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Studies on Enzyme - Producing Bacteria in the Digestive System of Starfish Collected from Manapad Estuary

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Abstract Even though studied in various phyla of oceanic invertebrates, the natural diversity of host-associated microbiota has little been studied in Echinodermata members such as starfish, sea urchins, and sea cucumbers. In the present investigation, a considerable amount of the population of amylase, cellulose, lipase, and protease-producing bacteria were detected in the gut of starfish collected from the Manapad estuary. The examination was carried out by standard microbiological methods with proper selective nutrient media. The present study reveals that the amylase-producing population was high (12 x 10^4 CFU/ml) in February and the cellulase-producing bacterial population was high (7 x 10^4 CFU/ml) in February and August. Lipase producing bacterial population was high (4.33 x 10^4 CFU/ml) in September and August (4 x 10^4 CFU/ml) and the proteolytic bacterial population was found to be the maximum in February (12.66 x 10^4 CFU/ml). In the present study, two isolates AK3 and AK8 which produces predominant enzymes are screened and on the nucleotide homology and phylogenetic analysis, the enzyme-producing bacterial strain AK3 was recognized and allotted as Enterococcus faecalis strain TKA2 (GenBank Accession Number: MZ823619). The strain AK8 was identified and assigned as Proteus sp. strain TKA1 (GenBank Accession Number: MZ823617). The attained result delivers scope for applications in the food and pharmaceutical industries.

Keywords Protoreasterlinckii, Manapad, Enzymes, Enterococcus Faecalis, Proteus Sp.

1. Introduction

Microorganisms related to metazoa have deep impacts on host health and development by altering host behavior, immunity, digestion, and reproduction [14]. These influences can be mediated by individual microorganisms or by complex communities over a wide range of mechanisms [15, 35]. Among the best well-studied non-human systems for animal symbiosis are marine invertebrates that comprise the sponges [16], corals [3], shipworms (Mollusca: Teredinidae) [10], Hawaiian bobtail squid (Euprymnascolopes) [27] and vestimentiferan tubeworms [11]. The complexity and spatial organization of the microbiota in these examples differ for each animal.

The bacterial flora within the GI tract of fish shows very broad and variable enzymatic potential, and these enzymatic masses may interfere positively in the digestive process of fish [29]. Fish gut bacterial isolates have been demonstrated to break down chitin [22, 18], cellulose [31, 2], p-nitrophenyl- b-N-acetylglucosamine and protein [5], starch [36], tannin [23] and phytate [30, 21].

Fajri et al. [12] isolated symbiotic bacteria from the starfish (P. nodosus) collected from the coastal area Takalar Regency, South Sulawesi, Indonesia. Their results confirmed the presence of microorganisms associated with starfish (P. nodosus) and the symbiotic bacteria were successfully isolated from the host.

Digestive enzymes aid in breaking down carbohydrates, fats, and proteins from the food we eat. Definite poor health conditions can affect the production of digestive enzymes and hence the replacement of digestive enzymes is a must. In this case, a search for microorganisms with digestive enzyme production is mandatory. The occurrence of proteolytic, cellulolytic, and amylolytic bacteria in the gut has been suggested as an omnivorous feeding aptitude of the fish by Creach [7].

The starfish Protoreasterlinckii feeds on a variety of benthic organisms and mainly feeds on sponges, sea anemones, soft corals, etc., and is also considered a rival of pearl oysters along the coast of the Gulf of Mannar. Being an omnivore species, the occurrence of protease, amylase, cellulase, and lipase-producing bacterial populations in the digestive tract of the starfish Protoreasterlinckii is justified.

Therefore, in this present study, we investigated the microbial communities producing enzymes such as amylase, cellulose, lipase, and protease from the gut of starfish Protoreasterlinckii.

2. Materials and Methods

Collection of starfish from Manapad estuary

Starfish Protoreasterlinckii (Order: Valvatida, Family: Oreasteridae) were collected at Manapad estuary every month in 2018 and the species identification was carried out under the supervision of Dr. T. Selvamohan, Assistant professor, Department of Zoology, Rani Anna Government College, Tirunelveli.

Enzymatic studies of bacteria isolated from the digestive system of starfish

In the laboratory, the starfish Protoreasterlinckii was dissected and the digestive system (gut) was separated using the bone cutter and forceps. The isolated gut was ground using mortar and pestle, then the colloidal substance was collected in the test tube. From that, 1ml of the sample was taken and dissolved in 9ml of distilled water for further analysis. To enumerate amylase, cellulase, lipase, and protease-producing bacteria, the diluted gut homogenate was poured on starch-agar, carboxymethylcellulose (CMC) agar, tributyrin agar, and skim milk agar media containing plates, respectively. These culture plates were incubated at 34 ℃ for 24 hours [24]. By multiplying the number of colonies formed on each plate by the reciprocal of dilution, colony numbers per unit sample volume of gut homogenate were determined [28]. Morphologically dissimilar, well-isolated colonies were randomly selected and streaked on Tryptone soya agar (TSA) slants. The slant cultured was then stored at 4oC in the refrigerator. All the isolates with apparently different morphological appearances were screened for the production of extra-cellular amylase, cellulase, lipase, and protease.

Screening of amylase-producing strains

For screening of amylase-producing strains, isolates were streaked on starch (1%) supplemented nutrient agar plates and incubated at 32 ℃ for 48 hours. The culture plates were then flooded with 1% Lugol's iodine solution [20] to identify amylase activity.

Screening of protease-producing strains

For extra-cellular protease production, the isolates were streaked on skim milk agar (4% gelatin) plates and incubated at 32 ℃ for 15 hours. The appearance of a clear zone around the colony indicated the presence of proteolytic activity [20].

Screening of lipase-producing strains

Lipase producers showed a clear zone surrounding their colony in 1% tributyrin plates [32].

