BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, March 2023. Vol. **20**(1), p. 211-221

Qualitative Analysis of Culturable Gut Microbes of Selected Cephalopod Species- A Comparative Study

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http://dx.doi.org/10.13005/bbra/3082

(Received: 06 November 2022; accepted: 21 January 2023)

Microbes present in the digestive tract of an organism are of great importance. The microbial qualitative and quantitative composition represents the physical, physiological conditions, habits, habitats of the organisms as well as their association patterns. The microbes of digestive system have prominent role not only in the sustainability of the organism but also in the food processing industry. The marine cephalopods are one of the preferred marine food resources, next to marine fisheries. The current study aims to understand the microbial content in the digestive system of consumable cephalopods such as sepia and cuttlefish. The commercially available squids from the market and cuttlefish samples from fishing area were collected and used for this study. The organisms were dissected in aseptic conditions and bacteria collected from the guts of these animals. Culturable bacteria were isolated and, identified using gram characteristics as well as 16s r RNA techniques based molecular identification. The identified bacteria were reported to Gen Bank submission. Bacterial representation in the gut microbiome of cephalopods is low and showed distinct difference between cuttlefish and squid species. This low number of bacterial composition may be due to the habitat conditions or the association of bacteria with the animal requires further studies to understand. The commercial value of cephalopods as protein rich food prioritizes the need to address proper treatment process which can alleviate their presence in Indian food industry scenario. The inter and intra-species relation of microbes and metazoans and the associated macromolecules can be used for pharma industry also in future

Keywords: Commercial value; Cephalopod; Digestive tract; Microbiome; 16 S ribosomal RNA.

Cephalopods are potential alternate protein rich food and have significant place in export commerce. They emerge as important contributors to the need of the country and future resources. They should be constantly monitored as they play a prominent role in trophic pyramid[1]. cephalopods require constant monitoring of their stock as they became a matter of concern due to climatic change or environmental disturbances [2-4]. The major catch of the squid species found to be higher (97%) from Indian waters alone, followed by Indo-Pacific Ocean periphery, Pink

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sea, Arabian sea, Mozambique to South China Sea, Philippines sea and Northern Taiwan. Cornea of the eyes has major contribution while distinguishing the species of cephalopods. Many studies have been conducted to study the biology, population dynamics, commercial exploitation, feeding habits and the energetic lifestyle of squids. Squid species are cannibalistic and big scale migrations occur during the scarcity of food [5-7]. The mass migration of squids permits them to take advantage of spatial and temporal marine production systems equipped with adaptations in the body structure viz. tentacles. They behave as ecological opportunists. Squid meat has an extraordinary nutritive value with high protein bioactive substances and omega 3 and 6 rich marine oil content which makes them most appropriate for human consumption [8,9]. Processing methods such as canning and drying followed by freezing are done prior to their sale in the domestic or overseas markets. The previous studies of cephalopods emphasized the ecology, catch, environmental impacts and food and feeding habits at national and international level [10-13]. Earlier studies on *Sepia officinalis*, *Octopus* sps., *Loligo vulgaris* and *Nautilus popilius mainly* focused on the cephalopod's digestive system physiology or morphology, motility and absorptive functions up to a limited extent [14]. Analyses of the cephalopod gut microbiome are limited in spite of their key phylogenetic position, ecological and commercial importance [15]. A unique set of microbial composition of the gut microbiota was observed in the study of six cephalopod species in comparison to their marine mollusk and fish counterparts[16]. Further, the gut microbiome of aquarium cultured cuttlefish indicated symbiotic relationship with that of the host [17]. The current study aims to assess the composition of digestive tract microbiome in selected cephalopods off India using the culture methods. In addition, the possible reasons for their unique pattern of representation in the cephalopods and their importance in the food industry were analyzed.

