International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2015): 6.391

Activity Patterns of Cell Free Supernatant of Antagonistic Microbial Strains in Rodents Host-Parasite Systems

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Abstract: ***Helminthes parasites are the major consequence in homeotherms and play very significant economic values for health, bioecology and zoonoses. The microbial interactions in the terrestrial ecosystem were demonstrated to be of antagonistic potential in relation to parasitic nematodes in rodent hosts, Rattus rattus. The endoparasitic roundworms of genus Pseudaspidodera harbored gram positive streptobacilli bacteria, Actinomyces on its body surface. The bacteria residing on the body surface of rodent's gut nematodes, provides a very good medium for better survival of roundworms by their microhabitat specific activities. The antagonistic activity of the positive coccii, Staphylococcus bacteria proved in vitro in the present investigation. The antagonistic bacterial strains were recovered from soil of giant fresh water prawn, Macrobrachium rosenbergii fisheries ponds in eastern regions of Uttar Pradesh, India. The growth and patterns of inhibitions by microbial metabolites exhibited interesting variations which explained characteristic interactions of antagonistic vs susceptible bacteria. Thus allelopathic correlations and interaction of antagonistic microbial metabolites reduced the population of Actinomyces bacterial strains, in turn caused unfavorable environment for roundworms, Pseudaspidodera cordinae in rodents gut. Therefore, infestation and establishment of helminthes in rodents became critical and hosts experienced healthier. So that the recovered bacterial strains can be apply as probiotic agents in terrestrial host-parasite systems.***

Keywords: Antagonism, *Staphylococcus, Actinomyces,* Probiotics, *Rattus rattus, Macrobrachium rosenbergii,* Host-parasite systems.

1. Introduction

Designation of the utterance "microbiology" and "microorganism" is not a trouble-free assignment. The branch of science deals very small organisms are so small that can't be visible unambiguously with the nude eye is known as microbiology but many earlier workers of microbiology are concerned with the functions or genetic compositions or molecular heterogeneity of microbial communities rather than observing external morphology and colony characteristics of particular microbial species with a microscope [1], [2]. Some microbiologists argue about microbiology with several diagnostic characteristics of microorganisms are very small size, unicellular organization and osmotrophic mode of feeding [3], [4]. Thus bacteria are very simple living beings, without a membrane-bound nucleus and sub-cellular components. Therefore, bacteria are described as prokaryotes and word derived from two Greek words pro and karyon, meaning primary nucleus. Notwithstanding their ease, bacteria have an enormous range of metabolic capacities and can be inhabited everywhere and cosmopolitan in most extreme environments on earth [5]. Bacterial cells can adopt three basic shapes and are of prime consequence in the classification and identification of bacteria. They are either round (coccus or a berry; pleural: cocci), rod-shaped (bacillus or a stick; pleural: bacilli) or spiral. Very small group microorganisms are the primary causative agents for several diseases are known as primary pathogens. One of the brain storming truth is the "*without microbes, life on Earth would not exist*" because they are responsible for cycling and biosynthesis of nutrients and make it obtainable for supplementary life forms [6], [7]. The recent outbreaks of food-borne disease caused by *Escherichia coli* and *Salmonella* have been well documented. The studies about bacteria associated with the parasitic helminthes are scarce and their identification, assessment, potential activities taken in consideration during the present study and comparisons were made with previous publications to determine that the microbes are antagonistic or pathogenic or not [8], [9], [10]. There is augmenting apprehension about the use and ill-treatment of antimicrobial drugs not only in human medicine and agriculture but also in aquaculture. The immense application of antimicrobials agents for disease control and growth promotion in animals, agriculture and fisheries aquaculture increases the discriminating anxiety exerted on the microbial world and encourages the natural emergence of bacterial resistance [11], [12], [13]. As a decent pronouncement comes in subsistence, recovered and experimentally proved by workers of microbiology, several definitions of probiotics have been proposed and defined probiotics as "a live microbial nourish supplement which optimistically affects the host animal by improving its intestinal equilibrium" [14]. The anticipated concepts of probiotics were applied in terrestrial and aquaculture system for an invariable interaction with the environments and hosts function by earlier workers [15], [16]. It has been confirmed that the aquaculture system is more susceptible to oscillations in the environmental conditions than the terrestrial animals or humans for health status. It has also been worked out that the dealings among microbiota, including probiots, allelopaths or antagonists and the host as well as parasites (helminthes) are not restricted to the intestinal territory means could also be active on the gills or the skin of host or parasites in its ambient environment [17]. The present investigation based on the most modern and decent definition of probiotics as "a live microbial adjunct which has a beneficial effect on the host by modifying the host-associated or ambient microbial community, by ensuring improved use of the feed or enhancing its nutritional value, by enhancing the host response towards disease, or by improving the quality of its ambient environment". The intensive interaction between

the culture environment and terrestrial host-parasite system taken in consideration for the betterment of rodents or mammalian health thereby, promote existence of healthy and disease free environment for mankind.

