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Research Article

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Analysis on inducement of antibiotic production in marine bacteria

Prem Anand, T[1*]. C. Chellaram[1], C. Felicia Shanthini[2] and R. Sivakumar[3]

[1]*Department of Biomedical Engineering, Vel Tech Multitech Engineering College, Chennai, Tamilnadu, India*
[2]*Department of Marine Science and Coastal Resource Management, Madras Christian College, Chenna, Tamilnadu, India*
[3]*Department of Electrical and Electronic Engineering, Vel Tech Multitech Engineering College, Chennai, Tamilnadu, India*

ABSTRACT

Competition amongst microbes for space and nutrients in the marine environment is a powerful selective force which has led to the evolution of a variety of effective strategies for colonizing and growing on surfaces. As the primary role of antimicrobial activity can be to antagonize competitors, bacteria may also produce antimicrobial compounds when they sense the presence of competing organisms. Bacterium - bacterium antagonistic interactions involving antibiotics are well documented in soils but work relating to this in marine environment is scanty. In this present study marine bacterial strains were induced for enhancement as well as for antibiotic production. In the present study out of the 75 antibiotic non-producer strains used for inducement study, 20 strains were induced. Inducements of antibiotic production by the bacterial strains were carried with heat killed and live Escherichia coli and Pseudomonas aeroginosa cells.

Key words: Marine bacteria, Antibiotic production, Southeastern India

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INTRODUCTION

The definition and function of secondary metabolites have been a controversy for decades. It was Bu'Lock [1] who first explicitly applied the term " Secondary metabolite" in microbiology and defined it as, "Given the generally acceptable view that there are basic patterns of general metabolism on which the variety of organic systems imposes relatively minor modifications, we can define secondary metabolites as having, by contrast, a restricted distribution (which is also species specific) and no obvious function in general metabolism". Secondary metabolites are often unique to a particular species or group of organisms and, while many act as antifeedents, sex attractants or antibiotic agents and many have no apparent biological role [2]. Nevertheless numerous secondary metabolites do impart known selective advantages on producers primarily of an ecological role. Bacterium - bacterium antagonistic interactions involving antibiotics are well documented in soils. In situ production of antibiotics in soil has been detected only in association with organically rich microenvironments like seeds, rhizospheres and straw fragments in soil [3-6].

Marine microorganisms have developed unique metabolic and physiological capabilities that not only ensure survival in extreme habitats but also offer the potential for the production of metabolites, which would not be observed, from terrestrial organisms [7] [8-10]. Competition amongst microbes for space and nutrients in the marine environment is a powerful selective force, which has led to the evolution of a variety of effective strategies for colonizing and growing on surfaces [11]. As the primary role of antimicrobial activity can be to antagonize competitors, bacteria may also produce antimicrobial compounds when they sense the presence of competing organisms [12]. Certain marine bacteria can be induced to produce antibiotics, however few attempts have been made to study such chemical communications between different bacterial species or how this might affect the

secretion of antimicrobial compounds [13-14].

In this present study marine bacterial strains (non-producers in control) were induced for antibiotic production. This type of study may help to find more novel molecules and screening can be widened to include non-producers also.

EXPERIMNTAL SECTION

Inducement of non-producer strains

A total 75 non-producer strains were chosen for the antibiotic inducement study. Clinical isolates of *E.coli* and *P. aeruginosa* were the terrestrial strains used for the assay and the study was carried out following the method of Spragg *et al.,* [14]. Five days cultures of *E. coli* and *P. aeruginosa* in nutrient broth were used to induce production of antimicrobial activity in the marine strains either as living or heat killed cells (121 °C, 15min).

Marine strains were inoculated separately in triplicate into three 30ml glass tubes containing 5 ml of marine broth 2216E (Himedia, Bombay, India). Heat killed or live *E.coli* cells (1ml) were placed inside a dialysis tubing and placed in contact with each marine strain in the test tubes. The control bottle contained only marine broth, marine producer strain and 1ml nutrient broth inside a dialysis tube. Similar set-up and experiment was carried out for *P. aeruginosa*.

