiotics are well documented in soils [9]. Although antagonism due to antibiotic production can be easily demonstrated in rich culture media under laboratory conditions but in low-nutrient environments it is not subject to experimental approach. Studies done with natural substrates may facilitate a better understanding of the problem. This approach has been extensively used in work with soil microbial populations [10-11], and has been taken to study competition among marine bacteria by Lemos*et al.*, 1991 [2]. There is little information about antagonistic interactions especially among population of marine bacteria [12-16].

The present study was carried out to understand the interactions due to antibiotic production by marine bacteria and their ecological role in the marine ecosystem. The principle objective is to find out the strains which have the competitive edge over other strains. Antibiotic producer and non-producer strain isolated form different species of seaweeds, biofilm and Opisthobranch surface were used for this study.

## MATERIALS AND METHODS

The interaction between marine strains were studied following the method of Lemos *et al.*, 1991 [2] with different types of mixed cultures, such as mixed cultures with two producer strains, two non-producer strains, three strains with different combination of producers and non-producers. All combinations were based on the different pigmentation of each strain in a particular mixed culture. All the strains used, grew well when they were cultured alone in seawater.

Mixed culture experiments were carried out in un supplemented seawater that was filtered and autoclaved. The bacteria were cultured in 250ml Erlenmeyer flasks containing 100 ml of seawater. Mixed cultures were performed by inoculating each flask with two or three different strains. Marine broth cultures (adjusted to an equal optical density) of each strain were used as inocula (0.2ml). The flasks were incubated in an orbital shaker (290 rpm) in room temperature. Samples were taken immediately after inoculation and then for every 5 hrs for two days. Cultures of

each strain growing alone in seawater served as controls. Appropriate serial dilutions of samples were plated on Zobell Marine Agar employing the conventional pour-plate technique. These plates were incubated for 3 days at room temperature and the number of colony forming units (CFU) of each strain was recorded. In mixed cultures, strains were distinguished by their pigmentation.

## RESULTS

All the producer strains were found to inhibit non-producer strain. The non-producer strain AA9 was inhibited by the producer strain AA7 after ten hours of co-culture and the non-producer strain was completely inhibited. The non-producer strain AA10 was found to be inhibited by producer strain AA5, in 20 hrs of co-culture and also the producer strain AB3 inhibited non-producer strain AB5 in 20 hrs. Complete inhibition of non-producer strain AC2 was noted at 10hrs by the producer strain AC3. The non-producer strains E2 and BFA1 were found to be inhibited by the producer strains AE2 and BFA7 at 20 and 15 hrs of co-culture respectively. The non-producer strains BFA23 and BFA10 were completely inhibited at 20hrs and 15 hrs of co-culture by producer strains BFA8 and BFA6. The producer strain OBSA1 was found to inhibit the non-producer strain OBSA15, and the non-producer was found to be completely inhibited at 20hrs. of co-culture . The non-producer strain OBSB8 was found to be inhibited by the producer strain OBSA2 at 10hrs co-culture.

Of the two producer strains in combination in co-cultures, one producer strain was found to be inhibited in all the cases. The producer strains AA7 and AC3 were found to inhibit the producer strains AB3 and AC3, complete inhibition was noted at 25 and 30 hrs of co-culture. The producer strains AE2 was found to inhibit the producer strain AD16 and in 30 hrs co-cultures complete inhibition was noted. The producer strain BFA8 was found to inhibit the producer strain BFA7 at 20 hrs. The producer strain OBSA1 was found to inhibit the producer strain OBSA2, the strain OBSA2 was completely inhibited at 25 hrs. In the combination of two non-producers both pair of strains used for the co- culture (AA9+AA10 & BFA1+BFA 13) grew well, no inhibition was noted in this combination.

In the co-culture of two producers and one non-producer, one producer strain and one non-producer strains were inhibited and the culture was dominated by one producer. The producer strain AK4 inhibited the producer strain AK6 (20 hrs) and the non-producer strain AK8 (10hrs). In the same combination, the producer strain A11 was found to inhibit both the producer strain AL3 and non- producer strain AL6. The strains AL3 and AL6 were found to be inhibited at 25 and 15 hrs of co-culture respectively. In the combination of one producer strain (AK10) and two non-producer strains (AL2 and AJ4), the producer strain inhibited both the non-producer strains. In the final combination of the three strains of non- producers (AA7, AB3 & AB5) none of the strains were inhibited.

