International Journal of Basic and Applied Biology
p-ISSN: 2394-5820, e-ISSN: 2349-5839, Volume 6, Issue 1; January-March, 2019, pp. 26-31

http://www.krishisanskriti.org/Publication.html

Studies on Production of Antibiotics by Marine Bacteria and Role of Quorum Sensing

Huma Maryam Mateen[1], Dr. Sunanda B. More[2] and Prof. Saiqua Khan[3]

[1,2,3]Yashwant Mahavidyalaya, Nanded, Maharashtra
E-mail: [1]irsh.mech@gmail.com, [2]drsbmore@gmail.com,
[3]saiquatadvi@gmail.com

Abstract—*Antibiotics are medicines used to prevent and treat bacterial infections. Antibiotic resistance occurs when bacteria change in response to the use of these medicines. These antibiotic resistant bacteria may infect humans and animals and the infection they cause are harder to treat than those caused by non-resistant bacteria. Antibiotic resistance is rising to dangerously high level in all parts of the world. New resistance mechanisms are emerging and spreading globally threating our abilities to treat common infectious disease. A growing list of infectious diseases such as pneumonia, tuberculosis, gonorrhea, food poisoning etc are becoming harder and sometime impossible to treat as antibiotics become less effective. That's why it is important to keep looking for new antibiotics because resistance will eventually develop for all antibiotics. In present study marine bacteria were isolated & analysed for their efficacy in antibiotic production against common clinical pathogens viz Salmonella typhi, E.coli, S.aureus, Proteus & Psendomonas. Out of 7 bacterial strains isolated and analyzed, two isolates exhibited significant antibacterial activity against E.coli & S. typhi. . Isolate designated as C3, produce antibiotic when co-cultured with E.coli and C7 produce antibiotic when cultured with S.typhi. Cell free extract of C7 & C3 which is later identified as Bacillus subtilis and Bacillus cereus (as per Bergey's manual) cultivated in the presence of heat killed S.typhi, E.coli cells. And antimicrobial activity was tested by disc diffusion method. Results of the present co-cultivation study depict interspecies quorum sensing pattern where E.coli induced antimicrobial compound production in C3 and S.typhi in C7. Present study on antibiotic production against S.typhi, E.coli in marine bacteria pave the way for the discovery of pathogen targeted / specific antibiotic production.*

1. INTRODUCTION

Antibiotics are medicines that kills bacteria or slows the growth of bacteria. They are used to cure diseases. Antibiotics were first produced in 1939. The term antibiotics was introduced by S.A Waksman in 1942. Antibiotic is very different from chemotherapeutic drugs, antibiotics are natural drugs that is produced by several fungi or bacteria but chemotherapeutics drugs are man-made substances. The serendipitous observation of penicillin antibiotics marked the beginning of the modern era of antibiotic discovery by Alexander Flemming in 1928.According to him *Penicillin notatum* were grown in appropriate substrate it would extrude a substance that has antibiotic properties [1]. Penicillin was active on *Pneumococci* and *Streptococci*. They have inhibitory effects on *Cornybacterium diphtheria* and *Treponema palladium*. Streptomycin were effective on Gram negative aerobic bacteria and *Mycobacterium tuberculosis*. The problems of infectious diseases suddenly increased when certain became antibiotic resistant [11]. *Mycoplasma*, *Clamydiae* or *Rickettsiae* were not affected by penicillin or streptomycin. Staphylococci species were the first pathogens to show resistance and their resistance spread throughout the world in a short period of time. This problem was solved when new antibiotics such as neomycin and colimycin were discovered. The isolation of peptide bogorol A from a marine bacillus culture was active against methicillin resistant *S.aureus* [3] and vancomycin resistant *Enterococcal* strains. An unusual peptide dicynthaurin was discovered from haemocytes of marine funicate *Haalocynthia aurantium* [8]. Halocin was discovered from *H.aurantium* [7]. This halocidin demonstrated significant potency against methicillin- resistant *S.aureus* and multidrug- resistant *Pseudomonas aeruginosa*.