Antibiotic activity screening

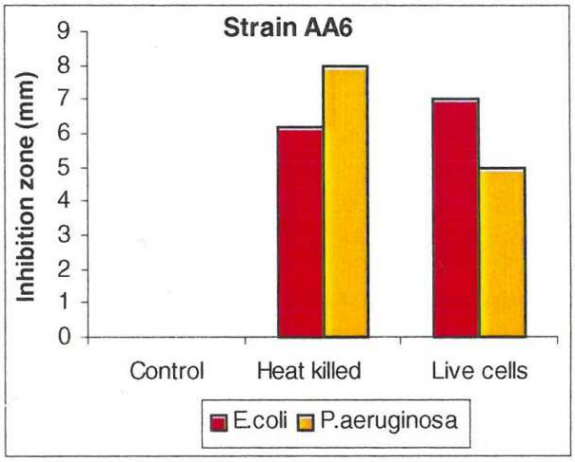
Antibiotic activity was screened in duplicate employing the standard disc diffusion assay. *E. coli* and *P. aeruginosa* were used as the test organisms. Paper discs (Whatman 6mm) were saturated with centrifuged (5000 rpm 10 mins) supernatant fluid (200 ul) from the test tubes and placed onto nutrient agar plates inoculated with the test organism. Plates were then incubated overnight at room temperature. Production of antimicrobial compounds was determined by measuring the inhibition zones from the edge of the disc to the edge of the clear zone.

RESULTS

Out of the 75 non-producer strains screened, 20 strains got induced for antibiotic production either by live or dead *E. coli* cells and *P. aeruginosa*. Potential (above 7mm) activity was exhibited by AA6, AB7, AE5, AF10, AI4, OBSA14 and BFA2 (Fig. 1 – 4). The induced strains AA1, AA6, AB7, AE5, AF10, H1, AK1 AL4 were isolated form seaweeds. The strain AA1 exhibited maximum inducement of 7 mm (Live cells) against *E. coli* and 5mm (Live cells) against *P. aeruginosa*. The strain AA6 exhibited a maximum inducement of 7 mm (Live cells) and 8 mm (Heat killed cells) against *E. coli* and P. *aeruginosa* respectively. A maximum inducement of 9.2 mm (Live cells) against *E. coli* and no activity (no inducement) was noticed against P.*aeruginosa* in the strain AB7. The strain AE5 maximum inducement of 8 mm (Live cells) against *E. coli* and 6.5mm (Live cells) P. *aeruginosa* was observed.

The strain AF10 exhibited maximum inducement of 10.5 mm (Live cells) against *E.coli* and 7mm (Live cells) against P. *aeruginosa*. Maximum of inducement of 6 mm(Live cells) and 7 mm (Heat killed cells) for *E. coli* and P.*aeruginosa* was noted in the strain AH1. In the strain AH8 a maximum inducement of 7 mm (Live cells) against*E. coli* and 4.2mm (Live cells) against P. *aeruginosa* was observed. The strain AK11exhibited a maximum inducement between 6.5 mm (live cells) and 7 mm (Heat killed cells) for *E. coli* and P. *aeruginosa* respectively (Fig 31). The strain AL4 exhibited amaximum activity (inducement) for 5.5 (Live cells) and 8 mm (Heat killed cells) against E. *coli* and P. *aeruginosa* and the strain OBSA 3 exhibited a maximum inducement of 5mm (Live cells) against E. *coli* and 7 mm (Heat killed cells) against P.*aeruginosa*. A maximum inducement of 7.5 mm (Heat killed) against E. *coli* and6mm (Heat killed) against P. *aeruginosa* was noted in the strain OBSA5 . In thestrain OBSA6 a maximum inducement of 5 mm (Live cells) and 2 mm (Live cells)against E. *coli* and P. *aeruginosa* was observed. The strain OBSA14 exhibited amaximum inducement of 9 mm (Heat killed) against E. *coli* and 5 mm (Heat killed) against P. *aeruginosa* and the strain OBSB6 was only induced against P. *aeruginosa and* the maximum inducement was 5 mm (Live cells). In the strain OBSB10 the inducement was 6.5 mm (Live cells) and 6.5 mm (Heat killed) against E. *coli* and P.*aeruginosa* respectively. The OBSA , OBSB and BFA, BFB strains were isolated from Opisthobranch surface and biofilm. The BFA2 strain exhibited a maximum inducement of 8.5 mm (Heat killed) and 4 mm (Heat killed) against E. *coli* and P.*aeruginosa* respectively. A maximum inducement of 7 mm (Heat killed) and 6.5 mm (Live cells) against E. *coli* and P. *aeruginosa* was noted in BFA20 strain. The BFA22 strain exhibited inducement only against P. *aeruginosa,* a maximum of 5 mm (Heat killed) activity was observed. The strain BFA 19 exhibited maximum inducement of 5 mm (Heat killed) and 7 mm (Heat killed) against E. *coli* and P. *aeruginosa* respectively and in the strain BFB20 inducement was observed only against E. *coli* (Live cells).