**Tab.1 Inhibition of producers and non-producers in mixed cultures**

| Mixed cultures | | |
|---|---|---|
| **Producer strains** | **Non-producer strains** | **Strains inhibited** |
| AA7 | AA9 | AA9 |
| AA5 | AA10 | AA10 |
| AB3 | AB5 | AB5 |
| AC3 | AC2 | AC2 |
| AE2 | AE9 | AE9 |
| BFA7 | BFA1 | BFA1 |
| BFA8 | BFA23 | BFA23 |
| BFA6 | BFB10 | BFB10 |
| OBSA1 | OBSA15 | OBSA15 |
| OBSA2 | OB SB 8 | OB SB 8 |
| AA7+AB3 | -- | AB3 |
| AB3+AC3 | -- | AC3 |
| AE2+AD16 | -- | AD16 |
| BFA7+BFA8 | -- | BFA7 |
| OBSA1+OBSA2 | -- | OBSA2 |
| -- | AA9+AA10 | None |
| -- | BFA1+BFA13 | None |
| AK4+AK6 | AK8 | AK6&AK8 |
| AL3+AL11 | AL6 | AL3&AL6 |
| AK10 | AL2+AJ4 | AL2 &AJ4 |

## DISCUSSION

The function of secondary metabolites in nature is a controversy raging for decades. Secondary metabolite production has also been hypothesized as 'elbowspace' to microbial species, which coexist in the same environment [17-18]. Long and Azam, (2001) [9] studied the antagonistic interactions among marine pelagic bacteria and reported that, "the perception that microbes are homogeneously distributed in seawater is changing to a perception that microbes are distributed heterogeneously. Bacterial species richness is also variable at the millimeter scale and the variability increases in response to increase in the concentration of particulate organic matter in seawater". They further report that for the heterogeneous distribution and potential for spatial structuring of bacterial populations, antagonistic interactions involving growth inhibition as one mechanism that may cause and maintain millimeter scale variations in the patterns of bacterial species composition.

It has been known for more than a century that some microorganisms inhibit or prevent the growth of others. Antagonisms between microorganisms were termed 'antibiosis' in 1890, eventually leading to the present day usage of antibiotic [19]. The results of the present study clearly show the antagonistic interaction of marine bacteria. Producers strains were found not only to inhibit non-producers but also other producer strain. In co-culture experiments the dominant strains completely inhibited the growth of the other strains within 35 hrs. *Lemos et al.*, (1991) [2], reported similar results and they found out most of the dominant producer strains inhibited the other producers within 10hrs of co-culture. In the present study complete inhibition time ranged from a minimum of 10 hrs to a maximum of 30hrs.

Lemos*et al.,* (1991) [2] reasoned that, even though for the results obtained, one could expect that the antibiotic producing strains would be dominant in their environment, but as previously reported by them [13], this is not the case. In nature, does this antibiotic producer have any advantage? [2], hypothesized that the production of the inhibitory substances would play a significant role in competition phenomena in some concrete microhabitats, such as algal surfaces, where these producer strains are relatively common. Rasool and Wimpenny (1982) [20] have calculated that *Streptomyces auerofaciens*, a producer of tetracycline, are able to produce enough antibiotic to prevent the growth of susceptible bacteria in a 10um radius around its hyphae.

Studies regarding antagonism in marine environment are very limited. The ecological significance of marine bacterial antagonism in pelagic waters is discussed by Long and Azam (2001) [9]. In mesotrophic and eutrophic waters or during phytoplankton blooms, heterotrophic bacteria on particles can count for large fractions of the bacterial activity (e.g., ectoenzymatic hydrolysis of organic particles and polymers and utilization of organic matter for respiration and growth). The cell specific levels of activity of particle attached bacteria are often 2 to 3 orders of magnitude greater than those of co-occurring free living bacteria. Attached bacterial hydrolytic enzymatic activity significantly influences the quantity and quality of biogenic matter that sinks from the upper water column into the ocean's depth. Since different bacteria express different arrays of hydrolytic enzymatic activities, changes in the bacterial species composition such as those potentially caused by microscale antagonism could alter the hydrolytic activity exerted by bacteria on organic particles.

Furthermore the species richness and diversity on particles could be influenced by bacterium-bacterium antagonisms and in turn this could affect the nature and biogeochemical transformation of the particles. Thus bacterium-bacterium antagonisms could be important variables in the ecology of the pelagic bacteria and in the bacterium-mediated carbon cycling in the ocean. Slattery *et al.,* (2001) [16] reported competition mediated antibiotic induction in the marine bacterium *Streptomycetes tejimariensis.* 12 of the 53 bacterial species induced istamycin production in *Streptomycetes tejimariensis*, which inhibited the competitor colonies. The results of the present study clearly demonstrate the competitive advantage of antibiotic producer strains to non-producers and this may play an ecological role in marine microhabitats. Previous reports of few studies carried out in marine bacterial competition were confined to algal epiphytic isolates and pelagic bacteria but in the present study strains isolated from biofilm and Opisthobranch surface were also used. Lemos*et al.,* (1991) [2] emphasized that further studies are necessary to establish the role of antibiotic substances in the control of marine bacterial populations and to determine the precise nature of the substances involved. But this can only be carried out with the collaborative efforts of experts in marine microbiology, ecology and chemistry.

