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EXOSYMBIONTS OF POLYCHEATS FROM KERALA COAST – POTENTIAL ANTAGONISTIC RESOURCE ?

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Abstract: Biological diversity of every ecosystem currently receives attention for conservation and sustainable utilization due to the increasing threats on the ecosystems from anthropogenic interventions. Proper sampling and identification of organisms are necessary to monitor biodiversity at any level. This paper documents Polychaete-associated Streptomycetes and anaerobic photosynthetic Purple Sulphur Bacteria (PSB) in the estuarine ecosystems of Kerala coast, India. The microbial population from the gut of *Namalycastis abiuma* species complex, *Namalycastis jaya* and their inhabiting soil samples were studied. The occurrences of these exo-symbionts were positively correlated with seasons. Among the polychaetes, *Namalycastis jaya* harbored the maximum PSB ($56 \pm 4.7 \times 0^4$ ml^{-1}) and *Streptomyces* density (18 ± 1.67) was recorded in the months of April and May. Even though soil showed considerably higher microbial density, only the extracts of the polychaete-associated microbes showed more potential than soil counterparts against aquatic fungal fish pathogen, *Saprolegnia parasitica.* Compared to PSB, *Sreptomyces* spp exhibited higher activity. Most potential strains were identified by using standard morphological and physiological techniques. Mass production of these microbial compounds and its usage as other pathogens remains to be studied.

Key words: *Namalycastis jaya*, Streptomycetes, Photosynthetic bacteria, Gut association, Antifungal.

INTRODUCTION

The interaction of microorganisms with aquatic biota is unique and diverse. Microbes present in the gut of polychaetes indirectly indicate their feeding habit, food preference and the type of nutrients required for their growth. This in turn, will enable one to infer their importance in nutrition. The association of microbes with tissues of the gastrointestinal tract of animals during evolution has resulted in a balanced relationship between resident microbes and the host. Numerous biochemical, physiological and immunological features, are considered to be the intrinsic characteristics of animal species, are actually the responses by the animal to the physical presence and metabolic activities of the normal indigenous micro biota. This microbial challenge has modified the course of evolution in animals resulting in the selection of animal microbe relationships. The gastrointestinal microbial community is considered by its high population density, wide diversity and complexity of interactions. Gut associated bacteria are widespread within nearly all invertebrate animals.

Phototrophic sulphur bacteria (PSB) are a specialized group of microorganisms that carryout the oxidation of sulphide under anaerobic conditions (Pfennig, 1977; Pfennig and Truper, 1981; Sucharita *et al*., 2010). It is shown that phototrophic bacteria play an important role in the preservation of the mangrove environment (Kabayashi *et al.*, 1978). The Genus *Streptomyces* was proposed by Waksman and Henric (1943) for aerobic and spore forming Actinomycetes. Streptomycetes are the largest antibiotic producing groups in the microbial world discovered so far. A general revision of the Genus *Streptomyces* was made with the ISP (International *Streptomyces* Project) Shirling and Gottlieb, 1966) as a result of which in the approved list *Streptomyces* spp. remains just 459 of the total initially described 1000. Studied on the occurrence and distribution of antagonistic Streptomycetes have been carried out in different region in around the world (Waker and Colwell, 1975; Okazaki and Okami, 1976; Dharmaraj and Alagarsamy, 2009; Magesh and Dhevendaran, 2012). The marine Polychaeta, *Namalycastis jaya*, Magesh

2012 and *Namalycastis abiuma* species complex (species group) were also collected from same region already reported from the southern coast of Kerala, India. The association between polychaetes *N. jaya* of with microorganisms are carryout in this first time and the results showed voluble sources of antibiotics against life threatening diseases.

MATERIALS AND METHODS

Location and Sample collection

The study was carried out from January 2008 to December 2008 (12 months) in retting zone located at Murukumpuzha of Trivandrum coast. A number of polycheates were collected and transported to laboratory in sterile and live condition with in sterilized polythene bag and bottles.

Morphological Observation of Polychaetes

The polychaetes associated with retting coconut husk were collected by breaking the coconut husk with hammer and chisel. Collection and identifications were made by previous contributions (Day, 1967 (1 & 2); Glasby 1999; Magesh *et al.*, 2012).