Screening of cellulase-producing strains

For screening of cellulase producers, isolates were grown in carboxymethylcellulose (1%) nutrient agar and incubated at 32 ℃ for 72 hours. After incubation, the positive colonies were detected by flooding the plates with 0.1% aqueous Congo red. After 20 minutes, the dye was decanted and the plates were flooded with 5M NaCl. After 20-30 minutes, the NaCl was decanted. The cleat zone around the colonies against the red background is an indication of cellulase activity [33].

Assay of qualitative extracellular enzyme activity

Qualitative extracellular enzyme activity was assessed based on the measurement of a clear zone (halo) around the colony as follows: + (low, 4-6mm halo diameter), ++ (moderate, 7-9mm halo diameter), +++ (high, 10-12mm halo diameter) and ++++ (very high, 13-16mm halo diameter). From the ability to produce the extracellular enzymes, highly qualitative organisms were selected and used for identification and other studies.

Identification of microbes producing extra-cellular enzymes

Morphological and biochemical tests were carried out as per the standard staining procedure of Cappuccino and Sherman [6]. Identification of the isolates by 16S rRNA sequencing was done by isolating the genomic DNA and amplifying the polymerase chain reaction (PCR) using 16S rRNA Forward primer: AGA GTT TGA TCC TGG CTC AG and 16S rRNA Reverse primer: ACG GCT ACC TTG TTA CGA CTT.

Report of microbial identification

The 16S rDNA gene sequence was used to carry out BLAST with the database of NCBI GenBank database. Based on the maximum identity score, the first ten sequences were selected and aligned using the multiple alignment software program Clustal W. The sequence was

submitted to GenBank and the distance matrix was generated using RDP database. The evolutionary history was inferred using the Maximum Parsimony method. The MP tree was obtained using the Subtree-Pruning-Regrafting (SPR) algorithm [26] with search level 0 in which the initial trees were obtained by the random addition of sequences (10 replicates). Branch lengths were calculated using the average pathway method [26]. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA6 [37].

3. Result and Discussion

Enumeration of amylase-producing bacteria from the gut of starfish

The amylase-producing bacteria were enumerated at 104 dilution factor. The amylase-producing population was high (12.00±1.000 x 10^4 CFU/ml) in February. Next to February, May showed the highest amylase-producing bacterial population of 9.00±0.000 x 10^4 CFU/ml. The amylase-producing bacterial population was minimum in July at 2.66±0.577 x 10^4 CFU/ml. Next to July, the month of September showed a minimal amylase-producing bacterial population (5.00±1.000 x 10^4 CFU/ml). The amylase-producing bacterial population from the gut of starfish *Protoreasterlinckii* collected from the Manapad estuary was given in figure 1. Dhage [9] suggested that amylase activity in the intestine of herbivorous carp is much more intense than in carnivorous fishes. Amylase is secreted by the entire intestine in the Indian major carps, *Catlacatla*, *Labeorohita*, and *Cirrhinusmrigala*, and its activity is high toward the proximal end [9]. Das and Tripathi [8] reported high amylase activity in the gastrointestinal tract of grass carp, which appeared to be the result of its omnivorous feeding habit. In the present investigation, a considerable population of amylolytic bacteria was detected in the gut of starfish *Protoreasterlinckii* and certain bacterial strains are known, extracellular amylase producers.