MATERIAL AND METHODS

Isolation of bacteria from Squid and cuttlefish

Squid and cuttlefish samples were procured from the commercial and fishing markets and transferred to the laboratory. Standard FAO catalogues of Cephalopods were used for the identification of these organisms [18,19]. The organisms were surface sterilized by washing with water, wipe them with absolute alcohol and 30 sec. immersion in the alcohol. The digestive tract was removed and placed in 10 ml of sterile saline solution after dissecting the organisms under aseptic conditions. The tract was teased and the contents were emptied with 5ml of sterile saline. 1ml of aliquot of gut homogenate was serially diluted with sterile saline up to 10^{-7} dilution. This 0.1 ml of homogenate was spread on Nutrient and Cetrimide agar media and incubated at 37°C for 24 hrs. Gram staining was done morphological characterization to distinguish between the Gram positive/Gram negative as well the shape of individual organisms. The florescence was checked under UV. The selected bacterial isolates from squid and cuttlefish were further transferred on Nutrient agar slants and sent for 16S rRNA based identification to Credora Life Sciences, Bangalore.

Molecular characterization of cephalopod gut associated bacteria

Identification of bacteria based on16S r RNA Sequencing

Hyper variable regions within the 16S r RNA gene represent a species unique signature used for bacterial identification. The CTAB method (N-cetyl, NNN Trimethyl Ammonium Bromide) was employed for the extraction of bacterial genomic DNA. The obtained DNA pellet was washed with 70% Ethanol, and dissolved in 20 µl sterile distilled water. The genomic DNA was read at wavelengths of 260°A and 280°A using UV-VIS spectrophotometer (Viva spec Bio -Spectrophotometer, Germany) for establishing purity and quantification.

The amplification of 16S region was performed using Polymerase Chain Reaction (PCR) technique. A reaction mixture of 20 µl comprising- Polymerase Chain Reaction buffer (PCR buffer) , ddNTP, dNTP mixture, Taq polymerase, primer, template DNA was used. Sterile nuclease free water was considered as negative control. PCR temperature profile comprised a denaturation stage (2 min 50 sec at 94°C), Annealing stage (30s at 48°C), Extension stage (1min 30 sec. at 72°C) followed by final extension stage of 6 min. Oligonucleotide primers of 500bp with a length of 20 nucleotides were

considered as 16 S forward primer of (5'-3') AGAGTTTGATCMTGGCTCAG, 50% GC and Tm Value 51.0°C and, 16S Reverse Primer Sequence (5'-3') of CGGTTACCTTGTTACGACTT with 60 % GC, Tm Value 56.0°C [20] were considered for the study. A comparison of 16S r RNA sequence of bacterial genome was carried out based on NCBI BLAST and bacterial phylogenetic status by multiple sequence alignment (CLUSTAL-O 1.2.4) . The closely related organism was identified and the sequences were deposited to the NCBI Gen Bank to obtain accession numbers.

Extraction and identification of proteins

A loopful of fluorescent bacteria (isolated from the squid) inoculum was added to 50ml of Nutrient broth. The culture medium was collected after 48 hrs of incubation at 37^0 C on shaker (500 rpm) conditions and centrifuged. Proteins were separated using salting and desalting techniques and further purified using dialysis from the spent medium [21]. The proteins were separated using Native PAGE. The protein band was further analyzed using Orbitrap HRLC-MS technique (Thermo EASY-Nlc and Q Exactive Plus - Orbitrap MS) at SAIF, IIT, Mumbai.

RESULTS AND DISCUSSION

Cephalopods are active marine predators. The earlier studies on their digestive system indicated many physiological adaptations in accordance with their habit and habitat. The physiology of digestive tract was monitored using non-invasive techniques. The welfare of the cephalopod in aquaculture and their digestive system shown functional relevance [22,23]. However, less priority was given to the studies aimed at contents of digestive tract of a cephalopods which play a major role in laboratory maintenance of animals as well as aquaculture practices[14] . A comparative study of guts of 6 cephalopod species and other mollusks, with that of marine fish was carried out to investigate the factors that shape the gut microbiota[15,16].

Isolation of bacteria from Squid and cuttlefish

The results of current microbial contents of the gut based on culture based studies indicated the presence of gram-negative bacteria rods with green florescence under UV 365nm. The bacteria from squid showed growth in Cetrimide agar. Qualitatively, only two different bacterial variants were observed in the gut of squid, and 4 different bacterial variants from the gut of cuttlefish during the study.