Enumeration of gut content from selected worm

Body surface of the live polychaetes were washed with sterile water and swabbed with 60-70% ethanol for surface sterilization. Then the polychaetes were homogenized with sterile distilled water. Worms were dissected and the gut behind the gizzard was equally divided into the three parts. The gut contents of the middle region were released by squeezing the intact worms. Gut contents of the same species were pooled to obtain samples (1 gm) taken for the isolation of Streptomycetes and anaerobic PSB.

Enumeration of Photosynthetic bacteria and Streptomycetes from polychaete

1 ml of 10^{-4} serially diluted polychaete samples were transferred into sterile petridishes containing selective medium, Glycerol asparagines agar (Pridham and Lyons 1961) and incubated at 28°C for 7-10 days. Remaining part of homogenized materials were transferred into sterile screw capped test tubes containing selective, PSB enrichment media (Pfenning Lippert, 1966; Arunasri *et al.*, 2005) and incubated with continues illumination of 200 watt bulb at room temperature for 10-15 days.

Identification of PSB

After 10 days of incubation, the positive tubes (green, red or purple) taken for further identification using the standard methods (Pfenning Lippert, 1966; Arunasri *et al.*, 2005).

Identification of Streptomycetes

After 7 days of incubation the total numbers of Streptomycetes colonies in each petridishes were counted using standard plate count technique. The identifications are made by standard procedures (Shirling and Gottieb, 1966; Locci, 1989).

RESULTS AND DISCUSSION

Polychaete, *Namalycastis* spp.

The positive association of Streptomycetes and PSB population in the gut of polychaete from retting soil of Murukkumpuzha, Kerala was noted. However, studies on the ecology of polychaete gut associated microbes especially, *Streptomyces* spp. and *Marichromatium* spp (PSB) are scarce. In the present investigation, selected polychaetes were identified as *Namalycastis abiuma* species complex and *Namalycastis jaya* by using standard procedures and taxonomic key (Magesh *et al.*, 2012). All polychaete samples shown positive association with *Streptomyces* spp. *and Marichromatium* spp. Among these isolates, selected 40 *Streptomyces* spp. and 10 PSB showed antifungal activity against aquatic fish pathogen, *Saprolegnia parasitica*. The antagonistic strains were identified in genus level.

Polychaete associated Streptomycetes

In this present study, the Colony Forming Unit of Actinomycetes population in polychaete, *Namalycastis abiuma* was ranged from 2 ± 0.12 to 17 ± 1.26. The minimum CFU of 2 ± 0.12 was recorded in the month of July and maximum Colony forming unit was recorded in the month of March. In the polychaete, *Namalycastis jaya* the minimum population density (2 ± 0.10) was recorded in July and the maximum population density (18 ± 1.67) was recorded in the month of April. In the sediments, the minimum density

(2 ± 0.15) was recorded in August and the maximum density (21 ± 2.10) was observed in March (Fig. 1).

The correlation was analysed between Actinomycetes population from *Namalycastis abiuma* species group and *Namalycastis jaya* with soil. The significant was at 5% level. The correlation between Actinomycetes population of *Namalycastis abiuma* species group and sediment samples (Fig. 2) were moderately positively correlated (r- 0.6208). The correlation between Actinomycetes population of *Namalycastis jaya* and soil samples revealed that they are significantly and positively correlated (Fig.3) with lower level (r - 0.3113). Generally, Actinomycetes populations varied broadly in their density in different body parts of invertebrates and most marine invertebrates harbour microorganisms within their tissues where they reside in the extra and intracellular spaces (Dhevendaran, 1984).

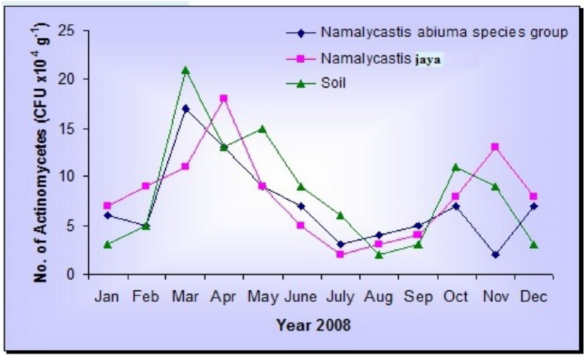

Fig. 1. Actinomycetes population in polychaetes, *N. abiuma* species complex, *N. jaya* & Soil during 2008