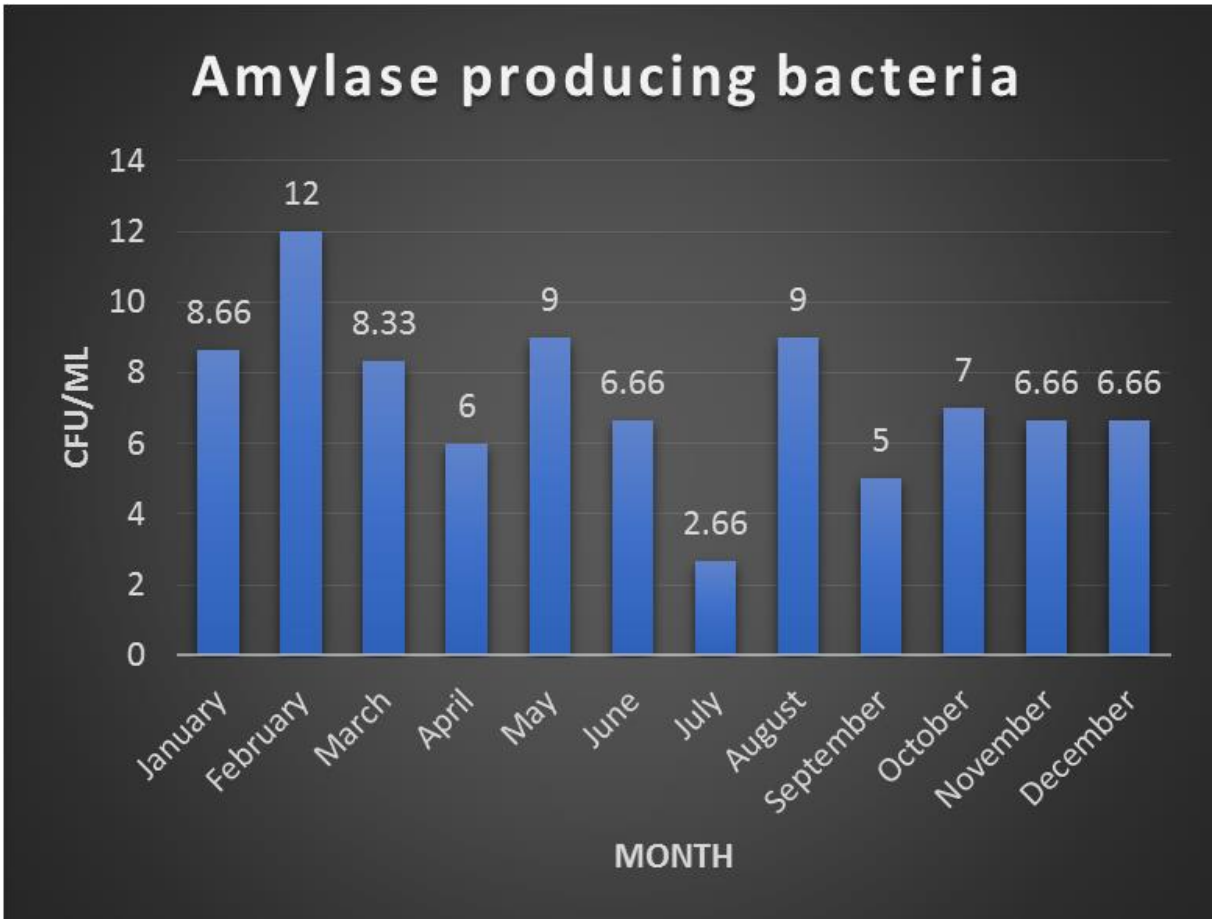

Figure 1. Amylase-producing bacteria from the gut of starfish

Enumeration of cellulase-producing bacteria from the gut of starfish

Carboxymethylcellulose (CMC) agar was used to enumerate the cellulase-producing bacteria from the gut of starfish *Protoreasterlinckii*. The cellulase-producing bacterial population was high (7.00±1.000 x 10^4 CFU/ml) in February and August. Next to February and August, January showed the highest cellulase-producing bacterial population of 5.33±0.577 x 10^4 CFU/ml. The cellulase-producing bacterial population was minimum in April, June, and November with 2.66±2.309 x 10^4 CFU/ml. Next to this, the month of September showed a minimal cellulase-producing bacterial population (3.00±000 x 10^4 CFU/ml). The cellulase-producing bacteria (CFU/ml) was given in figure 2. Barrington [4] and Yokoe and Yasumasu [38] believed that fish do not possess any cellulase, and Shcherbina and Kazlawlene [34] suggested the presence of microbial cellulase. In contrast to this, microbial intestinal cellulase activity was observed by Das and Tripathi [8] in grass carp and Saha and Ray [31] in rohu fingerlings. The presence of a considerable population of cellulolytic bacteria and their active role in extracellular cellulase production in the gut of starfish *Protoreasterlinckii* collected from the Manapad estuary has been confirmed in the present investigation. The results of the present study indicated that cellulolytic bacteria exist in the digestive tracts of starfish *Protoreasterlinckii* studied, which supports the hypothesis that bacteria might contribute to the utilization of cellulose in starfish.

Enumeration of lipase-producing bacteria from the gut of starfish

Tributyrin agar was used to enumerate the lipase-producing bacteria present in the gut of starfish Protoreasterlinckii, collected from the Manapad estuary. The lipase-producing bacterial population was high (4.33±0.577 x 104 CFU/ml) in September. Next to September, August showed the highest lipase-producing bacterial population of 4.00±0.000 x 104 CFU/ml. The lipase-producing bacterial population was minimum in December at 0.33±0.577 x 104 CFU/ml. Next to December, the month of November showed a minimal lipase-producing bacterial population (0.66±0.577 x 104 CFU/ml). In July, no lipase-producing bacteria were isolated. The total lipase-producing bacteria collected from the gut of starfish Protoreasterlinckii was given in figure 3. Al-Hussaini [1] observed the occurrence of lipase in cyprinids and the activity is more concentrated in the anterior intestine than in the posterior intestine. Das and Tripathi [8] noted the highest lipase activity in the hepatopancreas of both adult and fingerling grass carp. In the present perspective, microbial lipolytic activity was studied in the gut of starfish Protoreasterlinckii.

