EFFICACY OF ANTAGONISTIC ACTIVITY OF ENDOPHYTIC BACTERIA AGAINST SOME CLINICAL FUNGI

B.Megala and P.Thirumalaivasan

PG and Research Dept of Biotechnology,

Srimad Andavan Arts andScience College,

.Trichy – 621 107, Tamilnadu, India.

ABSTRACT

In the current investigation suggested that the endophytic bacteria from *Avicennia marina*of marine associated plant. The identification of endophytic bacteria by various biochemical tests were performed with the help of Bergey's manual of determinative bacteriology by 9th edition According to the bacteria, it has confirmed that the *Serratia*sp, *Bacillus megaterium*, *Bacillus subtilis*, *Pseudomonas* sp. and *Stenotrophomonas*sp were identified from *A.marina* leaves. The effect of antifungal activity of endophytic bacteria like *Bacillus subtilis*, *Bacillus megaterium*, *Stenotrophomonas*sp, *Pseudomonas* spand*Serratia*sp ofsecondary metabolites against some specific clinical fungi like *Aspergillusflavus, A. niger, A. terreus, A. sydowi*and*Penicillumcitrinum* were performed. Among the antagonistic bacteria. *Bacillus subtilis* with 100µl concentration of secondary metabolites were treated against pathogenic fungi *Aspergillus flavus, A. niger, A. terreus, A. sydowi* and *Penicillum citrinum* were excellent and significant results observed and extraordinary antifungal properties were performed and zone of inhibition were recorded higher then that of the other endophytic bacteria.

Key words: *Avicennia marina*, endophytic bacteria, Antifungal activity

INTRODUCTION

Endophytes are microorganisms that live inside living tissues of plants. In most cases, the microbial relationship with the host plant is symbiotic or mutualistic with no visible damage or morphological changes on their hosts (Schulz and Boyle, 2006). Because endophytes live in a steady environment inside the plant, they have more antagonistic potentiality than

microorganisms isolated from rhizosphere, plant surface, or soil (Dowler and Waver, 1974; Andrews, 1992).

Marine environment is currently pointed as one of the most important sources regarding natural products research field, since organisms from oceans have been exhibited remarkable biological, biochemical and biosynthetic potential (Gerwick and Moore, 2012; Mayer *et al*., 2010, 2011; Molinski*et al*., 2009; Newman and Cragg, 2014). Biodiversity is very expressive concerning high taxonomic levels: from 76 phyla described for *Eukariota*, about sixty can be found in marine areas, meanwhile forty for terrestrial or freshwater environments (Blunt *et al.*, 2013). Marine natural products research have often been mentioned as fundamental for discovery of new chemical structures, mainly for featuring unusual mechanisms of action (Molinski*et al.*, 2009). In a recent data, up to 2009, 2840 marine species have been investigated resulting on the isolation of 20,057 metabolites, which were published in 7795 articles. Despite exciting numbers, considering the total of 250,000 recognized marine species, it is estimated that only 1% of them have already been studied (Blunt *et al.*, 2013), (Purbowatiningrum Ria Sarjono, *et al.*, 2020), (Su *et al.*, 2020) which means there are an amazing roll of unexplored living beings comprising chemical and biological treasures.

Similarly, microbial natural products represent an extensive area for new therapeutic compounds search (Berdy, 2012; Cragg and Newman, 2013; Demain, 2014; Vederas and Li, 2009; Walsh and Fischbach, 2010). Relevant reviews emphasized microbial metabolites as targets for discovery and development of new drugs, mostly anticancer and antibiotics (Berdy, 2012; Butler *et al.*, 2013; Demain, 2014),(María, *et al.*, 2019), antifungals, antiparasitics, among others (Amedei and D'Elios, 2012). Microorganisms are very versatile and found everywhere, even in inhospitable habitats, in all ecosystems around the globe. It is preconized that less than 1% of all bacteria species and less than 5% of all fungi species are described, suggesting at least 10 million microbial species are unknown, remaining hidden in nature (Berdy, 2012). Besides, based on genetic researches, 90% of biosynthetic skill of microorganisms keeps unattainable, what ratifies the significance of microbial natural products research for drug discovery and, even for complete biodiversity knowledge and ecological relationships.