Fig.1 Antibiotic inducement of non-producer strains AA6 & AB7 against *E.coli* and *P.aeruginosa* using heat killed and live cells of *E.coli* and *P.aeruginosa*



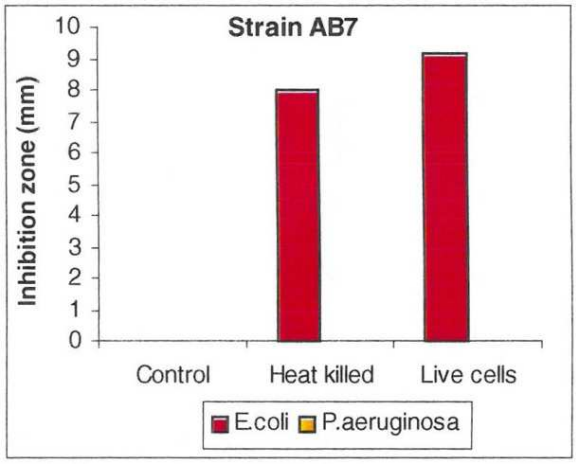
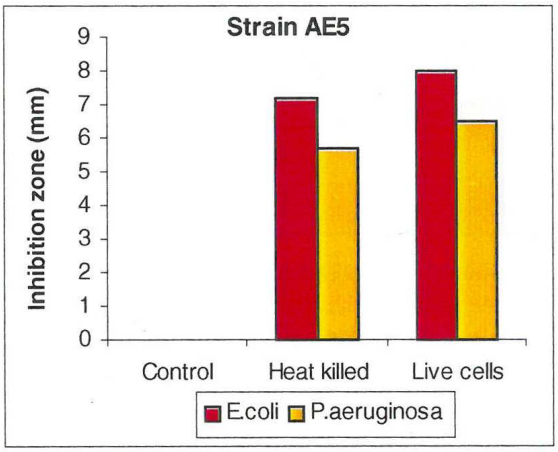


Fig.2 Antibiotic inducement of non-producer strains AE5 & AF10 against *E.coli* and *P.aeruginosa* using heat killed and live cells of *E.coli* and *P.aeruginosa*



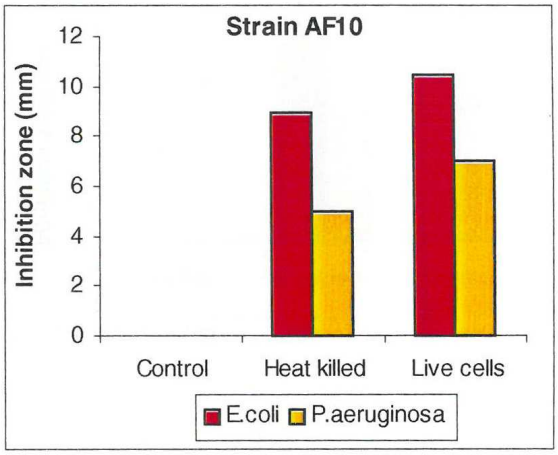
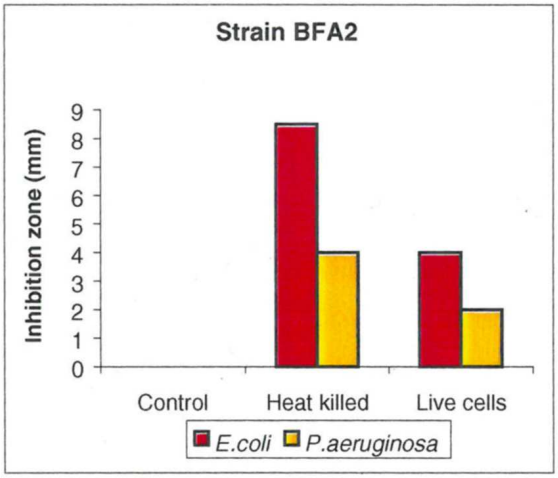