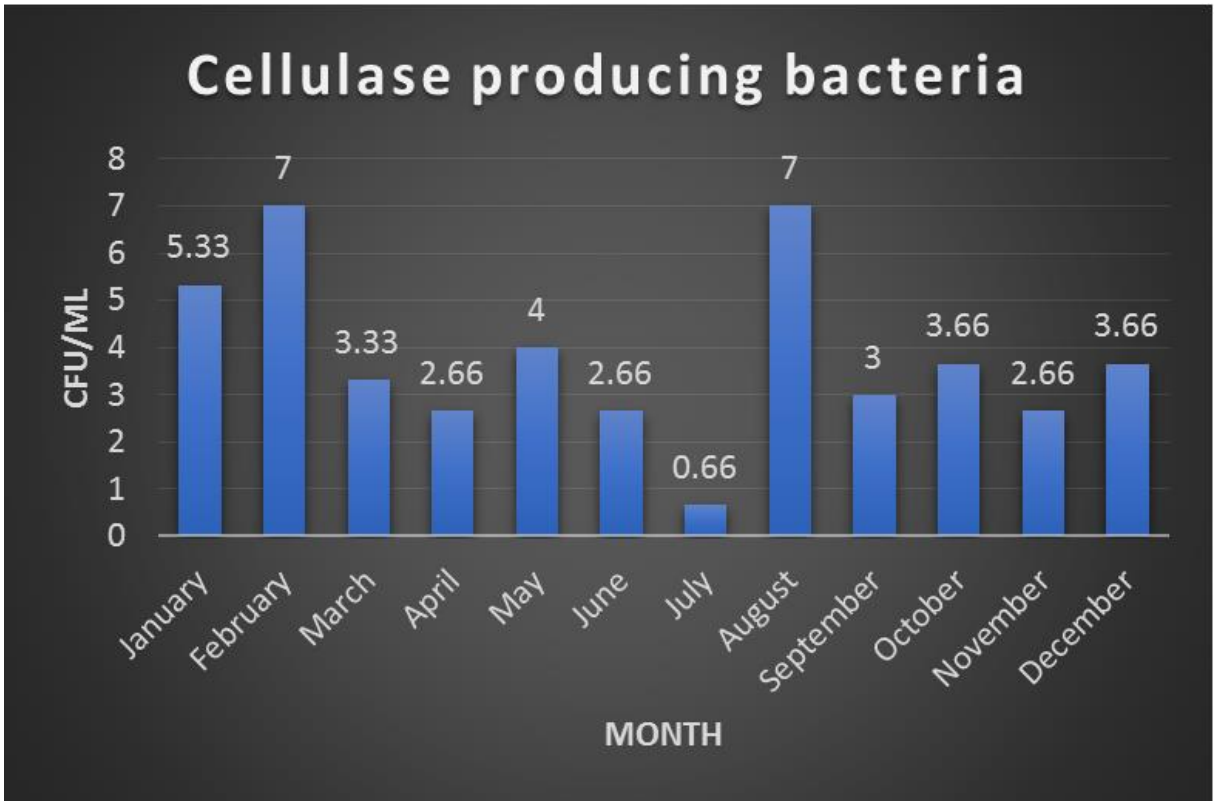

Figure 2. Cellulase-producing bacteria from the gut of starfish

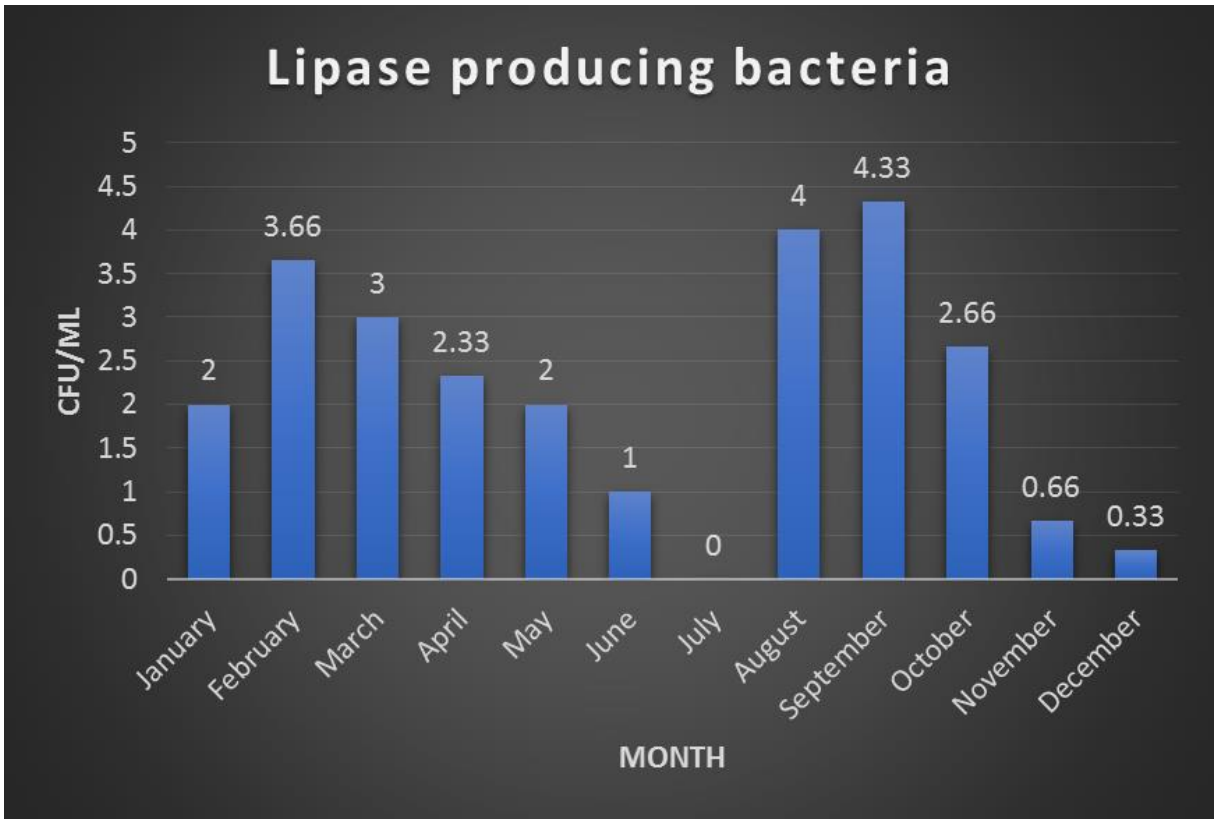

Figure 3. Lipase-producing bacteria from the gut of starfish

Enumeration of protease-producing bacteria from the gut of starfish

Skim milk agar was used to enumerate the protease-producing bacteria present in the gut of starfish Protoreasterlinckii, collected from the Manapad estuary. The protease-producing bacterial population was high (12.66±0.577 x 104 CFU/ml) in February. Next to February, January showed the highest protease-producing bacterial population of 10.33±0.577 x 104 CFU/ml. The protease-producing bacterial population was minimum in July at 2.33±0.577 x 104 CFU/ml. Next to July, the month of November showed a minimal protease-producing bacterial population (4.00±1.732 x 104 CFU/ml). The total protease-producing bacteria collected from the gut of starfish Protoreasterlinckii was given in figure 4. Ghosh et al. [13] suggested from their in vitro studies on enzyme-producing microbiota that bacteria isolated from the alimentary tract of rohu fingerlings were good producers of proteolytic enzymes. Bairagi et al. [2] quantified the proteolytic activity in the bacterial strains isolated from nine freshwater teleosts. Proteolytic bacteria were detected in the gut of the starfish Protoreasterlinckii examined in our study, and the maximum density in the proteolytic bacterial population was found in February and January.