Molecular characterization of cephalopod gut associated bacteria

The 16S r RNA amplicon BLAST sequence similarity with bacterial databases identified Proteobacterial species. The sequence based similarity studies using BLAST indicated the variants were closely related to *Alcaligenes and Stenotrophomonas* species (93.65% and 86.46%

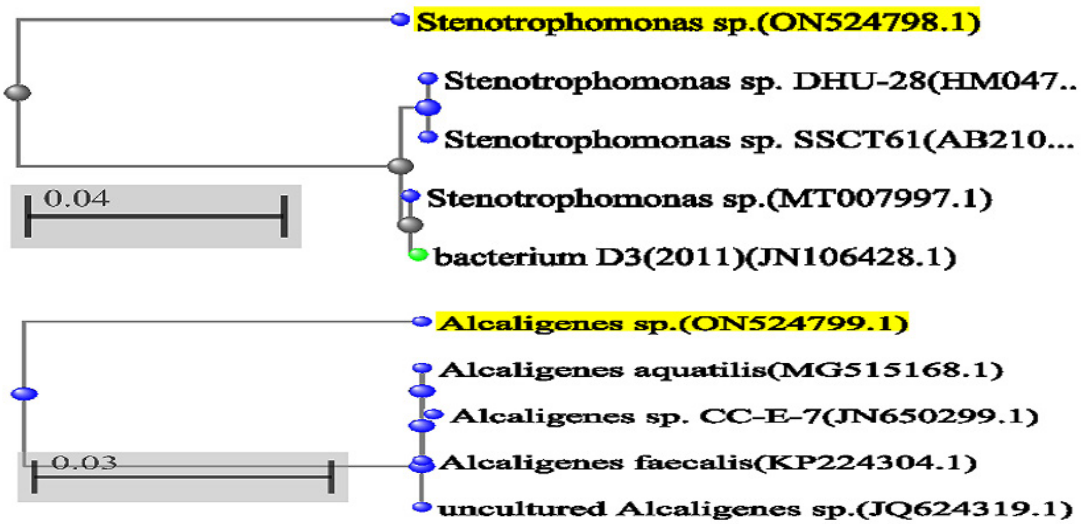

Fig. 1. Phylogenetic associations of *Stenotrophomonas* sp. Strain jpm 20 (a) , *Alcaligenes* sp. Strain sloli A09

Table 1. Heat map of *Stentrophomonas* sps. secreted Proteins extracted and characterized using LC-MS

Acession No	Coverage [%]	# Peptides/ uniqueseq/PSMS	# AAs	MW [kDa]	calc. pI
A0A2R7P3G6	–	–	–	–	–
A0A3N1K3U7	–	–	–	–	–
A0A3D0GD54	–	–	–	–	–
A0A4Z0DW87	–	–	–	–	–
A0A1W5DQU1	–	–	–	–	–
A0A1W5DRI2	–	–	–	–	–
A0A4V2FEE5	–	–	–	–	–
A0A1B2N6P9	–	–	–	–	–
A0A317BNT7	–	–	–	–	–
A0A239QDD8	–	–	–	–	–
A0A4V1WIG2	–	–	–	–	–
A0A1W5DR29	–	–	–	–	–
A0A0J8PQL4	–	–	–	–	–
A0A1W5DFW2	–	–	–	–	–
A0A1Y5Q6G1	–	–	–	–	–
A0A0Q4QD17	–	–	–	–	–
A0A4U9PMS0	–	–	–	–	–
A0A4Z0DRP5	–	–	–	–	–
A0A0F5ZPL3	–	–	–	–	–
A0A2T3WJN2	–	–	–	–	–
A0A1W5DIJ8	–	–	–	–	–
A0A1W5DU88	–	–	–	–	–
A0A0F5ZPP7	–	–	–	–	–
A0A1W5DRY1	–	–	–	–	–
A0A380AS26	–	–	–	–	–
A0A2W5KX19	–	–	–	–	–
A0A1W5DSI9	–	–	–	–	–
A0A3C0X491	–	–	–	–	–
A0A2T1JG43	–	–	–	–	–
A0A1W5DRR9	–	–	–	–	–
A0A4Z0DS73	–	–	–	–	–
A0A4Z0E2Z9	–	–	–	–	–
A0A355YHK4	–	–	–	–	–
A0A2D0AP22	–	–	–	–	–
A0A0R0E5D6	–	–	–	–	–
A0A1W5DS87	–	–	–	–	–
A0A4Z0DQS9	–	–	–	–	–
A0A2W5NZ56	–	–	–	–	–
A0A1B2N8H6	–	–	–	–	–
A0A4Q7R830	–	–	–	–	–
A0A1W5DS28	–	–	–	–	–
A0A0R0ALV5	–	–	–	–	–
A0A1W5DSR5	–	–	–	–	–
A0A4Z0DZN7	–	–	–	–	–
A0A1W5DHR6	–	–	–	–	–
A0A4S2D5F6	–	–	–	–	–
A0A2W5NWF3	–	–	–	–	–
A0A1W5DSJ9	–	–	–	–	–
A0A0R0CY49	–	–	–	–	–
A0A431UAC2	–	–	–	–	–
A0A4Z0DQY0	–	–	–	–	–