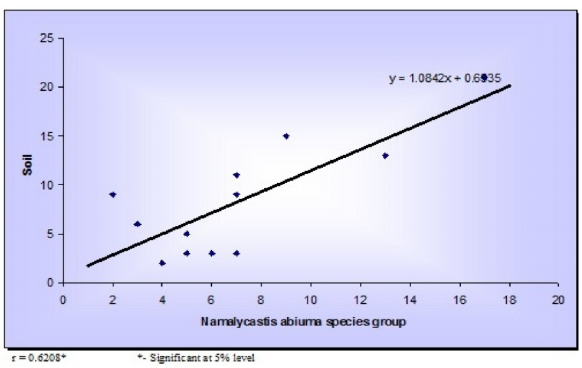

Fig. 2. Correlation between Actinomycetes population in *N.abiuma* species complex & Soil during 2008

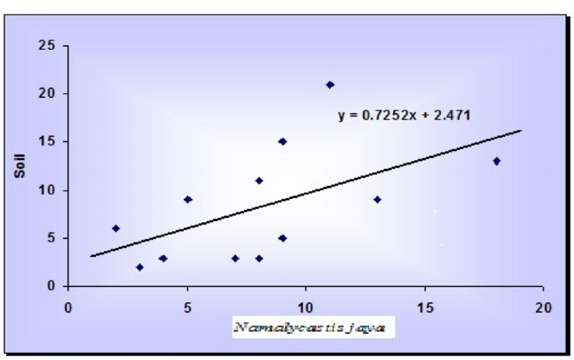

Fig. 3. Correlation between Actinomycetes population in *N. jaya* and Soil during 2008

Polychaete associated PSB

The density of PSB population isolated from the polychaete worms namely, *Namalycastis abiuma* species group and *Namalycastis jaya* was shown in the Fig. 4. In *Namalycastis jaya*, the maximum population ($56 \pm 4.7 \times 0^4$ ml^{-1}) and minimum population ($6 \pm 0.78 \times 10^4 ml^{-1}$) was recorded in the month of April and November respectively. During sample collection polychaete were not recorded in the month of July, August, October and December. In *Namalycastis abiuma* species complex, the minimum density ($6 \pm 0.52 \times 10^4$ ml^{-1}) was recorded in December and the maximum density ($49 \pm 4.67 \times 10^4$ ml^{-1}) was observed in March. In sediments, the maximum population ($61 \pm 5.20 \times 10^4$ g^{-1}) was recorded in the month of May and the minimum ($11 \pm 0.96 \times 10^4 g^{-1}$) in August. In June, no bacterial population was recorded in all samples. On the other hand, it has been reported that *Marichromatium purpuratum* was able to

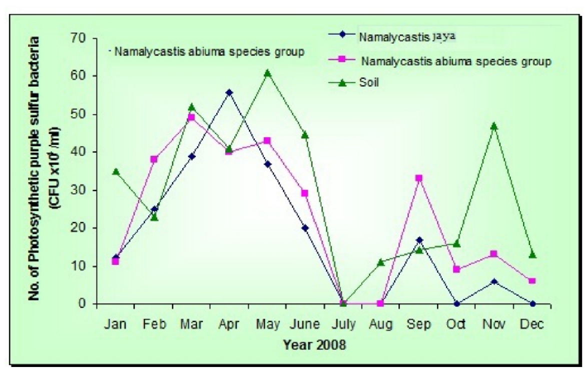

Fig. 4. PSB population in polychaetes, *N. abiuma* species comples and *N. jaya*. & in Soil during 2008

produce antimicrobial compounds with a broad spectrum activity against filamentous fungi, yeast as well as gram-negative and gram-positive bacteria (Burgess *et al*., 1991).

The correlation was analysed between PSB populations from *Namalycastis abiuma* species group and *Namalycastis jaya* with soil and it is significant at 5% level. Fig. 5 shows the correlation between the PSB populations isolated from *Namalycastis jaya* and soil samples and it showed moderately positively correlated (0.4549). Fig. 6 shown the correlation between PSB populations of *Namalycastis abiuma* species complex and soil samples and it also showed moderately positively correlated (r - 0.4581). Relatively, similar findings were also recorded in the sediments of Cochin (west coast of India) and Vellar estuary of East coast of India by Chandrika *et al*. (1990) and Dhevendaran, (1984) respectively.