Earlier bacteria were considered as an individual cell that itself had the capability to multiply in presence of nutrients. But recently, intercellular communication in bacteria has been discovered, which was once believed to be only in multicellular organisms. The advantage of this mechanism is that they will become more adapted to sessile environment, will have better nutrient supply and new modes of growth. This phenomenon of cell to cell communication is called Quorum Sensing. This intercellular communication is based on the small self-generated molecules called autoinducers. With the help of these autoinducers bacteria can regulate their behavior accordingly with the population density. The main principle of Quorum Sensing is when single bacteria releases autoinducers into the environment, their concentration is too low to be detected but in presence of other bacteria, the autoinducer reach to a threshold level and allows the bacteria to sense a critical cell mass and in response, activate or repress target genes. Quorum Sensing depends on population density [6]. On the other hand, extracellular signaling forms a basis for the control of molecules and cellular process and population behavior. Quorum sensing is of two types, firstly species

specific and secondly, interspecies. Species specific is more common in Gram negative bacteria which is mediated by acyl homoserine lactones(AHL) [5]. Quorum sensing regulates competence development, antibiotic synthesis, cell differentiation, sporulation, virulence factor induction and nutrient flux [4]. Recently quorum sensing was linked through proteomic analysis in order to increase pathogenic competence in strain of *Pseudomonas aeruginosa* [2]. Thus, we can say that although few researches have been done in this field but still it is interpreted that cell induced antibiotic production generates a high rate of antibody as compared to bacteria solely. Research is still going on.

It is important to keep looking for new antibiotics because resistance will eventually develop for all antibiotics, it is only a matter of time. That time can be delayed by prudent use of antibiotics but it cannot be delayed forever. The selection pressure imposed by antibiotic use and the ubiquity of antibiotic resistance gene in the environment ensure that resistant strains of pathogens will eventually emerge. All we can do is keep developing new antibiotics and prolong their useful life through prudent use. From that we can manage the problem of antibiotics resistance, but we can never solve it. Importance of this study is to discover and produce new antibiotics from marine microorganisms to restrain human pathogens and to compare the rate of antibiotic production by the bacteria solely and when it is induced with another bacteria Quorum Sensing forms the basis for cell induced antibiotic production. 75% of the earth's surface is covered with marine organisms. Thus the future of this marine technology is quite promising. Marine organisms turn out to be a source of anticancer compound for example, Didemin, which is isolated from *Carribean tunicle. Trideodemnum solidum* acts against leukaemia and melanoma. Marine bacteria are richly endowed with enzyme. *Thermococcus litoralis* archaebacteria produce viral DNA polymerase that can remain active for more than 2 hrs at 100^{o}C. Thus this shown that marine bacteria have the potential to synthesize self tolerant enzymes that can be used in food and pharmaceutical industries , as such they are used for therapeutic purposes .As in quorum sensing, Bacterial cells have the ability to show cell to cell communication in presence of another bacteria with their auto inducers. This allows the bacteria to sense a critical cell mass and in response activate or repress target genes. This coordinate behavior of cell-density-dependent has several advantages. In pathogenic microorganisms, the regulation of the virulence determinants throughout the infection process plays an important role in pathogenicity. The major goal of pathogens is to evade disease, and as such, quorum sensing is an important asset because it enables bacteria to appropriately time expression of immune response-activating products. Using quorum sensing, bacteria can amass a high cell density before virulence determinants are expressed, and the bacteria are able to make a concerted attack and produce virulence factors to overwhelm the host defenses.

2. MATERIALS AND METHODS

2.1 Materials

1) Marine water sample
2) Zorbell Marine Agar (ZMA)
3) Zorbell Marine Broth (ZMB)
4) Marine Agar
5) Muller Hinton Agar (MHA)
6) Nutrient Broth (NB)
7) Nutrient Agar (NA)
8) Luria Broth (LB)
9) Pathogenic micro organisms
10) *(Escherichia coli, Salmonella typhi,* Proteus, *Staphylococcus aureus,* Pseudomonas*)*

All chemicals and apparatus were provided by department of microbiology, Yashwant Mahavidyalaya, Nanded.

2.2 Methods:

2.2.1 Sample Collection

A study was done on 7 different isolates of marine bacteria. The marine water samples were kept at 4^{0}C until transferred to the laboratory. The sample was collected from Valappu Beach, Kerala respect.

2.2.2 Isolation of marine bacteria

5 ml of the marine water sample was inoculated in 50ml ZMB broth for culture enrichment. Then aliquots were streaked on the surface of the ZMA (3plates). These plates were incubated at 27^{0}C for 3-5 days. After incubation, 7 colonies were isolated based on their pigmentation, size, elevation and margine. These colonies were restreaked on marine agar plates for further growth.