MATERIALS AND METHODS

Collection of marine associated plant and isolation of bacteria;

Collection of marine associated plant *Avicennia marina* from sethubavachathiram of Thanjavur district, Tamilnadu, india

Isolation of endophyticbacteria such as *Bacillus subtilis.,Bacillus megaterium, Stenotrophomonas*sp, *Pseudomonas* sp*, and Serratia*spwere identified from the *Avicinnia marina* by using nutrient agar(NA) medium .

Clinical fungi

The clinical fungi like *Aspergillusflavus, A.niger, A.terrus, A.sydowi,* and *Penicillumcitrinum* were procured from Indian Biotrack Research Institute, Thanjavur. These clinical fungi were isolated from diabetic patient of Thanjavur medical college,Thanjavur.

Agar well – diffusion method (Kirby and Bauer 1996).

Agar well – diffusion method was followed for determination of antifungal activity. Nutrient agar (NA) plates were swabbed (sterile cotton swabs) with 24 hours culture old – broth culture of respective bacteria.. About 25,50;75 and 100µl of different concentration were added using sterilized dropping pipettes into the wells and plates were left for 1 hour to allowed a period of pre – incubation diffusion in order to minimize the effects of variation in time between the applications of different solutions of the plates were incubated in an upright position at 37˚C ± 2˚C for 24 hrs for clinical fungi.. Results were recorded as the presence or absence of inhibition zone. The inhibitory zone around the well indicated absence of tested organism and it was reported as positive and absence of zone is negative. The diameters of the zones were measured using diameter measurement scale. The effect of endophytic antagonistic bacteria were analysed against clinical fungi. *Aspergillusflavus, A.niger, A.terrus, A.sydowi,* and *Penicillumcitrinum*were performed. Triplicates were maintained and the average values recorded for antifungal activity.

RESULT AND DISCUSSION:

In the present investigation suggested that the effect of antifungal properties of endophytic bacteria. Huda mohammedahmed sheik, 2010)studied that the antimicrobial activity of certain bacteria and fungi isolated from soil mixed with human saliva against pathogenic microbes causing dermatological diseases**.** The antimicrobial activities of soil, saliva and mixture of both soil and saliva were tested against the three tested pathogenic organisms. The results revealed that soil alone showed moderate antimicrobial activity compared to saliva which did not produce any antagonistic effect. On the other hand, mixture of soil and saliva showed the highest antimicrobial activity. The same trend was observed for the cell free bacterial and fungal culture media. Thus, the pathogenic organisms showed higher sensitivity to the filtrates resulted from mixing soil and saliva. Based on the above mentioned results, all the experiments were carried out using the mixture of soil and saliva. This is may be due to that the alkalinity of saliva may favour the growth of antibiotic producing microorganism. Furthermore, saliva act as oral antiseptic because it contain lysozyme and prevent the growth of dental bacteria. Moreover, when saliva decreased the growth of pathogenic fungi increased leading to different oral diseases. Antimicrobial activities of all the 5 strains were checked against different test organisms. These strains showed their extensive inhibition effect particularly against gram-positive test bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and the test fungal strain (*Candida albicans*). On the other hand, *B. brevis* M1 66 and *B. brevis* T1 22 strains had an inhibitory effect against gram positive and gram-negative test bacteria (*Escherichiacoli* and *Proteus vulgaris*) as well as the test fungal strain.

In the recent investigation suggested that the efficacy of antagonistic activity of bacteria against some clinical fungi were performed. The effect of *Bacillus subtilis*with different concentration of 25,50,75,and 100µl was treated against *Aspergillusflavus, A.niger, A.terrus, A.sydowi,* and *Penicillumcitrinum* were determined. The higher concentration of *Bacillus subtilis*has maximum antifungal properties when compared with low concentration against fungi respectively. The effect of *Bacillus megaterium*culture filtrate of different concentrations of 25,50,75,and 100µl treated against *Aspergillusflavus, A.niger,A.terrus, A.sydowi, and Penicillumcitrinum*were performed respectively. The higher concentration of 100µl has extraordinary performance than that of the lower concentration respectively,(Table -1)

The effect of antifungal properties of endophytic bacteria S*tenotrophomonas*spwith different concentration of 25,50,75,and 100µl were treated against fungi. The higher concentrations of 100µl was moderate activity observed whereas *Pseudomonas* spalso the same trend of results were analysed against clinical fungi (Table-2 and 3)

The efficiency of antifungal properties of endophytic bacteria *Serratia*spagainst clinical fungi were determined with regarding minimum zone of inhibition even in the low concentration of endophytic bacteria were good performance

However, the endophyticbacteria had more potent for antagonistic properties against clinical fungi respectively.