A0A4Q4LAS4	–	–	–	–	–
A0A498CIE5	–	–	–	–	–
A0A023Y629	–	–	–	–	–
A0A0R0ANU2	–	–	–	–	–
A0A1W5DSQ8	–	–	–	–	–
A0A1W5DRL3	–	–	–	–	–
A0A4U3GQA6	–	–	–	–	–
A0A1D8YAH9	–	–	–	–	–
A0A4Z0E388	–	–	–	–	–
A0A3C0XAW0	–	–	–	–	–
A0A4Z0DSX7	–	–	–	–	–
A0A1Q4ESR5	–	–	–	–	–
A0A1B2N4G9	–	–	–	–	–
A0A3D0GES6	–	–	–	–	–
A0A0R0C9X0	–	–	–	–	–
A0A1W5DS47	–	–	–	–	–
A0A023XZR8	–	–	–	–	–
A0A4Z0DV97	–	–	–	–	–
A0A2J0SNC4	–	–	–	–	–
A0A2W5LFJ7	–	–	–	–	–
A0A1W5DGG2	–	–	–	–	–
A0A1W5DPF7	–	–	–	–	–
A0A4Z0E308	–	–	–	–	–
A0A4Z0DRP6	–	–	–	–	–
A0A5N7SB45	–	–	–	–	–
A0A4Z0DT03	–	–	–	–	–
A0A2Y9UCH2	–	–	–	–	–
A0A0U5I2Y5	–	–	–	–	–
A0A357N7P7	–	–	–	–	–
A0A4Z0DRX0	–	–	–	–	–
A0A246HIT6	–	–	–	–	–
A0A3S7KNA5	–	–	–	–	–
A0A4Z0E039	–	–	–	–	–
A0A4Z0DTP5	–	–	–	–	–
A0A0M1EJ90	–	–	–	–	–
A0A4Z0E332	–	–	–	–	–
A0A4Q1CX40	–	–	–	–	–
A0A3D0GCI2	–	–	–	–	–
A0A4Q4LIK9	–	–	–	–	–
A0A2W5LZC2	–	–	–	–	–
A0A2W5LSV3	–	–	–	–	–
A0A246HJA3	–	–	–	–	–
A0A2T1IS34	–	–	–	–	–
A0A4Z0DZ24	–	–	–	–	–
A0A0R0C9W0	–	–	–	–	–
A0A431U9V6	–	–	–	–	–
A0A4Z0DU32	–	–	–	–	–
A0A4Z0DXY9	–	–	–	–	–
A0A0R0CYG6	–	–	–	–	–
A0A355YD66	–	–	–	–	–
A0A4Z0E1X7	–	–	–	–	–
A0A355YHR5	–	–	–	–	–
A0A0R0BF08	–	–	–	–	–
A0A0R0DYW2	–	–	–	–	–
A0A4V2FEB3	–	–	–	–	–
A0A1D8YA83	–	–	–	–	–

