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# Phylogenetic diversity and predictive functional profile of bacteria associated with marine microalgae, *Isochrysis galbana* using next generation sequencing

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In aquaculture, microalgal-bacterial interaction has ecological significance, and thus demands better understanding for improvement of sustainability and productivity of large scale microalgal cultivation. Here, we assessed the bacterial diversity, including the uncultivable bacterial assemblage associated with the marine microalgae *Isochrysis galbana* using next generation sequencing approach. *Isochrysis* has been considered as one of the most favoured types of live feed in aquaculture and hence, chose *Isochrysis galbana* MBTDCMFRI S002. Total genomic DNA was extracted from *I. galbana* culture and 16S rDNA V3 region was sequenced with an Illumina MiSeq platform. A total of 30 different known bacterial genera were detected from 1190 identified operational taxonomic units. These bacterial phylotypes were affiliated to *Alphaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria*, *Actinobacteria*, *Acidimicrobiia*, *Bacteroidia*, *Flavobacteriia* and *Bacilli* classes. These 30 bacterial genera comprise only 4.62% of the total OTUs obtained and remaining 95.38% of the sequences do not exhibit any similarity against known bacterial genera in the taxonomic database. The functional profile of bacterial communities was predicted using PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) analysis. The results indicated that these associated bacterial communities are mainly involved in environmental as well as genetic information processing, membrane transport and nutrient metabolism. These functions may mediate their interaction with the phytoplankton host, and thus improve bacterial survival in microalgal habitat. Overall, the present study enhances the understanding of microalgal-bacterial interaction in terms of diversity and functional role of associated microbial community.

**Keywords**: 16S rDNA, Microalgal-bacterial interaction, Phycosphere bacteria, PICRUSt, Symbiosis

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Bacterial association plays a significant role in evolution and diversity of phytoplankton host[1]. It was reported that non-axenic cultures of microalgae harbours diverse groups of bacteria belonging to the classes *Gammaproteobacteria*, *Betaproteobacteria*, *Alphaproteobacteria* and *Bacilli*[2]. In addition, several uncultivated bacteria are noted in the phycosphere of microalgae[3]. The phycosphere associated bacteria could improve algal growth and metabolism by producing microalgal growth promoting factors such as siderophores, indole-3-acetic acid and vitamins[4]. Hence, it has been well-understood that algal-bacterial interaction could be explored to improve the quality and yield of phytoplankton host during their cultivation process[5]. Further, our previous studies highlight the aquaculture and biotechnological applications of phycosphere associated bacteria[6,7].

For a detailed investigation of microalgal-bacterial interaction, the first step is to study the bacterial diversity in microalgal habitat[8]. Microbial interaction studies are often hampered by difficulties in cultivating bacterial symbionts[1]. Thus, metagenomic technologies have become powerful tools for investigating interactions of microorganisms with their environment and host[3]. Traditional metagenomic sequencing was carried out using labour intensive techniques which include cloning, colony picking, plasmid extraction and sequencing, and, consequently, most studies analysed fewer than a hundred clones per sample[9]. Hence, only a minor fraction of microbial diversity was unravelled[10]. The recent advent of next generation sequencing has tremendously simplified routine metagenomic procedures[9]. These studies provided a more comprehensive exploration of bacterial communities in diverse ecosystems and revealed existence of several taxa not identified from former less sensitive approaches[10]. However, very few studies have focused on metagenomes of algal microhabitat[3,11,12]. The brown-golden marine microalga *Isochrysis* is an important phytoplankton that makes excellent live feed in aquaculture. In addition to high nutrient content, they have appropriate cell size and good survival rate during mass cultivation[13]. Hence, we selected *Isochrysis galbana* as a representative to better define microbial communities inherent to microalgae using next generation sequencing technologies. Additionally,

PICRUSt analysis was used to predict functional profile of associated microflora.

## Materials and Methods

*I. galbana* culture MBTDCMFRI S002 (GenBank Accession No. JF708124) maintained at Microalgae culture collection of Marine Biotechnology Division, ICAR-Central Marine Fisheries Research Institute (CMFRI), Cochin (Kerala, India) was used for this study. The total genomic DNA was extracted from 10 mL liquid culture of *I. galbana* at late growth phase following modified phenol-chloroform enzymatic extraction method[14,15]. Sequencing and analysis of V3 region of 16S rDNA amplicon was performed with an Illumina MiSeq platform at AgriGenome Labs Private Limited, Cochin. Clustal O program was used to construct a consensus V3 region sequence after removing spacer and conserved regions from original paired-end data. After removing chimeras (UCHIME), reads from all samples were pooled and clustered into Operational Taxonomic Units (OTUs) (similarity cut off = 0.97; Uclust program)[16,17]. Entire downstream analysis was carried out using QIIME program[18]. The representative sequence for each OTU was aligned against Greengenes core set of sequences (PyNAST) and reference chimeric data sets[19,20]. Finally, classification was performed against SILVA OTUs database using RDP classifier. Sequence data was deposited in the Sequence Read Archive of NCBI GenBank (Accession No. SRR6740228). Functional profiles of bacterial communities were predicted using PICRUSt analysis.