Qualitative selection of bacteria for the production of enzymes isolated from the gut of starfish

Extracellular enzyme production by bacterial strains isolated from the gut of starfish Protoreasterlinckii collected from Manapad estuary was assayed qualitatively and given in Table 1. The initial screening of enzymes-producing bacteria was done by selecting the predominant colony either in the production of amylase, cellulase, lipase, and protease and marked as AK1-AK12. The strains AK3 and AK8 produce the enzymes in abundance. Therefore, these two bacterial strains are selected for bacterial identification.

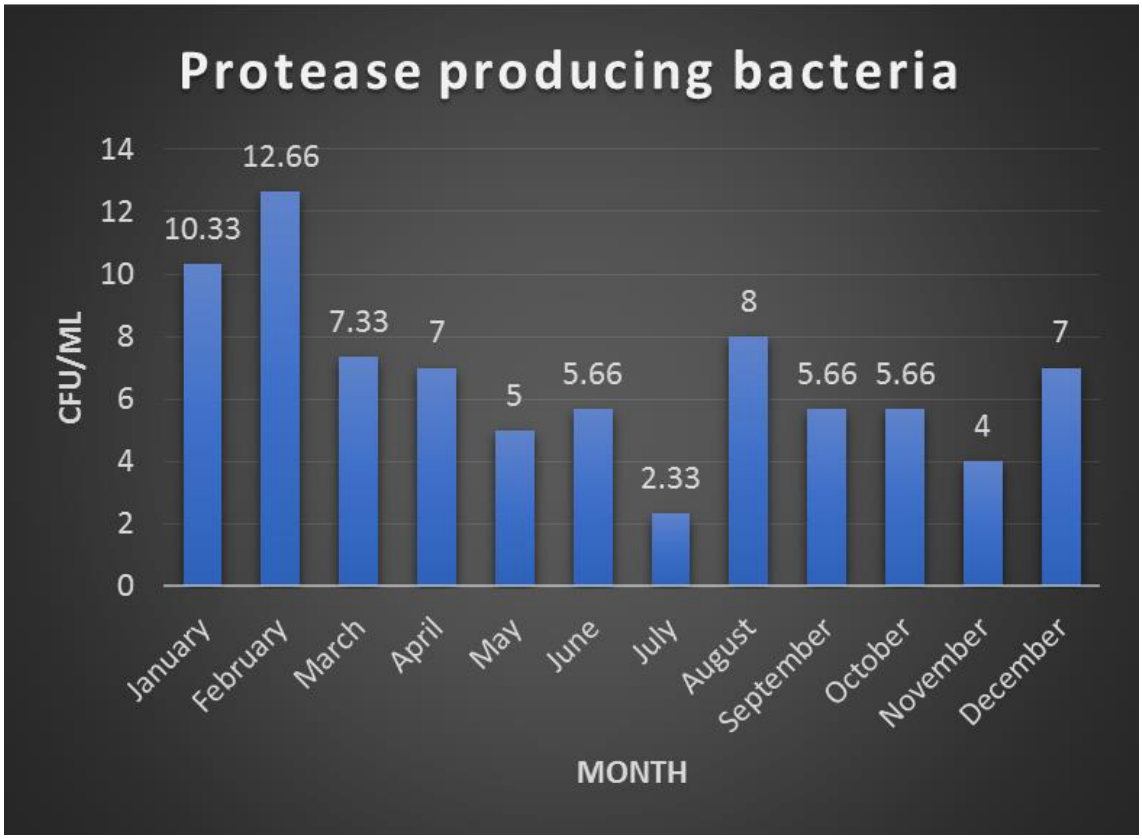

Figure 4. Protease-producing bacteria from the gut of starfish

Table 1. Qualitative analysis of enzymes producing bacteria from the gut of starfish

Bacterial strains	Amylase	Cellulase	Lipase	Protease
AK1	++	-	+	++
AK2	+	-	-	+
AK3	-	++++	++	+++
AK4	+	+	-	+
AK5	+	-	-	+
AK6	+	+	-	+
AK7	-	-	-	-
AK8	++++	-	+++	++++
AK9	+	-	-	-
AK10	++	-	++	++
AK11	-	++	-	++
AK12	+	-	+	+

++++, very high (13-16mm halo diameter); +++, high (10-12mm halo diameter); ++, moderate (7-9mm halo diameter); 1, low (4-6mm halo diameter); -, nil

Identification of bacteria producing extra-cellular enzymes isolated from the gut of starfish

The morphological and biochemical characterization of strains AK3 and AK8 were given in Table 2. The strain Ak3 was Gram-positive, non-motile and non-sporingcocci. It showed positive results for the VogesProskauer test and the Nitrate reduction test. All the other tests showed negative results. The strain AK8 was a Gram-negative, motile and non-sporing rod. The biochemical tests such as the Indole production test, VogesProskauer test, and Oxidase test showed negative results and all the other biochemical tests showed negative results.

More recently, culture-independent approaches have been taken to characterize the microbial community associated with adult sea stars of Acanthaster cf. solaris (Crown-of-thorns star), Asteriasamurensis (Japanese common star), and Patiriapectinifera (Blue bat star) [25, 17]. To gain more taxonomic information on the selected strains (AK3 and AK8), the 16S rDNA of the strains was partially sequenced.

The 16S rRNA sequence of isolated strains AK3 and AK8 was subjected to alignment using the Basic Local Alignment Search Tool (BLAST) to find the regions of local similarity between the sequences. With the help of BLAST, the sequences were aligned and phylogenetic trees were created for the isolates AK3 and AK8 using BLAST pairwise alignments. The phylogenetic trees for AK3 and AK8 were drawn as rectangular cladograms and were given in Figures 5 and 6. The IcI|Query_29647 indicates the query search result for AK3 and IcI|Query_5685 indicates AK8.