A0A4Z0DUQ4	–	–	–	–	–
A0A1W5DRJ6	–	–	–	–	–
A0A4Z0E493	–	–	–	–	–
A0A0R0DWS3	–	–	–	–	–
A0A4Z0DZF0	–	–	–	–	–
A0A397M9Y6	–	–	–	–	–
A0A0R0DZT9	–	–	–	–	–
M5D7E1	–	–	–	–	–
A0A4Z0E1N0	–	–	–	–	–
A0A1B2N4U5	–	–	–	–	–
A0A4Z0E0Y5	–	–	–	–	–
A0A4Q7RU54	–	–	–	–	–
A0A3D4TK95	–	–	–	–	–
A0A0R0CFM5	–	–	–	–	–
A0A4Z0DYQ0	–	–	–	–	–
A0A498CHN1	–	–	–	–	–
B8LAU6	–	–	–	–	–
A0A4Z0DRJ7	–	–	–	–	–
A0A4Q4LE00	–	–	–	–	–
A0A0R0CKP9	–	–	–	–	–
A0A3N1R039	–	–	–	–	–
B4SIW5	–	–	–	–	–
A0A0R0AIG5	–	–	–	–	–
A0A0R0CMQ8	–	–	–	–	–
A0A2R7QQI7	–	–	–	–	–
A0A4Z0DRF1	–	–	–	–	–
A0A4Z0DZL8	–	–	–	–	–
A0A3D5JS11	–	–	–	–	–
A0A4Z0DR01	–	–	–	–	–
A0A0M0P1V9	–	–	–	–	–
A0A4Z0DWL6	–	–	–	–	–
A0A2T3X2F7	–	–	–	–	–
A0A0R0E4I2	–	–	–	–	–
A0A4R4IC51	–	–	–	–	–
A0A0R0D8D1	–	–	–	–	–
Q7X0G1	–	–	–	–	–
A0A4Z0DZF3	–	–	–	–	–
A0A0R0DIQ7	–	–	–	–	–
A0A3N1K6Q6	–	–	–	–	–
A0A4Z0DZV7	–	–	–	–	–
A0A4Z0E1M1	–	–	–	–	–
A0A1D8Y7J2	–	–	–	–	–
A0A2J0UGT4	–	–	–	–	–
A0A0R0BSW6	–	–	–	–	–
A0A4T1DKR3	–	–	–	–	–
A0A246HIC6	–	–	–	–	–
A0A0R0CXC6	–	–	–	–	–
A0A1W5DGS1	–	–	–	–	–
A0A0R0DHF7	–	–	–	–	–
A0A0U5HY80	–	–	–	–	–
A0A0R0D0D2	–	–	–	–	–
A0A0R0CHQ6	–	–	–	–	–
A0A5N0KMT0	–	–	–	–	–
A0A4Z0E174	–	–	–	–	–
A0A0R0C2W5	–	–	–	–	–
A0A4Q1CRR0	–	–	–	–	–

A0A1Q4EXN0	–	–	–	–	–
A0A5N0KRC7	–	–	–	–	–
A0A353U758	–	–	–	–	–
A0A1W5DSD8	–	–	–	–	–
A0A4Z0E2D3	–	–	–	–	–
A0A0Q4QUV2	–	–	–	–	–
A0A2J0UAU1	–	–	–	–	–
A0A4Z0DY99	–	–	–	–	–
A0A0R0AFI0	–	–	–	–	–
A0A0K2IF90	–	–	–	–	–
A0A0Q4QGX2	–	–	–	–	–
A0A0K2IUH4	–	–	–	–	–
A0A4Z0DZE2	–	–	–	–	–
A0A0Q4QF94	–	–	–	–	–
A0A4Z0DQM8	–	–	–	–	–
A0A498C240	–	–	–	–	–
A0A1D8YEE6	–	–	–	–	–
A0A4Z0E2G8	–	–	–	–	–
A0A0R0CSI7	–	–	–	–	–
A0A0R0CHY8	–	–	–	–	–
A0A4Z0DSG8	–	–	–	–	–
A0A4Z0E1S1	–	–	–	–	–
A0A2Y9U3Z2	–	–	–	–	–
A0A4Z0E0I4	–	–	–	–	–
A0A4Z0E192	–	–	–	–	–
A0A4Z0DYN4	–	–	–	–	–
A0A2T1IP77	–	–	–	–	–
A0A2M9QZ64	–	–	–	–	–
A0A4Z0E0R4	–	–	–	–	–
A0A0R0CV94	–	–	–	–	–
A0A2J0UX67	–	–	–	–	–
A0A4V2FGS0	–	–	–	–	–
A0A2M9QX77	–	–	–	–	–
A0A4Z0E095	–	–	–	–	–
A0A1D8Y994	–	–	–	–	–
A0A4Z0E1V8	–	–	–	–	–
A0A2D2W2A6	–	–	–	–	–
A0A0R0AAH8	–	–	–	–	–
T5KP48	–	–	–	–	–
A0A4R7US30	–	–	–	–	–
A0A4Z0DQY3	–	–	–	–	–
A0A4Z0DYU7	–	–	–	–	–
A0A1A6Y0L8	–	–	–	–	–
A0A4Z0E1H0	–	–	–	–	–
A0A4Z0DQK3	–	–	–	–	–
A0A023Y783	–	–	–	–	–
A0A0M1EV12	–	–	–	–	–
A0A149Q5G4	–	–	–	–	–
A0A3N1KP13	–	–	–	–	–
A0A363S4I9	–	–	–	–	–
A0A2J0T2Q8	–	–	–	–	–
A0A0D0JM52	–	–	–	–	–
A0A0R0BJ61	–	–	–	–	–
A0A4Z0E112	–	–	–	–	–
A0A4Z0E327	–	–	–	–	–
A0A2W5LHA7	–	–	–	–	–