Table 1: Effect of antifungal activity of endophytic bacteria against clinical fungi

Name of the fungi	Zone of inhibition (mm)							
	Bacillus subtilis				***Bacillus megaterium***			
	25µl	**50µl**	**75µl**	**100µl**	**25µl**	**50µl**	**75µl**	**100µl**
Aspergillusflavus	4.66±1.55	5.00±1.66	6.01±2.00	7.00±3.33	3.04±1.04	4.02±2.10	5.16±3.12	6.50±3.15
A.niger	16.0±5.33	19.6±6.53	20.6±6.86	21.3±7.76	10.6±1.06	11.8±2.08	12.5±2.10	14.6±3.11
A.terreus	11.0±3.66	14.6±4.86	18.3±6.10	17.3±5.76	9.05±1.07	11.5±2.09	12.8±2.11	13.0±3.13
A.sydowi	10.1±1.33	8.00±2.66	11.6±3.86	17.0±5.66	6.03±1.06	7.02±2.08	7.04±3.10	9.04±3.16
Penicillumcitrinum	11.6±3.86	13.3±4.43	16.0±5.33	17.0±5.66	7.05±1.03	8.06±2.05	10.4±2.11	11.5±3.08

Values are expressed by mean ± S.D

Table 2: Effect of antifungal activity of endophytic bacteria against clinical fungi

Name of the fungi	Zone of inhibition (mm)							
	***Stenotrophomonas*sp**				***Pseudomonas* sp**			
	25µl	**50µl**	**75µl**	**100µl**	**25µl**	**50µl**	**75µl**	**100µl**
Aspergillusflavus	3.01±1.02	3.74±1.92	4.01±2.15	4.91±2.69	3.12±0.64	4.01±1.02	4.56±1.15	5.12±2.01
A.niger	3.15±1.01	3.91±1.59	4.19±2.00	4.93±2.53	4.01±1.03	4.47±1.17	5.11±1.29	5.54±1.50
A.terreus	3.00±1.00	3.94±1.41	4.11±2.07	4.97±2.91	3.06±0.99	3.16±1.17	3.99±1.56	4.19±2.01
A.sydowi	2.91±0.99	3.17±1.19	3.91±1.96	4.15±2.19	3.12±1.02	3.78±1.96	4.12±2.03	4.73±2.19
Penicillumcitrinum	3.12±1.03	3.79±1.93	4.02±2.01	4.29±2.91	3.16±1.00	3.79±1.19	4.19±1.89	4.97±2.13

Values are expressed by mean ± S.D

Table 3: Effect of antifungal activity of endophytic bacteria against clinical fungi

Name of the fungi	Zone of inhibition (mm)			
	***Serratia*sp**			
	25µl	**50µl**	**75µl**	**100µl**
Aspergillusflavus	1.66±0.55	2.00±0.96	2.71±0.99	3.00±1.19
A.niger	1.00±0.33	1.76±0.53	1.91±0.86	2.03±1.00
A.terreus	1.03±0.66	1.96±0.86	2.12±0.90	2.37±1.01
A.sydowi	1.07±0.33	1.25±0.61	1.91±0.86	2.00±1.00
Penicillumcitrinum	1.06±0.86	1.39±0.91	1.50±1.00	2.40±1.07

Standard deviation ± Standard error

ACKNOWLEDGMENT

The authors are grateful to the Principal, PG and ResearchDept of Biotechnology, SrimadAndavan Arts and Science College, Trichy.and Indian Biotrack Research Institute, Thanjavur for providing laboratory facilities during the period of investigation.

REFERENCE

Amedei, A., D'Elios, M.M., (2012). New therapeutic approaches by using microorganism-derived compounds.*Curr. Med. Chem*. 19, 3822-3840

Andrews, J.H. (1992) Biological control in the phyllosphere. Annual Review of Phytopathology 30, 603–635.