Fig.3 Antibiotic inducement of non-producer strains BFA2 & OBSA14 against *E.coli* and *P.aeruginosa* using heat killed and live cells of *E.coli* and *P.aeruginosa*



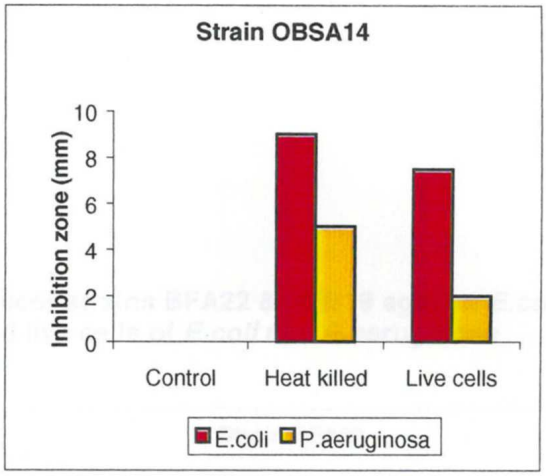
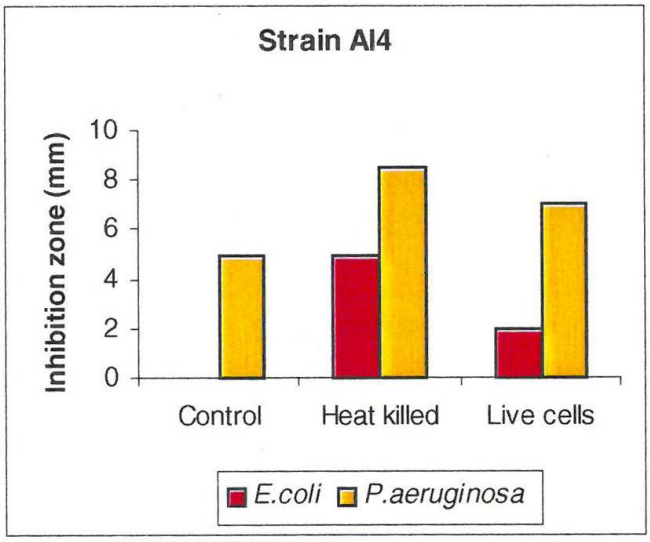


Fig.4 Antibiotic inducement of non-producer strain AI4 against *E.coli* and *P.aeruginosa* using heat killed and live cells of *E.coli* and *P.aeruginosa*



DISCUSSION

The production of antimicrobial compounds by marine bacteria is usually assayed under straightforward growth conditions and only strains, which constitutively produce such compounds, can be successfully screened [14]. However, as the primary role of antimicrobial activity can be to antagonize competitors, bacteria may also produce antimicrobial compounds when they sense competing organisms. However few attempts have been made to study such chemical communication between different bacterial species or how this might affect the secretion of antimicrobial compounds [14-16].