Table 2. Morphological and Biochemical Characterization of strains AK3 and Ak8 isolated from the gut of starfish

S.no	Morphological and Biochemical test	AK3	AK8
1	Gram staining	Positive cocci	Negative rod
2	Motility	Non Motile	Motile
3	Spore staining	Non Sporing	Non Sporing
4	Indole production test	Negative	Negative
5	Methyl red test	Negative	Positive
6	VogesProskauer test	Positive	Negative
7	Citrate utilization test	Negative	Positive
8	Nitrate reduction test	Positive	Positive
9	Urease test	Negative	Positive
10	Oxidase test	Negative	Negative
11	Catalase test	Negative	Positive
12	H_2S production test	Negative	Positive

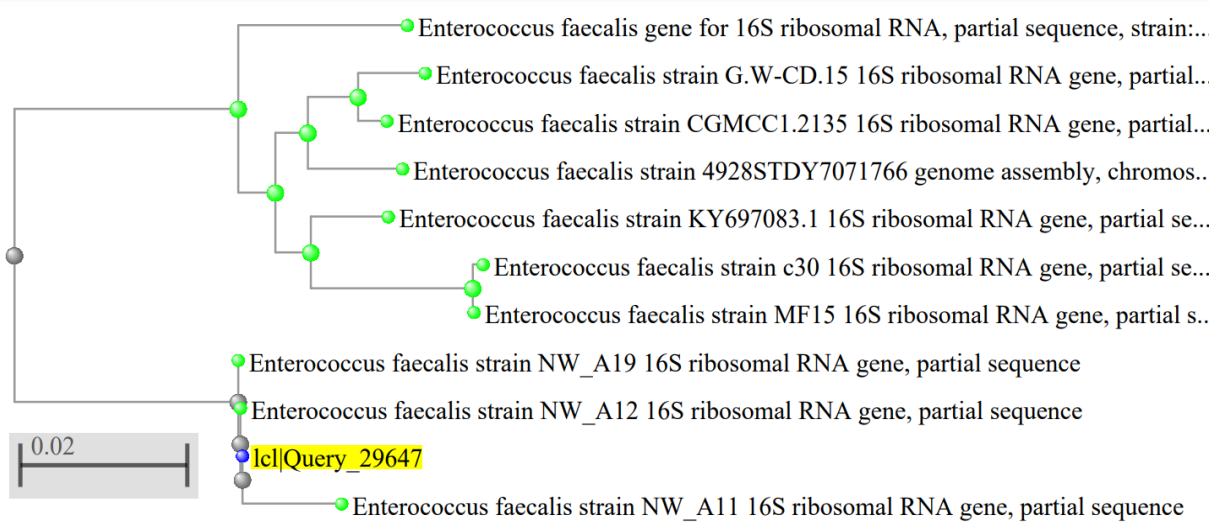

Figure 5. Phylogenetic tree (Rectangular Cladogram) of isolate AK3

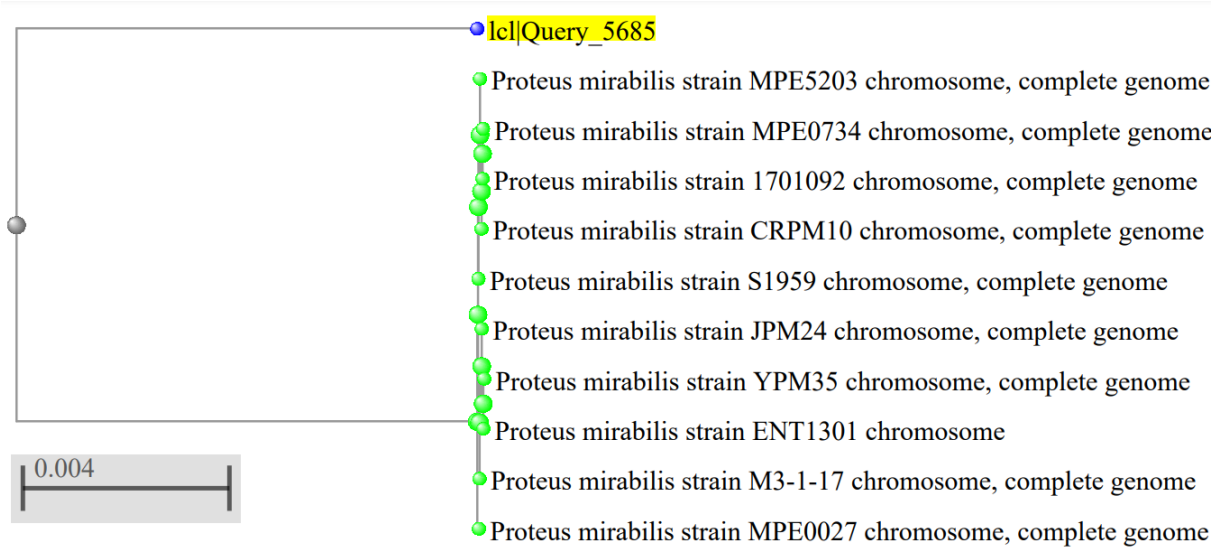

Figure 6. Phylogenetic tree (Rectangular Cladogram) of isolate AK8

Table 3. Identification of bacterial strains isolated from the gut with partial sequence of 16S rRNA genes referenced to accession no. in GenBank

S.no	Isolate	Organism with accession number	Closest relative (obtained from BLAST search)	Similarity (%)
1	AK3	*Enterococcus faecalis strain* TKA2 (MZ823619)	*Enterococcus faecalis strain* NW_A12(MG543825)	100
2	A15	*Proteus* sp. strain TKA1 (MZ823617)	*Proteus mirabilis* strain MPE5203 (CP053685)	98.16