2. Methodology

Isolation and characterization of bacterial strains: One group of bacterial strain, *Staphylococcus* (coded as 226) were gram positive coccii served as targets for inhibiting the growth of other gram positive streptobacilli bacterial strain, *Actinomyces* (coded as 229) isolated [18] from the soil of giant fresh water prawn, *Macrobrachium rosenbergii* fisheries ponds [19] and body surface of nematode, *Pseudaspidodera cordinae* [20] residing in rodent's, *Rattus rattus* gut respectively at Allahabad, Uttar Pradesh, India. The round worms were collected, processed and identified [21]. Catalase, oxidas, haemolysis, pathogenesity and culture specific activities were critically worked out [22] for the characterization of pathogenic [23] and antagonistic bacterial strains [24].

Colony counting: Samples were aseptically inoculated on nutrient agar (Himedia) to recovered pure culture [13]. From a single colony broth culture were created and 0.1ml of each isolate was serially diluted (10^{-1}-10^{-10}) after 24hrs of incubation at 32-35°C. The colonies obtained were counted by the below mentioned formula [25]:

Colony Forming Units (CFU) = [Number of colonies x dilution factor] per ml of culture

Antagonism of cell free culture supernatant: Antagonism of bacterial strain 226 against the target microbial culture 229 was detected on the basis of efficacy and inhibiting activities of cell free supernatant by means of zone of clearance around the colony through disc diffusion assay [26]. Inhibition around the disc (soaked with metabolites) was detected after 24hrs using a Hi-antibiotic zone scale (Himedia). Gram positive antagonistic bacilli strains was grown in nutrient broth (Himedia) for 36hrs and cells were pelleted by centrifugation at 10000 rpm for 10 minutes at 4°C for the collection of cell free supernatant containing microbial metabolites responsible for the antagonistic as well as allelopathic activities against the target susceptible strains [27], [28]. Simultaneously the growth of bacterial strains (Antagonists) were analyzed and estimated at regular interval of 2hrs up to 58hrs by the application of 3ml of culture media using a spectrophotometer ELICO and the absorbance was recorded at 600nm [29]. The reading obtained was plotted along with the diameters of the zone of clearance obtained [30].

3. Results and Discussion

Strain 226: The colonies of strain were smooth, circular, entire, convex, dry and whitish in color with rotten smell on nutrient agar culture medium. The rate of growth was very good on nutrient agar and nutrient broth both with CFU $4.38x10^5$/ml. The biochemical test revealed that the strains were negative in oxidase and positive in catalase activities and gram staining. Thus by these diagnostic feature, the colonies were characterized as *Staphylococcus* recovered from soil of prawn fisheries ponds.

Strain 229: The colonies of this strain were mucoid for 24hrs during incubation and later on it spread throughout the culture medium as whitish mat within 72hrs due to swarmed growth of the strain with light smell form spores. The growth on nutrient agar and broth was very good at 36-38°C and optimum growth was recorded at 48hrs of incubation. Biochemically it was a catalase and haemolysis positive (pathogenic) and gram positive strain with CFU $3.98x10^5$/ml. These are recovered from body surface of round worm *P. cordinae* [20] and identified as *Actinomyces*.

Synergistic microbial relationship with *P. cordinae*: Severe infection of aspidoderid nematodes in the intestine of wild rat, *R. rattus* were recorded during the period of investigations (infection prevalence 67% and mean intensity 11.5) from different urban locality of Allahabad, Uttar Pradesh, India. The inflammatory and necrotic diseases in the intestine of wild rats were promoted by the above described pathogenic gram positive streptobacilli i.e. strain 229 (*Actinomyces*) which positively influenced the population density and abundance of round worms in gut environment, thereby pathogenic to mammalian hosts indirectly. The wide-reaching augment in bacterial confrontation to antibiotics has motivated investigations into the applications of microbes or bota as probiotics [31]. The *in vitro* inhibition of pathogenic bacterial strains by the antagonistic strain 226 were tested and applied *in vivo* found to be significant for negative growth of roundworms in the mammalian rodent model organism during the schoolwork. Therefore, the recovered strains 226 (*Staphylococcus*) from soil of fisheries ponds proposed to be a probiotic agents in rodents host-parasite system in the present investigation. The relevance of antagonistic bacterial strains against the pathogenic microbes by the allelopathy or probiotics in aquaculture reflects significant positive interactions; therefore, it needs substantial hard work of investigation. The impact and correlations of allelopaths and probiots on gastrointestinal microflora and fauna leftovers inadequately described so far [32], [33], [34]. The current investigations showed that the probiotics not only related to intrinsic environment, therefore, these strains that have such activities can reside in the hatchery tanks or large aquaculture ponds by displacement of deleterious normal bacteria like the definition probiotics given by earlier workers as: "a probiotic is a mono- or mixed culture of live microorganisms that, applied to animal, affect beneficially the host by improving the properties of the indigenous microflora" [35], [36]. The dweller microbes assistance from a fairly constant niche/habitat in the gastrointestinal tract terrestrial livestock in host parasite system [37] and in aquatic animals [38] may well thought-out as methods of biological control, that termed the restraint or the abolition of pathogens by the introduction of other organisms, like microbes [39]. The inhibition of growth or killing of pathogenic strains involve a diffusible, bacterial toxin and was not associated with increased numbers of live bacteria within the intestine of the rodents hosts unlikely to be due to viable bacteria and bacterial invasion to tissues [40]. The peak zone of inhibition to 229 was recorded at 32-36hrs and quantitative evaluation of antagonist 226 against the 229 was