**ACKNOWLEDGMENT**

Authors thank sincerely to Department of Science and Technology (DST) and Indian council of Medical Research (ICMR), New Delhi for the finance support.

**REFERENCES**

1. Atlas, RM, Principles of microbiology. 2nd Edition, Mc Graw Hill, 1997, NY, 739-805.
2. Lemos, ML, Dopazo, CP., Toranzo, AE., Barja, JL, Competitive dominanceof antibiotic producing marine bacteria in mixed cultures. *J. Appl. Bacteriol*., 1991, 71, 228-232.
3. Chellaram, C., Edward, JKP, Improved recoverability of bacterial strains from soft coral, Lobophytum sp. for antagonistic activity, *Journal of Pure and Applied Microbiology*, 2009, 3 (2), 649-654.
4. Anbuselvi, S., Chellaram, C., Jonesh, S., Jayanthi, L., Edward, JKP,.Bioactive potential of coral associated gastropod, *Trochus tentorium* of Gulf of Mannar, Southeastern India, *Journal of Medical Sciences*, 2009, 9 (5), 240-244.
5. Chellaram, C., Sreenivasan, S., Anand, TP., Kumaran, S., Kesavan, D., Priya, G. Antagonistic bacteria from live corals, Tuticorin coastal waters, Southeastern India, *Pakistan Journal of Pharmaceutical Sciences*, 2009, 24 (2), 175-181.
6. Fredrickson, A.G., and Stephanopoulos, G, Microbial competition. *Science*, 1981, 213, 972-984
7. Williams, ST., Viclkers, JC, The ecology of antibiotic production, *Microbial. Ecol*., 1986, 12, 43-52.
8. Gram, L., Inhibitory effect against pathogenic and spoilage bacteria of *Pseudomonas* strains isolated from spoiled and fresh fish. *Appl. Environ. Microbiol*., 1983, 59, 2197-2203.
9. Long, RA., Azam, F., Antagonistic interactions among marine pelagic bacteria, *Appl. Environ. Microbiol*., 2001, 67 (11), 4975 - 4983.
10. Gottlich, D., The production and role of antibiotics in soil. *J. Antibiotics*, 1976, 24, 987-1000.
11. Williams, ST, Are antibiotics produced in soil? *Pedobiologia*, 1982, 23, 427-435
12. Chellaram, C., Prem Anand, T., Kesavan, D., Priya, G., Chandrika, M., Gladis, C, Enhanced cultivability of antagonistic bacterial strains from soft coral *Sinularia* sp., Gulf of Mannar, southeastern India, *African Journal of Microbiology Research*, 2011, 5 (12), 1521-1526.
13. Lemos, ML., Toranzo, AE., Barja, JL, Antibiotic activity of epiphytic bacteria isolated from intertidal seaweeds. *Microb. Ecol*., 1985, 11, 149-163
14. Prem Anand, T., Chellaram, C., Kumaran, S., Felicia Shanthini, C. Screening for antibiotic producing marine bacteria against fish pathogens, *International Journal of Pharma and Bio Sciences*, 2011, 2 (1), 314-325.
15. Burgess, JG., Jordan, EM., Bregu, M., Spragg, AM., Boyal, KG, Microbial antagonism, a neglected avenue of natural products research, *J. Biotechnol*., 1999, 70: 27-32.
16. Slattery, M., Rajbhandari, I., and Wesson, K, Competition-mediated antibiotic induction in the marine bacterium *Streptomyces tenjimariensis. Microbial. Ecol*. 2001, 41(2), 90-96.
17. Zahner, H., Drautz, H., Weber, W, In: Bioactive microbial products: search and discovery, (eds) Bulock, J.D., Nisbet, L.J., Winstanley, D.J. Academic Press, 1982. NY, 51-70.
18. Chellaram, C., Sreenivasan, RS., Jonesh, S., Anand, TP., Edward, JKP.In vitro antibiotic bustle of coral reef associated gastropod, Drupa Margariticola (Broderip, 1832) of Tuticorin coastal waters, Southeastern India, *Biotechnology*, 2009, 8 (4), 456-461.
19. Bentley, R, From antibiosis to antibiotics a brief account of early work. Biochemist, 1990. 12 (5), 14-17.
20. Rasool, KA., Wimpenny, JWT., Mixed continuous culture experiments with an antibiotic producing *Streptomycetes*and *E.coli*. *Microbial. Ecol*., 1982, 8, 267-272.