## Results and Discussion

A total of 582020 sequence reads were obtained, and after filtering 490869 reads were retained. From 490869 reads 3084 OTUs were identified. From identified OTUs, further analysis was carried out using 1190 OTUs (1894 singletons). It was noted that 95.38 % of the sequences do not exhibit any similarity against known bacterial genera in the taxonomic database and were categorized as unknown. A total of 30 different known bacterial genera affiliated to *Alphaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria*, *Actinobacteria*, *Acidimicrobiia*, *Bacteroidia*, *Flavobacteriia* and *Bacilli* classes were detected (Table 1). Interestingly, these 30 bacterial genera comprise only 4.62% of the total OTUs

Table 1 — Phylogenetic diversity of bacteria associated with *Isochrysis galbana*

| Phylum | Class | Order | Family | Genus |
|---|---|---|---|---|
| *Proteobacteria* | *Alphaproteobacteria* | *Rhodospirillales* | *Rhodospirillaceae* | *Reyranella* |
| | | | | *Fodinicurvata* |
| | | *Rhodobacterales* | *Rhodobacteraceae* | *Tropicimonas* |
| | | | | *Ruegeria* |
| | | | | *Labrenzia* |
| | | | | *Roseobacter* |
| | | *Caulobacterales* | *Hyphomonadaceae* | *Oceanicaulis* |
| | | | | *Maricaulis* |
| | | | *Caulobacteraceae* | *Brevundimonas* |
| | | *Rhizobiales* | *Methylobacteriaceae* | *Methylobacterium* |
| | | | *Rhizobiaceae* | *Rhizobium* |
| | | | *Phyllobacteriaceae* | *Nitratireductor* |
| | | *Sphingomonadales* | *Sphingomonadaceae* | *Sphingomonas* |
| | *Gammaproteobacteria* | *Alteromonadales* | *Alteromonadaceae* | *Alteromonas* |
| | | | | *Marinobacter* |
| | | | *Idiomarinaceae* | *Idiomarina* |
| | | *Oceanospirillales* | *Alcanivoracaceae* | *Alcanivorax* |
| | | | *Oceanospirillaceae* | *Pseudospirillum* |
| | | | *Halomonadaceae* | *Salinicola* |
| | | *Enterobacteriales* | *Enterobacteriaceae* | *Escherichia* |
| | | | | *Klebsiella* |
| | | *Pseudomonadales* | *Moraxellaceae* | *Psychrobacter* |
| | *Deltaproteobacteria* | *Myxococcales* | *Sandaracinaceae* | *Sandaracinus* |
| *Actinobacteria* | *Actinobacteria* | *Actinomycetales* | *Corynebacteriaceae* | *Corynebacterium* |
| | | | *Propionibacteriaceae* | *Propionibacterium* |
| | | *Micrococcales* | *Micrococcaceae* | *Rothia* |
| | *Acidimicrobiia* | *Acidimicrobiales* | *Acidimicrobiaceae* | *Ilumatobacter* |
| *Bacteroidetes* | *Bacteroidia* | *Bacteroidales* | *Bacteroidaceae* | *Bacteroides* |
| | *Flavobacteriia* | *Flavobacteriales* | *Flavobacteriaceae* | *Muricauda* |
| *Firmicutes* | *Bacilli* | *Lactobacillales* | *Streptococcaceae* | *Streptococcus* |

obtained. The percentage OTUs of identified bacterial genera was depicted in Fig. 1. Further, the microbial diversity was analysed by calculating Shannon, Chao1 and observed species metrics. The rarefaction curve for each of the metric is provided in Fig. 2. The chao1 metric estimates the species richness while Shannon metric is the measure to estimate observed OTU abundances, and accounts for both richness and evenness. The observed species metric is the count of unique OTUs identified in the sample. Functional role and metabolic capability of bacterial flora associated with *I. galbana* has been predicted by PICRUSt analysis (Suppl. Table S1. *All supplementary data are available only online along with the respective paper at NOPR repository at http://nopr.res.in*).

It is widely accepted that more than 99% of the microorganisms present in many habitats are not readily culturable. Hence, in order to get extensive information about complex microbial communities present in any environment, culture independent approaches are indispensable[21]. In our previous study, only two bacterial genera; *Alteromonas* and *Labrenzia* were obtained from selected microalgal culture by culture dependent method[22] whereas the next generation sequencing approach, as described in this study, has revealed the occurrence of several other bacterial genera in the same microalgal habitat. In addition to these known bacterial groups, numerous unknown bacterial groups were also associated with *I. galbana.* Thus, the obtained results clearly specify the existence of diverse groups of cultivable and uncultivable bacteria in the phycosphere of *I. galbana*. Most of the bacterial groups identified in this study have previously been found in association with microalgae. It was reported the consistent association of bacteria belonging to *Alphaproteobacteria* and *Gammaproteobacteria* with different groups of microalgae[23]. Similarly, bacterial genera such as *Marinobacter*, *Alteromonas*, *Labrenzia* and *Sphingomonas* were formerly identified as algal associates[22-26].

