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Research Paper

ALLELOPATHIC ACTIVITIES OF SPECIFIC MICROBIAL METABOLITES IN THE INLAND PRAWN FISHERIES OFF EASTERN UTTAR PRADESH, INDIA

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ABSTRACT **The gram positive bacterial strain of Streptococcus spp. recovered from Macrobrachium rosenbergii is a pathogenic strain. The in vitro allelopathic activities of all 22 bacterial strains of same habitat were tested against Streptococcus spp. Out of aforementioned strains only one showed antagonistic activity and identified as gram positive rod, Clostridium spp. The in vitro allelopathic interactions of these two bacterial strains were demonstrated to be a potential antagonist in the inland water fisheries. This study was carried out for the determination of natural allelopathic and antibacterial activity of antagonistic bacterial strain against the pathogenic bacterial strains of fresh water aquaculture system of eastern Uttar Pradesh, India.**

KEYWORDS :Macrobrachium rosenbergii, Allelopathic activity, Antibacterial

INTRODUCTION

The enhanced demand for aquatic food products has imposed development and strengthening of aquaculture activity in most regions of the world. This intensification has led to an increased use and misuse of drugs and chemicals in aquaculture systems that lead to emergence of food safety concern. Microbial prod- ucts are seen as, among other things, alternatives to the prophy- lactic use of chemicals (Moriarty, Decamp, and Lavens, 2005). However the poor qualities of probiotics in the market and the misapprehensions regarding their function have raised doubts on their efficacy. The enhanced use of antibiotics and other chemical compounds may illicit and stimulate as leads to the resistance among pathogenic bacterial strains intern posing a great challenge to human beings (Jebasingh and Murugan,2011). The inhibitory effect of biochemical compounds against the other flora and fauna is called allelopathy. The word allelopathy derived from two Greek words allel and pathos and uttering the meaning as “let the one suffer that looks like you”. In other terms we can say that allelopathy is the direct or indirect interactive impact of the biochemical metabolites of microbial strains against the others that escape into the environment. In aquatic systems the definition of allelopathy so broad and comprises all chemical interactions among unicellular organisms (Cembella, 2003; Gross, 2003;Legrand, Rengefors, Fistarol, and Granéli, 2003; Granéli, 2006; Ianora, Boersma, Casotti, Fontana, Harder, Hoffmann, Pavia, Potin, Poulet, and Toth, 2006; Tillmann, John, and Cembella, 2007). At present forage is no longer taken in consideration by the heterotrophs only for taste and instantaneous nutritional requirements for survival, but also in terms of their utility of give specific health benefits beyond their fundamental nutritive quality. Now a days gross part of the active running food markets are influenced by the healthy food products aiming towards up healing the balance between micro flora of gut and surrounding mediums. The antagonistic possessions of bacterial strains can be employed in various sys- tems like aquaculture, agriculture, and epidemiology, biological control of pests and in eradication of disease causing or pathogenic organisms (flora and fauna). The pattern and specificity of the allelopathy with the corresponding pathogenic susceptible strains is based on the lowering of pH values, inhibiting properties of their metabolites, including lactic acid, hydrogen peroxides and bacteriocidin and subsidizing to the inhibition of pathogenic bacterial strains has been worked out by the earlier workers (Servin, 2004; Ligockaa, Paluszak and Hermann, 2005; Vuyst and Leroy, 2007; Szala, Paluszak, and Motyl, 2012). Decent and innovative approaches have been used since yesterdecades as alternative to antibiotics in control and treatment of several gastrointestinal, infectious, microbial and parasitic diseases. Aforementioned applications of approaches are using live bio-therapeutic agents such as bacterial isolates and metabolites to be secreted by them present in the cell free supernatant. The antiseptic bacteriocins are produced by several *Lacto bacillus* strains in their secretory metabolites have been used against the gram positive food borne pathogenic strain of *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus subtilis* and spores of *Clostridium perfrigens*. Therefore, now a days microbial antago- nists with alleopathic bustle may concerned much attention for the use as native and natural biopreservatives in food and fisheries industries (Saranya and Hemashenpagam, 2011; Kaynar and Beyatli, 2012). However, studies relating to the antibacterial properties of these organisms have been limited and not fully exploited for use. In this study we report the benefits of carefully selected strain of gram positive bacilli, *Clostridium* spp. on the growth performance of giant fresh water prawn, *Macrobrachium rosenbergii* as biological control agent in aquaculture by their al- lelopathic activity against the pathogenic bacterial strain, *Streptococcus* spp. Such a strategy could well form the basis of a rationale for the search of potential antagonistic and allelopathic antibacterial agents that could help to bring down disease incidence in the scampi culture pond and inland water fisheries.