2.2.3 Screening for anti- microbial activity

2.2.3.1. Disc diffusion method

Bacterial strains from 7 isolated colonies were inoculated in 10ml of sterile NB and it was kept on shaker at 110rpm for 48hrs. Five flasks, each containing 5ml of sterile LB, were taken. The pathogens were inoculated in flaks & kept it on shaker at 110rpm at 37^{0}C for 6 hrs. The 100 µl of these pathogens were spread on the NA plates. The NB containing cultured strains were centrifuged at 6000 rpm for 10 min for separating their supernatant. The filter paper disc was dipped in the crude extract of supernatant of the cultured bacterial strains and it was kept on the MHA plates. The Plates were then kept for 48 hrs. for incubation. After incubation, zones were observed around the filter paper disc.

2.2.3.2. Enhancement of anti-microbial compound synthesis by cross cell induction

The pure bacterial strains, which has shown antimicrobial activity against pathogens, were used in this method for enhancement of their antimicrobial compound synthesis by cross cell induction. For this method, 25 ml of Luria Broth were taken in each test tube such that 2 sets (for 2 bacterial strains) can be prepared. Each set contains 3 test tubes. 1st test tube was used as blank which contains no pathogen and only bacterial strain. In 2nd test tube bacterial strain was inoculated with heat killed pathogens and in 3rd test tube bacterial strains are inoculated with live pathogens. These sets of test tubes were kept for 3 days for incubation. After incubation, the broth culture was centrifuged at 5000 rpm for 10 min at 40C. Then again above disc diffusion method steps were repeated.

2.2.3.3. Biochemical identification of bacterial strains

Biochemical identification of the selected strains was performed by physical and biochemical characterization. For physical characterization, Gram staining was performed to detect their Grams nature.

For biochemical characterization, different tests were performed viz., Catalase Test, Oxidase Test, IMVIC Test, etc. The isolated culture C3 and C7 were aseptically inoculated in Biochemical media and incubated at 37^0C for 24 hrs. At the end of incubation period, series of required reagents were added in different tubes as per standard data to carry out different biochemical tests.

3. RESULT

3.1 Colony Characters and morphology

Colony character and morphology which has been observed during isolation of marine bacteria is explained in table no 1 below for 7 isolated colonies.

Table 1: Colony Characters and morphology

Sr. No	Characters	C-1	C-2	C-3	C-4	C-5	C-6	C-7
1	Size	4mm	3mm	3mm	4mm	3mm	2mm	3mm
2	Shape	Circular	Circular	Circular	Circular	Irregular	Circular	Circular
3	Margine	Entire	Entire	Entire	Undulate	Entire	Entire	Undulate
4	Colour	White	White	Pale yellow	White	Pale yellow	White	Orange
5	Elevation	Raised	Raised	Raised	Umbonate	Flat	Raised	Umbonate
6	Opacity	Opaque	Translucent	Translucent	Opaque	Translucent	Opaque	Iridescent
7	Consistancy	Smooth	Smooth	Sticky	Waxy	Dry	Smooth	Soft smooth
8	Grams nature	G+ve Hollow Rods	G-ve Short Rods	G+ve Endospor Rods	G+ve Short Rods	G-ve Rods	G+ve Endospord Rods	G+ve Endospord Rods

3.2 Screening of antibacterial activity

3.2.1 Disc diffusion method

The results of screening of antimicrobial activity by disc diffusion method is described in table no 2 below.

Table 2: Screening of antimicrobial activity by disc diffusion method

Strains	S.typhi	E.coli	S.aureus	Proteus	Pseudomonas
C1	-	-	-	-	-
C2	-	-	-	-	-
C3	-	+	-	-	-
C4	-	-	-	-	-

C5	-	-	-	-	-
C6	-	-	-	-	-
C7	+	-	-	-	-

Strain C7 have shown antibiotic activity for *S.typhi* whereas C3 shown for *E.coli*. For further analysis of this isolated marine bacteria, we have selected C3 and C7 strain.

3.2.2 Enhancement of antimicrobial compound synthesis by cross signaling induction

Two pure strains of bacteria C3 and C7 has shown antimicrobial activity, these strains are taken for antimicrobial compound by cross signaling induction of the cells against those pathogens whom they have shown antimicrobial activity. In our present study, we studied 2 strains in their logarithmic phase with the co-cultivation of the respective pathogen *E.coli*, and *S.typhi* and studied their antimicrobial activity. The antimicrobial activity was measured as zone of inhibition measured in millimeter.