Based on the nucleotide homology and phylogenetic analysis, the enzyme-producing bacterial strain AK3 was identified and assigned as Enterococcus faecalis strain TKA2 (GenBank Accession Number: MZ823619). The nearest homolog species of this strain was found to be Enterococcus faecalis strain NW_A12 (GenBank Accession Number: MG543825). The isolate AK3 showed 100% similarity with Enterococcus faecalis strain NW_A12. The strain AK8 was identified and assigned as Proteus sp. strain TKA1 (GenBank Accession Number: MZ823617) and its nearest homolog species was found to be Proteus mirabilis strain MPE5203 (GenBank Accession Number: CP053685) and the similarity was 98.16%. Information regarding the close homologs of the strains is provided in Table 3. The high relative abundance of Helicobacter-related taxon found previously in the coelomic fluid of Asteriasamurensis was significantly less (<1% relative abundance) among the sea star taxa presented in the study of Nakagawa et al. [25]. The results of Jackson et al. [19] corroborate these findings with the addition of Chromatiales and Enterobacteriales also making up observable differences between sample types. Our present study also proves the strain AK3 is 100% related to Enterococcus faecalis strain NW_A12 (GenBank Accession Number: MG543825) and the strain AK8 is 98.16% similar to the strain Proteus mirabilis strain MPE5203 (GenBank Accession Number: CP053685).

4. Conclusions

In conclusion, this study, to the best of our knowledge, is the first of its kind in the Manapad coastal area, Tamil Nadu, India to isolate two bacteria namely Enterococcus faecalis strain and Proteus sp. strain for the production of industrially important enzymes such as amylase, cellulose, lipase, and protease. This study is significant as it would pave the way for future research on digestive enzymes. Therefore, we recommend our study for further investigations, especially related to cellulase activity, since these topics are not fully understood and merit further studies.

REFERENCES

[1] Al-Hussaini A. H., "On the functional morphology of the alimentary tract of some fish in relation to differences in their feeding habits: Cytology and morphology", Quartarly Journal of Microscopical Science, 90, 323–354, 1949. https://doi.org/10.1023/A:1021355406412

[2] Bairagi A., Ghosh K. S., Sen S. K., Ray A. K.," Enzyme producing bacterial flora isolated from fish digestive tracts", Aquaculture International, 10(2), 109–121. 2002. https://doi.org/10.1023/A:1021355406412

[3] Baker A. C., "Flexibility and specificity in coral-algal symbiosis: Diversity, ecology, and biogeography of Symbiodinium" Annual Review of Ecology, Evolution, and Systematics, 34(1), 661–689, 2003. https://doi.org/10.1146/annurev.ecolsys.34.011802.132417

[4] Barrington E. J. W., "The alimentary canal and digestion", In M. E. Brown (Ed.), The physiology of fishes, Vol. I (pp. 109–161), Academic Press, 1957.

[5] Belchior S. G., Vacca G., "Fish protein hydrolysis by a psychrotrophic marine bacterium isolated from the gut of hake (Merlucciushubbsi)", Canadian Journal of Microbiology, 52(12), 1266–1271, 2006. https://doi.org/10.1139/w06-083

[6] Cappuccino J. G., Sherman N., "Microbiology: A laboratory manual", (6th ed) (pp. 15–224), Pearson Education, Inc., 2002.

[7] Creach P. V., "The proteolytic enzymes of poisons", In CNRS (Ed.), Nutrition in Poecilothermes (pp. 375–471), CNRS, 1963.

[8] Das K. M., Tripathi S. D., "Studies on the digestive enzymes of grass carp, Ctenopharyngodonidella (V)", Aquaculture, 92, 21–32, 1991. https://doi.org/10.1016/0044-8486(91)90005-R

[9] Dhage K. P., "Studies of the digestive enzymes in the three species of the major carps of India", Journal of Biological Sciences, 11, 63–74, 1968.

[10] Distel D. L., Morrill W., MacLaren-Toussaint N., Franks D., Waterbury J., "Teredinibacterturnerae gen. nov., sp. nov., a dinitrogen-fixing, cellulolytic, endosymbiotic gamma-proteobacterium isolated from the gills of wood-boring molluscs (Bivalvia: Teredinidae)", International Journal of Systematic and Evolutionary Microbiology, 52(6), 2261–2269, 2002. https://doi.org/10.1099/00207713-52-6-2261

[11] Dubilier N., Bergin, C., Lott C., "Symbiotic diversity in marine animals: The art of harnessing chemosynthesis", Nature Reviews. Microbiology, 6(10), 725–740, 2008. https://doi.org/10.1038/nrmicro1992

[12] Fajri M. D., Lallo S., Sartini S., "Preliminary studies on isolation and screening of antibiotic producing symbiotic bacteria from starfish (Protoreasternodosus) collected from coastal area Takalar Regency, Indonesia", Journal of Experimental Biology and Agricultural Sciences, 9(2), 183–188, 2021. https://doi.org/10.18006/2021.9(2).183.188

[13] Ghosh K., Sen S. K., Ray A. K., "Characterization of bacilli from the gut of rohu, Labeorohita, fingerlings and its significance in digestion", Journal of Applied Aquaculture, 12(3), 33–42, 2002. https://doi.org/10.1300/J028v12n03_04

[14] Gil-Turnes M. S., Hay M. E., Fenical W., "Symbiotic marine bacteria chemically defend crustacean embryos from a pathogenic fungus", Science, 246(4926), 116–118, 1989. https://doi.org/10.1126/science.2781297

[15] Hadfield M. G., "Biofilms and marine invertebrate larvae: What bacteria produce that larvae use to choose settlement sites", Annual Review of Marine Science, 3, 453–470, 2011. https://doi.org/10.1146/annurev-marine-120709-142753

[16] Hentschel U., Piel J., Degnan S. M., Taylor M. W., "Genomic insights into the marine sponge microbiome", Nature Reviews, Microbiology, 10(9), 641–654. 2012. https://doi.org/10.1038/nrmicro2839