Materials and Methods

The bacterial samples were collected from the water and soil of the pond in which *Macrobrachium rosenbergii* was being cultured in Allahabad, U.P. Simultaneously bacterial sample were collected to body surface of giant freshwater prawn, *M. rosenbergii* by swabbing. Bacterial samples were cultured in nutrient- broth for 24hrs and 0.1ml of culture aseptically inoculated in nutrient-agar. The pure culture of each isolates was created, seri- ally diluted and colonies obtained were counted after Upadhyay, Malhotra and Malhotra (2009).Antagonism of bacterial strain 158 against target microbial cul- ture 144 was detected by disc diffusion assay (Chang, Liu and Shyu, 2000). The sterilized discs of whatmann paper were placed aseptically on cultured pathogenic susceptible bacteria, previ- ously soaked with cell free broth culture of test organism (antagonist) followed by incubation of culture at 360C and periodically examined for the zone of clearance around the disc in cultured medium as a positive indication of inhibitory activity. The growth pattern of target strains was calculated after every 3hrs for 58hrs by spectrophotometer (ELICO) at 600nm**.** The growth curve was plotted along the diameters of the zone of clearance.

Results

The samples of bacterial pure cultures isolated from scampi, pond water and soil were subjected to different biochemical tests for their numeric evaluation and preliminary identification. An antagonism assay by disc diffusion showed that cell free supernatant of sample 158 inhibited growth of sample 144 with zone of clearance ranged

between 0.6 to 2.1cm in diam- eter (Fig.1). No growth of antagonistic bacteria was commenced between 0.0 to 4.0hrs (Fig. 2). The exponential growth was take place from 6 to 42hrs as assessed by the rapid change in opti- cal density (OD). However, very little change was noticed in OD516 IJSR - INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH between 42 to 58hrs. This was the stationary phase. In this phase maximum antagonistic activity was observed at 38hrs (Fig.2). Maximum zone of clearance (2.1cm) was obtained at 38hrs and minimum obtained at 0.0hrs.

Strain 158: The colonies were smooth, circular, with regular edges, convex, shiny and mucoid. The rate of growth was very good. After 24hrs, culture did not settle down in broth but caused turbidity. These were gram positive rods with endospores, motile and isolated from fresh water scampi pond's water of selected sites. These were identified as *Clostridium* spp.

Strain 144: The colonies were evoked blue in color, rough, dry and formed spores. The growth on nutrient agar was extremely slow and optimum growth commencing after 72hrs of incuba- tion. Very little or no growth was recorded in nutrient broth. Gram staining revealed these to be the chains of cocci *i.e.* positive streptococci. The strains were recovered from body surface of *M. rosenbergii* and identified as *Streptococcus viridians.*

Discussion

Since the first antibiotic from bacterium was described in 1966, the number of new compounds has increased constantly during the years. Even though only a few compounds from organisms might be interesting for the pharmaceutical market today. Some bacterial species are already used as biocontrol, and are added to aquaculture stocks. Most metabolites from bacteria found so far were isolated from marine bacterial species. In recent studies wide array of bacteria were tested for bacterial antagonis- tic effects, and it was demonstrated that this trait appeared to be a widespread feature in marine habitats, and was present in many bacterial groups. The production of secondary metabolites by bacteria, namely, *Roseobacter* has been reported previously, and some organism of this group is thought to be either probi- otic or antibiotic in different aquaculture conditions. The bacterial strain 158 inhibited the growth of other strain 144 when the initial count of the antagonist i.e.158 was far greater than that of the prawn pathogen, 144. This indicated that the antagonist must be available at significantly higher levels than the pathogen, and the degree of inhibition increased with enhanced level of antagonist. Therefore, as a potential probiotic, strain 158 has to be supplied in sufficient quantity and on a regular basis into the system. One of the most viable criteria for considering the bacterium as a candidate to be used in biocontrol program in prawn culture system is its non-pathogenicity (Verschuere et al., 2000). Culture 158 has not been recognized and reported as prawn pathogen previously. The present work strengthens the candidature of strain 158 as an "antagonistic/alleloparhic probiotic" in prawn rearing systems.

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CONCLUSION

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