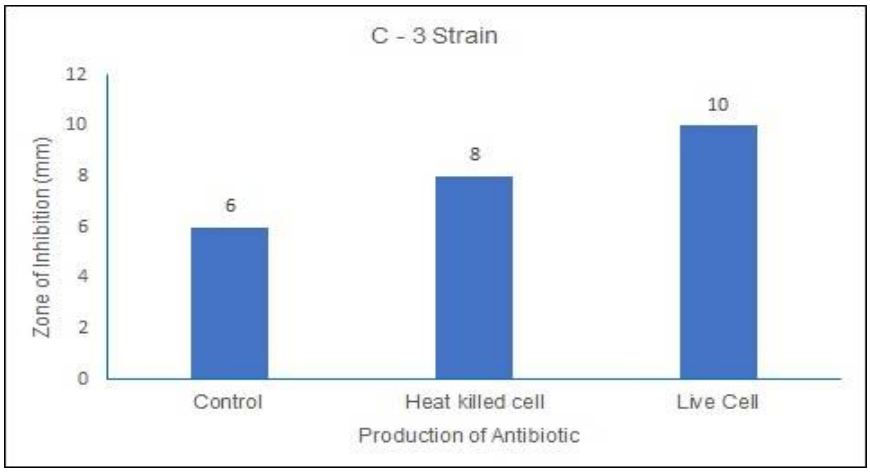


Figure 1: Production of antibiotic by C – 3 strain in response to *E.coli*

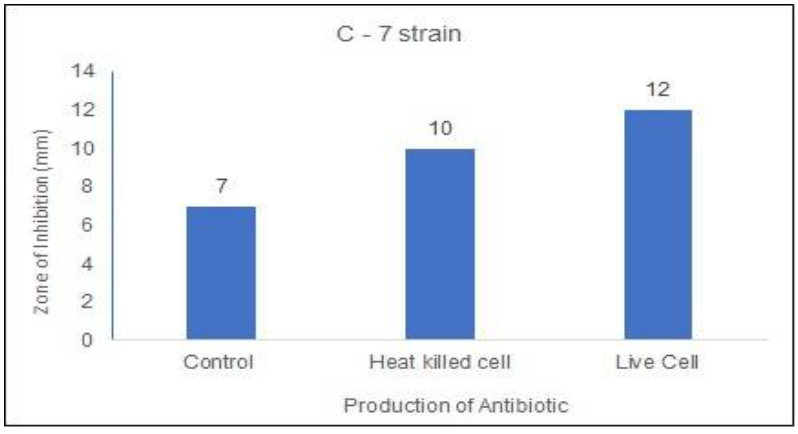
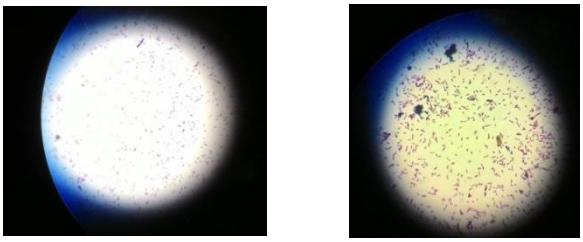


Figure 2: Production of antibiotic by C – 7 strain in response to *S.typhi*

3.3 Characterisation of Bacterial Strains:

3.3.1 Physical Characterisation

The Gram staining was performed on C3 and C7 strain and both were found to be Gram positive endospore containing rods, as shown in figure no 3 below.



a) Microscopic view of C3 b) Microscopic view of C7

Figure 3: Gram staining of C3 and C7

3.3.2 Biochemical test results

Table 5: Biochemical Identification of strains

Test	C3	C7
Citrate	+	+
Nitrate reduction	-	+
Catalase	+	+
Arginine	+	-
Sucrose	+	+
Mannitol	-	+
Glucose	+	+
Trehalose	+	+
Indole	-	-
MR	-	-
V.P.	+	+
Oxidase	+	-

As per physical and biochemical test results, isolated strain C3 was found to be Gram positive endospore containing rods. C3 is non-fermentative and tested negative for nitrate reductase. It did not grow in anaerobic medium leading us to hypothesize that it was an obligate aerobe. This was substantiated by positive results for both oxidase and catalase activities, it led to the tentative identification of C3 as *Bacillus cereus*.