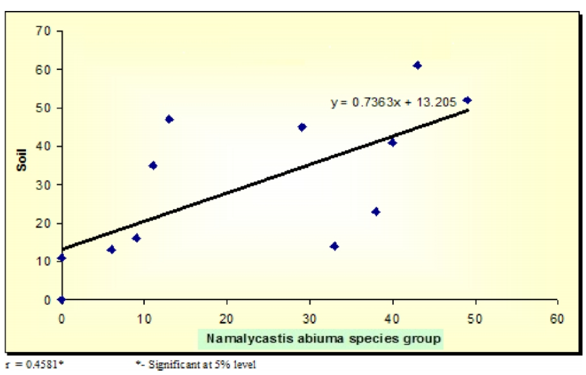

Fig. 5. Correlation between PSB population in *N.abiuma* species complex & Soil during 2008

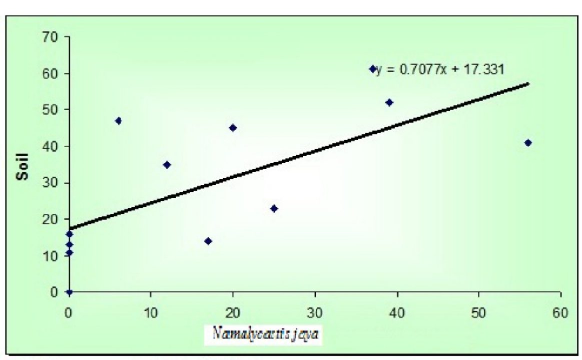

Fig. 6. Correlation between PSB population in *N. jaya* & Soil during 2008

Antagonistic activity

In the present study, primarily 177 Actinomycetes and 39 PSB strains (polychaete, gut associated) were randomly selected and tested for antifungal activity against the fungi, *Saprolegnia parasitica* (MTCC 718). Totally 50 strains (40 *Streptomyces* spp. and 10 PSB) showed antifungal activity. Similarly such occurrence (Fish associated Actinomycetes) of Actinomycetes could be beneficial to the fishes either for production of enzymes and vitamins which are useful for fishes (Sivakumar et al., 2007). There are possibilities for some metabolite from marine animals which might have originated from symbiotic bacteria within the host rather than the host animals. This has been discussed intensely for many years (Proksch et al., 2002; Piel, 2004). Gut contents harboured more population density when compared to other parts of the fishes and other invertebrates like polychaetes. The reason for this could be that the gut is exposed to variety of microbial populations along with diverse food particles and more numbers of microbes can be retained in the guts. Streptomycetes isolates showed Grey, green, cream and white colour of aerial mycelium and cream, pale yellow, yellow, pink and black on the substrate mycelium. All strains are Gram positive, non-motile and rectiflexible spore chain and only 7 isolates were exhibited positive results of melanin production. Based on the morphological obsevation, the selected PSB isolates were (All strains are Purple red coloured, rod shaped, gram negative and motile bacteria) identified as *Marichromatium* spp. The details of identification of microbes were described in materials and method of this paper.

The mode of association was not well known but both the host and microbes are benefitted because it has been believed that the *Streptomyces* and *Marichromatium* (PSB) were known to control pathogens (Burgess *et al*., 1991; Magesh and Dhevendaran, 2012; Selvakumar and Dhevendaran, 2011) and it was assumed that polychaete gut might serve as an excellent habitat for the microbes. Marine invertebrates have developed highly specific relationship with numerous microorganisms and these associations are of recognized ecological and biological importance (Strahl *et al*., 2002).

CONCLUSIONS

The symbiotic relationship of microbes with the aquatic organisms preferably the Polychaetes would be of much importance for the biotechnological applications especially Antibiotic production. *Streptomyces* spp. and PSB were identified by genus level. Exosymbionts, both *Streptomyces spp.* and *Mrichromaium* spp. of polycheats from Thiruvananthapuram, Kerala coast undoubtedly proved a promised source of potential antagonistic strains. Further research on mass production of these Antagonistic microbial compounds and its usage as other pathogens remains to be studied. However, these microbial sources are potent enough to utilize them on the commercial scale in the days to come.

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