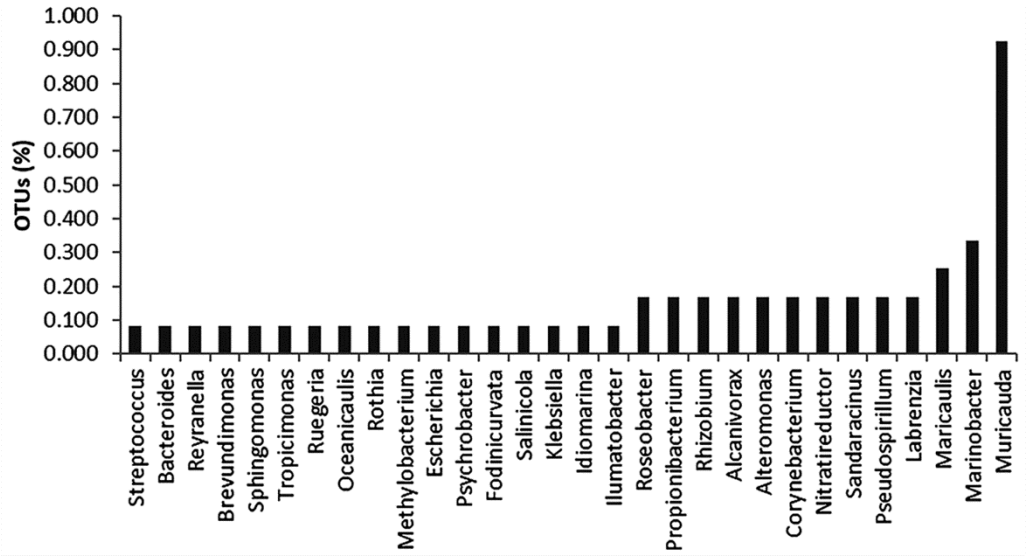


Fig. 1 — The percentage OTUs of identified bacterial genera

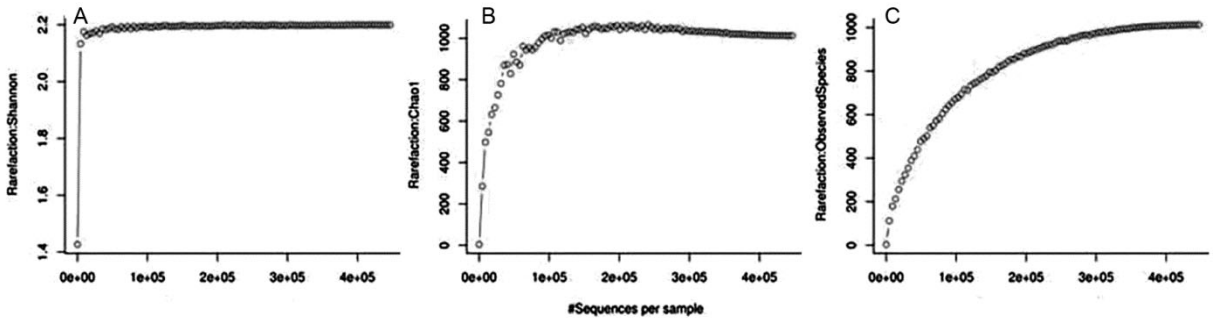


Fig. 2 — The rarefaction curve for (A) Shannon metric; (B) Chao1 metric; and (C) observed species metric

The major functional modules identified by the analysis include environmental information processing, membrane transport, nutrient metabolism and genetic information processing. These functions might have a significant role in microbial interaction with phytoplankton host. It was found that most number of genes fall into environmental and genetic information processing which reflects the expected abundance of these functions in nature[1]. Many genes that function in nutrient metabolism (carbohydrate, amino acids and nucleotides), membrane transport (ABC transporters) and signal transduction were also identified. Improved bacterial survival in any habitat can be facilitated by nutrient uptake and metabolism[27]. Similarly, signalling between microalgae and bacteria likely a precursor for specific interactions and might enhance bacterial fitness in their phycosphere[23]. In addition, the presence of extensive energy acquiring mechanism including photosynthesis was also detected in the algal microhabitat. It was noticed that these associated bacteria were able to degrade xenobiotic compounds. These results signify the possibilities of exploring microalgal associated bacteria for gene mining and bioprospecting.

## Conclusion

The present study reports metagenomes associated with marine microalgae, *Isochrysis galbana*. It has demonstrated that phycosphere of *I. galbana* alone harbours a great diversity of microorganisms. By characterising functional profile of associated bacteria, several functional modules with potential positive effects on bacterial interaction with *I. galbana* were identified. Thus, next generation sequence based metagenome analyses in combination with function-based studies significantly enhance our understanding of biodiversity and genetic potential of microbial communities present in microalgal habitat. In summary, the current study gives detailed insight into algal-bacterial interaction in terms of diversity and functional profile of associated bacteria which can be further utilised for various applications.

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## Conflict of Interest

Authors declare no competing interests.

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