Also in C7, isolated strain was found to be Gram positive motile bacillus, having endospore containing rods often occurring in short chains. It was fermentative and tested positive for nitrate reductase. This was substantiated by positive results for both catalase and V.P test. It led to the tentative identification of C7 as *Bacillus subtilis* according to Burgey's manual.

4. DISCUSSION

It has been well established that antibiotic production can be induced or enhanced by exposing producing strains with competing organisms. As the concept of the survival of the existence, the marine bacteria can also compete for the nutrients and available space for growing for which they are forced to secrete some molecules that can help them for the existence of that environment and rest to eliminate. The results of the present co-cultivation study confirm that the isolated C3 and C7 produced antimicrobial compounds in the presence of *E.coli* and *S.typhi* against whom they are reactive. Results of the present co-cultivation study depict interspecies quorum-sensing pattern where *E.coli* induced antimicrobial compound production in C3 and *S.typhi* induced in C7. Mixing of live/ heat killed *S.typhi* and *E.coli* with bacteria will facilitate the transfer of signal molecules produced by the

inducer. In the present study, strain C3 and C7 when cultured alone in a shake flask produced very less amount of antibacterial compound where as C3 and C7 produced high amount of antibacterial compound when co-cultured with live and /or heat killed cells of *S.typhi* & *E.coli.*

5. ACKNOWLEDGEMENTS

The authors would like to express their gratitude towards Department of Microbiology Yashwant Mahavidyalaya, Prof. S. M. More and Mr. D. J. Pitlewad.

REFERENCES

[1] Amann, R.I., Ludwig, W. & Schleifer, K.H. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol, (1995);Torsvik, V., Goksoyr, J. & Daae, F.L. High diversity in DNA of soil bacteria. Appl. Environ. Microbiol, (1990).

[2] Arevalo-Ferro C, Buschmann J, Reil G, Gorg A,Wiehlmann L, Tummler B, Eberl L, Riedel K: Proteome analysis of intraclonal diversity of two *Pseudomonas aeruginosa* TB clone isolates. Proteomics (2004), 4:1241 -1246.

[3] Barsby T, Kelly M T, Andersen R J,Tupuseleiamides and Basilisksmides, new acyl dipeptides and antifungal polyketides produced in culture by a *Bacillus laterosporus* isolate obtained from a tropical marine habitat. J. Nat.Prod. (2002),65,1447-1451

[4] Cvitkovitch DG, Liu YH, Ellen RP: Quorum sensing and biofilm formation in Streptococcal infections. J Clin Invest (2003) 112:1626-1632.

[5] Fuqua C, Parsek MR, Greenberg EP: Regulation of gene expression by cell-to-cell communication: Acyl-homoserine lactone quorum sensing. Annu Rev Genet (2001), 35:439-468

[6] Hiramatsu K, Hanaki H, Ino T, Yabuta K, Oguri T, Tenover FC: Methicillin resistant *Staphylococcusaureus* clinicalstrain with reduced vancomycin susceptibility. J Antimicrob Chemother (1997), 40:135-136

[7] Jang, W, S, Kim, K N, Lee, Y .8, Nam, M.H, Lee,I H, Halocidin: a new antimicrobial peptide from hemocytes of the solitary tunicate *Halocynthia aurantinm*. FEBS Lett,(2002),521, 81-86

[8] Lee, J.H, Lee Y S, Kim C H, Kim C R, Hong T, Menzel L, Boo L M, Pohl J, Sherman M A, Waring A, Lehrer R I. Dicynthaurin: an antimicrobial peptide from haemocytes of the solitary tunicate, *Halocynthia aurantium* . Biochem. Biophys. Acta (2001)1527, 141-148.

[9] Patterson.G. L and Bolis, C M Fungal cell- wall polysaccharides elicit an antifungal secondary metabolite(Phytoelexin) in the Cynobacterium *Syctonema ocellatum.* Journal of Phycology (1997)33, 54-60

[10] Rejitha Nair, Studies on production of antibiotics by induction of marine bacteria with human pathogen (2012)

[11] Walsh, C.T. Antibiotics: Actions, Origins, Resistance (ASM Press, Washington; 2003).