found to be sufficient to eradicate the microbes from gut environment were estimated by disc diffusion assay and analyzed through advanced numerical tools (Fig. 1). It is therefore, a pertinent question whether it is possible to modify the composition of a microbial community in the field by the exogenous addition of a probiotic? This is particularly important; a long-term exposure is required for the probiotic effect. It is not easy task to reply on this aspect without going in detail because no more literature available to provide real evidence for this in aquacultural practices [41], [42] and host-parasite system [43], [44], [45], [46], [47]. The findings corroborated the inoculation of high doses of lactic acid bacteria to establish microbial communities in fish juveniles provoked a temporary change in the composition of the intestinal microbial community within a few days after the intake and strains showed a sharp decrease and were lost from the gastrointestinal tract in most of the fish [48], [40], [50].

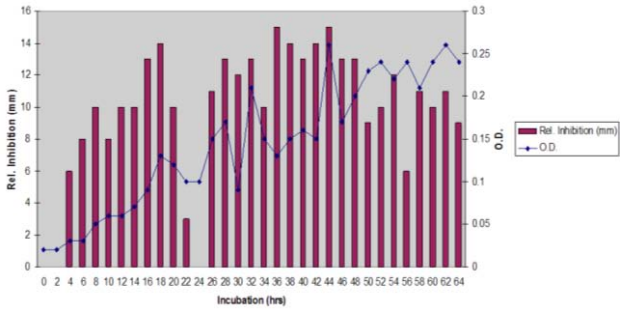

Figure 1: Growth curve vs antagonism of strain 226 (*Staphylococcus*) from soil of fresh water prawn fisheries ponds against the strain 229 (*Actinomyces*) from body surface of aspidoderid roundworm *Pseudaspidodera cordinae* recovered from gut of *Rattus rattus*.

Bacterimia

Production, qualitative and quantitative antagonistic activities were validated *in vivo* by the establishment of long-term experiment with model organism during the period of investigation. The inflammatory and necrotic CFU value of pathogenic strain 229 called as bacterimia (analogous to parasitimia) was $2.4x10^7$ estimated during the experiment and found to significant numerically. Bacteriocin or metabolite pathogenic activities of target microbes were worked out by the *in vitro* application of colony overlay assay [51], [52]). Simultaneously one of the bacterial strain, 226 isolated from the other habitat i.e. soil of fresh water prawn fisheries ponds, found to be positive in antagonistic action against the pathogenic strain 229 *in vitro*. Significant eradication or loss of pathogenic strain from intrinsic habitat was estimated in other experiment by the stool analysis of the host at a seven days regular interval. Thereby, as a potential probiotic, 226 have to be supplied in sufficient quantity and on a regular basis into the system for proper and healthy immune as well as terrestrial/rodents host-parasite system. One of the chief criteria for taking into consideration the bacterium as a contention to be used in biocontrol program in host-parasite system was its non-pathogenicity supported by the findings of earlier workers [53], [54], [55], [56], [57]. Before a culture can be used as a probiotic, it is necessary to confirm that no pathogenic effects can occur in the host. Therefore, the target species should be challenged with the candidate probiotic, under normal or stress conditions. This can be done by injection challenges, by bathing the host in a suspension of the candidate probiotic, or by adding the probiotic to the culture [58], [59]. The effect of present probiotics have been tested *in vivo* as well and candidate probiotics or antagonistic metabolites added to the rodents host-parasite system and their effect on growth and/or survival parameters of pathogenic roundworms assessed [60], [61], [62], [63]. Thus the newly recovered microbial strain 226 (*Staphylococcus*) from soil of fisheries ponds in the present investigation proposed to be a probiotic agents in rodents host-parasite system because of its non-pathogenesity in the terrestrial host system as well as supply of sufficient quantity of potential probiotics or microbial metabolites as antagonist on a regular basis into the system for betterment of rodents or mammalian health thereby, promote existence of healthy and disease free environment for mankind.

4. Acknowledgements

Author is thankful to Prof. S. K. Malhotra, Head, Department of Zoology, University of Allahabad, Allahabad, Uttar Pradesh, India for laboratory facility during the period of investigation. SKU is grateful to Prof. Asha khanna, Head, Department of Zoology, St. Aloysisus Autonomous College, Jabalpur, Madhya Pradesh, India for her help in identification of bacterial strains.

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