A0A4Z0DYA1	–	–	–	–	–
A0A427CF66	–	–	–	–	–
A0A2U4HFE5	–	–	–	–	–
A0A0R0AMU9	–	–	–	–	–
A0A4Z0E3J7	–	–	–	–	–
A0A2W6G348	–	–	–	–	–
A0A2R7R082	–	–	–	–	–
A0A3N1QLM7	–	–	–	–	–
A0A0R0CQ61	–	–	–	–	–
A0A4Z0DXA0	–	–	–	–	–
A0A4Z0DR66	–	–	–	–	–
A0A2M8VN75	–	–	–	–	–
A0A0M0P3Z8	–	–	–	–	–
A0A0U5AYD8	–	–	–	–	–
A0A2W6UQ55	–	–	–	–	–
A0A0R0ARZ9	–	–	–	–	–
A0A4Z0DXN9	–	–	–	–	–
A0A149QFI1	–	–	–	–	–
A0A2J0SNK9	–	–	–	–	–
A0A0R0CUE4	–	–	–	–	–
A0A5K1NT45	–	–	–	–	–
A0A4Z0E2X8	–	–	–	–	–
A0A4Z0E2D4	–	–	–	–	–
A0A0R0E0H4	–	–	–	–	–
A0A0R0BRS4	–	–	–	–	–
A0A2W6HCD0	–	–	–	–	–
A0A3S5GHZ1	–	–	–	–	–

respectively) represented Class Betaprotebacteria and Gammaproteobacteria. The bacterial species' nucleotide sequences were submitted to NCBI (Accession no. s - ON 524799 and ON 52498). The Gen Bank Accession no. ON524798 represented the *Stenotrophomonas* sp. strain jpm20 16S ribosomal RNA gene, partial sequence of 948bp. Similarly, the second isolate sequence submitted was given Gen Bank Accession no. ON 524799 represented as *Alcaligenes* sp. strain Sloli A09 16S ribosomal RNA gene partial sequence (Fig.1). Earlier studies on squid indicated the presence of *Photobacterium* sps. a bioluminescent bacterium [24]. Of the above two species *Stenotrophomonas* has been identified as a bioluminescent bacterium which produced a florescent protein in the medium during culturing.

