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Research Note

Bacteria Associated with Solitary and Colonial Ascidians from Thoothukudi Coast, Tamil Nadu

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Solitary and colonial ascidians were collected from submerged structures along the coast of Thoothukudi and the bacteria associated with the surface and whole body homogenate were studied quantitatively and qualitatively. Solitary ascidians were identified as *Cnemidocarpa areolata, Herdmania momus, Microcosmus exasperates* and *Phallusia nigra* and colonial ascidians as *Didemnum psammathodes, Diplosoma swamiensis, Eudistoma viride* and *Lissoclinum fragile.* Epibiotic bacterial load varied from 3.0×10^2 to 5.2×10^5 cfu sqcm^{-1}, while the bacterial load from whole body homogenate of ascidians ranged from 10^4 to 10^6 cfu g^{-1}. A total of 590 bacterial isolates were characterized up to generic level. The generic composition was dominated by *Bacillus* sp. and *Arthrobacter* sp.

Ascidians are marine sessile filter feeding animals commonly called sea squirts and are cosmopolitan in distribution. They are classified into two groups, the solitary ascidians which live as individuals and the colonial ascidians which live together as colonies (Meenakshi, 2010). The secondary metabolites synthesized by ascidians contain interesting pharmacological substances exhibiting antimicrobials, antiviral, anti-inflammatory and cytotoxic activities (Rinehart et al., 1983, Anand & Edward, 2002, Ogi et al., 2008). Ascidians have a wide geographic distribution and around 3000 solitary and colonial ascidians have been described (Tait et al., 2007). Along the Indian coast 359 species of ascidians belonging to 38 genera have been reported (Meenakshi, 2010). The Gulf of Mannar along Thoothukudi coast is rich in ascidians and 85 species have been reported (Meenakshi, 2010). Ascidians have great potential as protein rich quality food (Meenakshi, 2010). Ascidian (*Halocynthia roretzi*) is enjoyed as food in Japan, particularly in the Hokkaido and Johoku districts because of its protein, carbohydrate and other micronutrients. The tunic of the pyurid ascidian, *Microcosmus hartmeyeri* and mantle bodies of *H. roretzi* and *H. aurantium* are farmed for food in Japan (Nanri et al., 1992). In Europe, *Micocosmus sabatieri* and *M. vulgaris* are consumed and *Pyura chilensis* is an important food item in Chile (Davis, 1995). The present study was undertaken to make an qualitative and quantitative assessment of the bacteria associated with ascidians.

Ascidians were collected from Thoothukudi Fishing Harbour, V. O. Chidambaram port and Tharuvaikulam from submerged rocks, country crafts and also from trawl catch. They were identified according to standard keys (Meenakshi, 2003) and analysed for their protein content (AOAC, 1995). The qualitative and quantitative bacterial flora from surface swab (10 cm^2) and whole body homogenate were estimated by standard methods and the bacterial isolates were identified up to generic level (Le Chevallier et al., 1980 and Surendran et al., 2003).

Solitary ascidians were identified as *C. aerolata*, *H. momus, M. exasperates*, *P. nigra* and colonial ascidians as *D. psammathodes*, *D. swamiensis*, *E. viride* and *L. fragile*. Among these ascidians *D. swamiensis* alone has been reported as native while others are non – native but established in our waters and characterized either as cryptogenic or invasive. *D. psammathodes*, *E. viride*, *L. fragile* and *H. momus* have been characterized as cryptogenic and *P. nigra*

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and *M. exasperatus* are reported as invasive (Ali et al., 2009). The status of *Polycarpa* sp. and *C. areolata* could not be ascertained from published literatures. The occurrence of 359 species belonging to 38 genera and 10 families of ascidians were reported to be distributed along Indian coast (Meenakshi, 2010). The coast of Gulf of Mannar especially along Thoothukudi coast was reported to be rich in ascidians (Ali et al., 2009), where they have recorded 28 species, while in another study, 85 species were reported from Thoothukudi coast (Meenakshi, 2010).

Protein content of all ascidians was around 5% on wet weight basis and was comparable to those reported earlier (Karthikeyan et al., 2010; Tamilselvi et al., 2010) thus it a low proteinaceous organism. Detailed nutrient profile needs to be done to consider them as a food source. Quantitative examination of cultivable bacterial flora associated with the surface of ascidians revealed variation ranging from ND to 10^5 cfu sq cm^{-1} (Table 1). The cultivable bacterial load from the whole body homogenate was in a higher range of 10^4 to 10^6 cfu g^{-1}. The lower count on the surface could be due to the fact that many epiphytic bacteria associated with marine organisms were shown to produce antibacterial secondary metabolites which inhibit settlement of potential competitors (Jensen & Fenical, 1994; Bernan et al., 1997). The lowest count of 10^2 cfu sq cm^{-1} was observed with *E. viride* and a similar

Table 1. Quantitative bacteria from the surface swab and whole body homogenate of ascidians