[17] Høj L., Levy N., Baillie B. K., Clode P. L., Strohmaier R. C., Siboni N et al., "Crown-of-thorns sea star, Acanthaster cf. solaris, have tissue characteristicmicrobiomes with potential roles in health and reproduction", Applied and Environment Microbiology, 2018. https://doi.org/10.1128/AEM.00181-18

[18] Itoi S., Okamura T., Koyama Y., Sugita H., "Chitinolytic bacteria in the intestinal tract of Japanese coastal fishes", Canadian Journal of Microbiology, 52(12), 1158–1163, 2006. https://doi.org/10.1139/w06-082

[19] Jackson E. W., Pepe-Ranney C., Debenport S. J., Buckley D. H., Hewson I., "The microbial landscape of sea stars and the anatomical and interspecies variability of their microbiome", Frontiers in Microbiology, 9, 2018. https://doi.org/10.3389/fmicb.2018.01829

[20] Jacob M. B., Gerstein M. J., "Handbook of microbiology", D Van Nostrand Co. Inc. Princeton, NJ, 1960.

[21] Khan A., Mandal S., Samanta D., Chatterjee S., Ghosh K., "Phytase-producing Rhodococcus sp. (MTCC 9508) from fish gut: A preliminary study", Proceedings of the Zoological Society, 64(1), 29–34, 2011. https://doi.org/10.1007/s12595-011-0004-1

[22] MacDonald N. L., Stark J. R., Austin B., "Bacterial microflora in the gastrointestinal tract of Dover sole (Soleasolea L.), with emphasis on the possible role of bacteria in the nutrition of the host", FEMS Microbiology Letters, 35(1), 107–111, 1986. https://doi.org/10.1111/j.1574-6968.1986.tb01508.x

[23] MandalS., Ghosh K., "Isolation of tannase-producing microbiota from the gastrointestinal tracts of some freshwater fish", Journal of Applied Ichthyology, 29(1), 145–153, 2013. https://doi.org/10.1111/j.1439-0426.2012.02054.x

[24] Mondal S., Roy T., Sen S. K., Ray A. K., "Distribution of enzyme producing bacteria in the digestive tracts of some freshwater fish",ActaIchthyologicaet Piscatoria, 38(1), 1–8, 2008. https://doi.org/10.3750/AIP2008.38.1.01

[25] Nakagawa S., Saito H., Tame A., Hirai M., Yamaguchi H., Sunata T., Aida M., Muto H., Sawayama S., Takaki Y., "Microbiota in the coelomic fluid of two common coastal starfish species and characterization of an abundant Helicobacter-related taxon", Scientific Reports, 7(1), 2017. https://doi.org/10.1038/s41598-017-09355-2

[26] Nei M., Kumar S., "Molecular evolution and phylogenetics", Oxford University Press, 2000.

[27] Nyholm S. V., McFall-Ngai M. J., "The winnowing: Establishing the squid–Vibrio symbiosis", Nature Reviews, Microbiology, 2(8), 632–642, 2004. https://doi.org/10.1038/nrmicro957

[28] Rahmatullah S. M., Beveridge M. C. M., "Ingestion of bacteria in suspension Indian major carps (catlacatla, Labeorohita) and Chinese carps (Hypophthalmichthysmolitrix, Aristichthysnobilis)", Hydrobiologia, 264(2), 79–84, 1993. https://doi.org/10.1007/BF00014095

[29] Ray A. K., Roy T., Mondal S.,RingÃ¸ E., "Identification of gut-associated amylase, cellulase, and protease-producing bacteria in three species of Indian major carps", Aquaculture Research, 41, 1462–1469, 2009. https://doi.org/10.1111/j.1365-2109.2009.02437.x

[30] Roy T., Mondal, S., Ray A. K., "Phytase-producing bacteria in the digestive tracts of some freshwater fish", Aquaculture Research, 40(3), 344–353, 2009. https://doi.org/10.1111/j.1365-2109.2008.02100.x

[31] Saha A. K., Ray A. K., "Cellulase activity in rohu fingerlings", Aquaculture International, 6(4), 281–291, 1998. https://doi.org/10.1023/A:1009210929594

[32] Sangiliyandi G., Gunasekeran P., "Extracellular lipase producing Bacillus licheniformis from an oil mill refinery effluent", Indian Journal of Microbiology, 36, 109–110, 1996.

[33] Sarbahi D. S., "Studies on the digestive enzymes of goldfish Carassiusauratus (Linn.) and large mouth black bass, Micropterussalmoides (Lacepede)", Biological Bulletin, 100(3), 244–257, 1951. https://doi.org/10.2307/1538534

[34] Shcherbina M. A., Kazlawlene O. P., "The reaction of the medium and the rate of absorption of nutrients in the intestine of carp", Journal of Ichthyology, 11, 81–85, 1971.

[35] Shin S. C., Kim S. H., You H., Kim B., Kim A. C., Lee K. A., Yoon J. H., Ryu J. H., Lee W. J., "Drosophila microbiome modulates host developmental and metabolic homeostasis via insulin signalling", Science, 334(6056), 670–674, 2011. https://doi.org/10.1126/science.1212782

[36] Sugita H., Shibuya K., Hanada H., Deguchi Y., "Antibacterial abilities of intestinal microflora of the river fish", Fisheries Science, 63(3), 378–383, 1997. https://doi.org/10.2331/fishsci.63.378

[37] Tamura K., Stecher G., Peterson D., Filipski A., Kumar S., "MEGA6: molecular evolutionary genetics analysis version 6.0", Molecular Biology Evolution, 30(12), 2725–2729, 2013. https://doi.org/10.1093/molbev/mst197

[38] Yokoe Y., Yasumasu I., "The distribution of cellulase in invertebrates. Comparative Biochemistry and Physiology", 13, 323–338, 1964. https://doi.org/10.1016/0010-406x(64)90027-1