Previous studies on laboratory maintained aquarium cuttlefish *(Sepia officinalis)* at Marine Biological Laboratory, Woods Hole indicated the presence of symbiotic bacteria in the gut of these animals. The simple microbiome of two bacteria amplicon variants (ASVs) represented the entire digestive tract [18] and belonged to *Vibrionaceae and Piscirickettsiaceae* of Gammaproteobacteria. During the current study, 4 isolates represented the entire digestive tract of cuttlefish were identified by the 16S r RNA amplicon based BLAST similarity (>83%) search revealed bacteria belonged to Flavobacteria, Gammaproteobacteria, and Betaproteobacteria classes of Proteobacteria. The 16s R RNA genes of bacterial variants indicated a BLAST similarity of variant 1 with *Myroides odoratimimus* strain pgdne3 (93.09 %); Variant 2 with *Pseudomonas putida* strain BPA1 (92.61%) ; variant 3 with >85% to an Uncultured bacterium clone Shelves_B_90 and, variant 4 showed with *Pseudomonas viridiflava* strain CE9 (88.35 %). The representative 16S r RNA gene sequences of the variants were submitted to Gen Bank. The Gene bank accession numbers ON 838558, ON838559, ON 838560 and ON624237 were assigned partial 16s RNA genes of to *Pseudomonas fluorescens* (664 bp); *Burkholderiales bacterium* (788 bp); *Syntrophobacterales bacterium* (217bp) and, *Pseudomonas* sp. strain ansj 3 (707bp).

Extraction and identification of proteins

The Liquid Chromatography-Mass Spectrometry (LC-MS) analysis indicated a spectrum of total 240 proteins isolated by protein analysis (Table-1). The heat map indicated the accession number of proteins extracted, number of peptides, overall molecular weight and number of amino acid present. Of which overall molecular weight and number of amino acid present showed variations. Among these, 29 unidentified proteins were further identified and analyzed using the UNIPROT and PDB Data bases and BLAST.

The study indicated few of the unidentified proteins represented Type IV secretion systems' macromolecules. The proteins of T4SS are characterized as type IV secretion protein which can be utilized for inter-bacterial killing. Earlier studies of *Stenotrophomonas* sps expressed proficiency in killing competitor bacterial species especially *Escherichia coli, Klebsiella pneumonia, Salmonella typhii and Pseudomonas aeruginosa* (gram –ve)[25]. The invasive proteins (A0A4Z0DSX7), a metallic protein similar to that of an immunity protein (PDB id.6PDK) along with type IV secretion system protein virB5 Extraction and identification of proteins (A0A0U5I2Y5) were some of the proteins encountered based on the LC_MS based analysis. The VirB2 and VirB5 proteins mediate host-cell targeting. They behave like adhesins and, initiate target binding to specific host receptors [26]. The above reason can be a possible reasonable explanation for the unique representation of only 2 species of bacteria in the squid digestive tract.

Research analysis of gut microbiota of six species of wild cephalopods by Illumina Mi Seq sequencing of 16S r RNA gene amplicons indicated that each cephalopod gut consisted of a distinct consortium of microbes. The gut microbial composition reflects host phylogeny[15,16]. Previous studies indicated that healthy octopuses and squids are associated mainly with *Vibrio* sps followed by members of other genera such as *Pseudomonas, Aeromonas, Staphylococcus* or *Streptococcus sps*. [27]. The presence of *Stenotrophomonas* sp., *Alcaligenes* sps, *Pseudomonas* sps, and *Myroides* sps require detailed study to assess their role as opportunistic pathogens that can affect human life or probable probiotics, or in bioremediation, production of antibiotics or other industrial uses[28,29].

The presence of florescent bacteria offers protection and of symbiotic relation which can be seen in benthic organisms such as squid and cuttlefish. The current study emphasized the observation of 2 species in squids and 4 species in cuttlefish could be due to their phylogenetic characteristics. A careful processing of these cephalopods is necessary as the external microbial contamination can be removed as well as, the internal microbial population can be source of food spoilage as well as food poisoning.

CONCLUSION

The comparative study of composition of gut microbes from squid and cuttlefish confirmed that the composition of microbes was different in both the cephalopod species of benthic habitat. The distinct change supports that the pattern follows the phylogeny. The secreted proteins that are associated with *Stenotrophomonas* sps. control the other selected bacterial populations indicating the possible reason for the low representation of other species. These macromolecules that express bactericidal activity can be further used in the drug development studies as well. Since the squids are delicacy, the qualitative and quantitative bacterial load should be assessed and, warrants for proper treatment process prior to their use as food.

ACKNOWLEDGEMENT

The authors thank SVKM Management and I/C Principal Dr. Krutika Desai for the facilities provided. A special mention in appreciation of extended technical co-operation from Credora Life Sciences and SAIF of IIT Mumbai during the study.

Conflict of Interest

There is no conflict of interest.

Funding Source

There are no funding sources.

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