S.No.	Ascidian	Solitary/ Colonial	Surface swab (cfu sq cm^{-1})	Whole body homogenate (cfu g^{-1})
1	*Phallusia nigra*	S	3.0×10^4	2.4×10^6
2	*Cnemidocarpa areolata*	S	1.4×10^4	9.4×10^6
3	*Herdmania momus*	S	5.2×10^5	1.2×10^6
4	*Microcomus exasperatus*	S	6.9×10^3	1.2×10^6
5	*Eudistoma viride*	C	3.0×10^2	1.7×10^6
6	*Didemnum psammothodes*	C	ND	1.9×10^6
7	*Diplosoma swamiensis*	C	ND	2.7×10^5
8	*Lissoclinum fragile*	C	ND	7.5×10^4

ND – Not done, S – solitary, C- colonial

Table 2. Bacterial flora in surface swab of ascidians (percentage)

S.No.	Genus	*Eudistoma viride* (42)	*Herdmania momus* (32)	*Microcosmus exasperatus* (28)	*Phallusia nigra* (15)
1.	*Arthrobacter*	4.8	12.5	10.7	-
2.	*Bacillus*	47.6	87.5	78.6	-
3	*Moraxella*	-	-	3.6	-
4.	*Pseudomonas*	14.3	-	3.6	6.7
5.	*Vibrio*	33.3	-	3.6	53.3
6.	*Alcaligenes*	-	-	-	20
7.	*Flavobacterium*	-	-	-	6.7
8.	*Sarcina*	-	-	-	6.7
9.	*Staphylococcus*	-	-	-	6.7

Figures in parenthesis indicate the total number of bacteria isolated

Table 3. Bacterial flora in whole body homogenate of ascidians (percentage)

S.No	Genus	*Didemnum psammathodes* (49)	*Diplosoma swamiensis* (48)	*Eudistoma viride* (44)	*Cnemidocarpa areolata* (51)	*Herdmania momus* (32)	*Lissoclinum fragile* (33)
1.	*Arthrobacter*	12.2	16.7	11.4	7.8	25	15.2
2.	*Bacillus*	83.7	72.9	86.4	68.6	59.4	69.7
3	*Moraxella*	2	-	-	-	-	-
4.	*Vibrio*	-	6.3	2.3	19.6	15.6	15.2
5.	*Alcaligenes*	-	-	-	1.9	-	-
6.	*Flavobacterium*	2	4.2	-	1.9	-	-

Figures in parenthesis indicate the total number of bacteria isolated

observation was also documented earlier by Ramasamy & Murugan (2003) who have reported a 30 times greater bacterial load on *D. psammathodes* than on *E. viride*. Studies by Mary et al. (2016) have shown that the methanol crude extract of *E. viride* could inhibit the growth of *Vibrio cholerae* and *V. harveyi*. As shown in Table 2, the epibiotic bacterial flora associated with ascidians were dominated by Gram positive bacteria (87.6%) mostly consisting of the genera *Bacillus* and *Arthrobacter.* Other Gram positive genera such as *Staphylococcus* and *Sarcina* were also recorded but represented poorly. Certain colonial ascidians also sequester acids in their tunics and the reduced pH could be detrimental to the growth of microorganisms (Pisut & Pawlik, 2002). Certain studies suggest that gram positive bacteria generate spores and this will ensure their survival within the marine environment (Ettoumi et al., 2009). Among the gram positive bacteria *Bacillus* was the dominant species. *Bacillus* species found normally in mud and sediments of marine environment has been found commonly associated with marine benthos including ascidians (Romanenko et al., 2001). Although *Bacillus* was the most dominant genus associated with ascidians in the present study, Romanenko et al., 2001 reported the dominance of *Pseudoalteromonas* followed by *Bacillus i*n the internal organs of marine ascidians from Russian waters. The generic composition of the isolates from whole body homogenates of ascidians have also revealed the dominance of *Bacillus* sp. from all samples of ascidians studied (Table 3). Antibiotics including cyclic peptides, cyclic lipopeptides and novel thiopeptides have been reported from *Bacillus* sp. (Anand et al. 2006). Moreover *B. firmus*is involved in biogeochemical cycles and diverse degradation process (Ettoumi et al., 2009). Other Gram positive bacterial genera included *Arthrobacter*, *Micrococcus* and *Staphylococcus*. Studies by Taylor et al. (2007) have documented the predominance of gram negative bacteria in the marine environment. The Gram negative bacterial composition among epibiotic bacteria was quite diverse with members of *Flavobacterium*, *Alcaligenes*, *Moraxella*, *Pseudomonas* and *Vibrio*. Earlier studies have reported *Flavobacter*, *Vibrio*, *Pseudoalteromonas* and *Pseudomonas* to be common invertebrate associated bacterial genera (Tait et al., 2007). Gram negative bacterial population was represented by the genera *Vibrio* in most samples and *Flavobacterium* and *Moraxella* in a few in our study. Ananthan & Sathishkumar, 2016 reported on the inhibitory activity of bacterial isolates collected from ascidians of Gulf of Mannar that belonged to *Vibrio* sp., *Bacillus* sp. and *Marinobacter* sp. The bacteria associated with ascidians have been studied since these microorganisms have shown to produce novel bioactive compounds that are not found in terrestrial strains (Blunt et al., 2008).

The present study has shown the diverse bacterial population associated with ascidians, however the ecological role of the microorganisms associated with ascidians could not be defined. Further studies are required to determine the exact nature of the association between bacteria and the ascidians. The results could serve as base data to study the relationship between bacteria and ascidians and harness the bioactive compounds that might be produced as a result of these relationships.

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