Berdy, J., (2012). Thoughts and facts about antibiotics: where we are now and where we are heading. J. Antibiot. 65, 385-395.

Blunt, J.W., Buckingham, J., Munro, M., (2013).Taxonomy and marine natural products research. In: Fattorusso, E., Gerwick, W.H., Taglialatela-Scafati, O. (Eds.), Handbook of Marine Natural Products. , 1 ed. *Springer, New York,* 3–54.

Butler, M.S., Blaskovich, M.A., Cooper, M.A., 2013. Antibiotics in the clinical pipeline in 2013.J. Antibiot. 66, 571-591.

Cragg, G.M., Newman, D.J., (2013). Natural products: a continuing source of novel drug leads. Biochim.*Biophys.Acta Gen. Subj*. 1830, 3670-3695.

Demain, A.L., (2014). Importance of microbial natural products and the need to revitalize their discovery.*J. Ind. Microbiol. Biotechnol*. 41, 185-201.

Dowler, W. and Waver, D. (1974) Isolation and characterization of *Pseudomonas* 8 from apparently healthy peach trees.*Phytopathology* 65, 233–236.

Gerwick, W.H., and Moore, B.S., (2012). Lessons from the past and charting the future of marine natural products drug discovery and chemical biology. Chem. *Biol*. 19, 85-98.

Huda and Ahmed, (2010) Antimicrobial activity of certain bacteria and fungi isolated from soil mixed with human saliva against pathogenic microbes causing dermatological diseases. 17(4), 331-339.

María J. Ek-Ramos, Ricardo Gomez-Flores, Alonso A. Orozco-Flores, Cristina Rodríguez-Padilla, Guadalupe González-Ochoa and Patricia Tamez-Guerra, (2019). Bioactive Products From Plant-Endophytic Gram-Positive Bacteria. *Front. Plant Sci.,*

Mayer, A.M., Rodriguez, A.D., Berlinck, R.G., Fusetani, N., (2011). Marine pharmacology in 2007-8: marine compounds with antibacterial, anticoagulant, antifungal, anti-inflammatory, antimalarial, antiprotozoal, antituberculosis, and antiviral activities; affecting the immune and nervous system, and other miscellaneous mechanisms of action. *Comp. Biochem. Physiol. C Toxicol.Pharmacol*. 153, 191-222.

Mayer, A.M.S., Glaser, K.B., Cuevas, C., Jacobs, R.S., Kem, W., Little, R.D., McIntosh, J.M., Newman, D.J., Potts, B.C., Shuster, D.E., (2010). The odyssey of marine pharmaceuticals: a current pipeline perspective. *Trends Pharmacol. Sci*. 31, 255-265.

Molinski, T.F., Dalisay, D.S., Lievens, S.L., Saludes, J.P., (2009). Drug development from marine natural products.*Nat. Rev. Drug Discov*. 8, 69-85.

Newman, D.J., and Cragg, G.M., (2014). Marine-sourced anti-cancer and cancer pain control agents in clinical and late preclinical development. *Mar. Drugs;* 12, 255-278.

PurbowatiningrumRia,QisthyHanifati,AnggitSaputra,NiesSuciMulyani, AgustinaLulustyaningati, Ngadiwiyana, Ismiyarto, DewiKusrini, and Nor Basid, (2020). Isolation, Characterization, and Identification of Endophytic Bacteria by 16S rRNA Partial Sequencing Technique From Leaves of carica papaya and Its Potential as an Antioxidant. *AIP Conference Proceedings*2237, 020053

Schulz, B. and Boyle, C. (2006) What are endophytes Microbial Root Endophytes. *Springer Berlin Heidelberg, Berlin, Heidelberg*, 1-13.

Su, Y., Cheng, M.J., Der Wu, M. *et al.* (2020). New Metabolite from the Endophytic Fungus *Corynesporasmithii*. *Chem Nat Compd* .

Vederas, J.C., and Li, J.W.H., (2009). Drug discovery and natural products: end of an era or an endless frontier?. Science 325, 161-165.

Walsh, C.T., Fischbach, M.A., (2010). Natural products version 2.0: connecting genes to molecules. *J. Am. Chem. Soc*. 132, 2469-2493.