In the present study out of the 75 non-producer strain used for inducement study, 20 strains were induced. Potential (above 7mm) activity was exhibited by AA6, AB7, AE5, AF10, AI4, OBSA14 and BFA2. Variations in inducement of strains were noted against the two pathogens and some strains were induced to produce antimicrobial compounds against both or against either one of the pathogens. The strains responded against both the pathogens were AA1, AA6, AE5, F10, AH1, AH8, AK11, AL4, OBSA5, OBSA14, OBSB 10, BFA2 and BFA 20. The strains responded only against *E.coli* were AB7 and BFB20 and against *P.aeroginosa* was BFA22 and OBSB6, Burgess *et al.*, [17] reported inducement of antibiotic production by strains which did not normally produce antibiotics. They used cell free supernatants to enhance antimicrobial production as well as for inducement of antibiotic production. This type of studies was limited and their importance was stressed by both Spragg *et al.*, [14] and Burgess *et al.*, [18]. Long and Azam [19] reported production of inhibitory compounds by attached and free living bacteria and found significantly greater percentage of attached bacteria than free living bacteria produced inhibitory compounds (66.7 and 40.7 %). But the actual antibiotic-producing bacteria may be higher if inducement considerations are taken into account.

Once again the point emphasized by Spragg *et al.*, [14,20] can be repeated, "in order to find more novel structures, new way of screening for these compounds must be applied" and inducement studies can thus become part of a screening program.

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REFERENCES

[1]. JD Bu'Lock, The biosynthesis of national products: an introduction to secondary metabolism, Mc Graw Hill, London.**1965.** 54-56
[2]. RJP Cannell, How to approach the isolation of a natural product. In : Methods in Biotechnology Vol.4 Natural Products Isolation, (ed) Cannell, R.J.P., Humana Press, NJ, USA. **1998.** 39-43
[3]. G Bruehl,; R Mittar; B Confer, *J. Plant Sci.* **1969**, 49, 235-246.

[4]. LS Thomashow; DM Weller; RF Bonsall; LS Pierson, *Appl. Environ. Microbiol*. **1990**, 56, 968-912.
[5]. J Wright, *Ann. Appl. Biol*. **1965a**, 44, 461-466.
[6]. J Wright, *Ann. Appl. Biol*. **1965b**, 44, 561-566.
[7].T Prem Anand; C Chellaram; S Kumaran; C Felicia Shanthini, *International Journal of Pharma and Bio Sciences*, **2011,** 2 (1), 314-325.
[8].TP Anand; CF Shanthini; C Chellaram, *International Journal of Pharma and Biosciences*, **2012**, 3 (2), 359-368.
[9].C Chellaram; T Prem Anand; S. Kumaran; R.S. Sreenivasan, *Pakistan Journal of Pharmaceutical Sciences*, **2011**, 24 (2), 153-158.
[10]. C Chellaram; T Prem Anand; C Felicia Shanthini; B Arvind Kumar; Sidharth P Sarma. International Conference on Biotechnology and Food Sciences (ICBFS'2012), APCBEE Proceedia, **2012**, 2, pp-37-42.
[11]. JG Burgess, EM Jordan; M Bregu; AM Spragg; KG Boyal, *J. Biotechnol*. **1999**, 70, 27-32.
[12]. GL Patterson, CM Bolis, *J. Phycol*. **1997**, 33, 54-60.
[13]. MA Spragg; KG Boyd; MO Hubble; JG Burgess, Proceedings of the Fourth under water science symposium, Society for Under Water Technology, London, **1997**, pp-147-157.
[14]. AM Spragg; M Brega; KG Boyd; JG Burgess, *Lett. Appl. Microbiol*. **1998**, 27, 142-146.
[15].T Prem Anand; C Chellaram; A Nafiza Parveen; C Felicia Shanthini, *Journal of Chemical and Pharmaceutical Research*, 5 (4): 16-21. **2013.**
[16]. G Priya; C Chellaram, (2011) *Journal of Chemical and Pharmaceutical Research*, **2011**, 3 (3), 154-158
[17]. JG Burgess; H Miyashita; H Sudo; T Matsunaga. *FEMS Microbiol. Lett*. **1991**, 84, 301-306.
[18].JG Burgess; EM Jordan; M Bregu; AM Spragg; KG Boyal. *J. Biotechnol*. **1999**, 70,27-32.
[19]. RA Long; F Azam, *Appl. Environ. Microbiol*. **2011**, 67 (11), 4975 - 4983.
[20]. C Chellaram; P Raja; A Alex John; S Krithika, *Pakistan Journal of Biological Sciences*, **2013**, 16 (9), 431-434