Use of Metagenomics as an Approach to Relations between Algae and Bacteria: A Review

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Abstract In this review, we present many different classes of ecological relationships in which are registered interactions among algal and bacterial organisms. The uses of molecular techniques as metagenomics to test hypothesis, evaluate subjacent processes and searching for patterns in microbial communities are included and discussed. Our documental research suggests that great advances have been accomplished in this discipline until now, and many of these advances were through metagenomics application. Nevertheless, some questions and aspects of relations algae-bacteria remain not answered yet and metagenomics could be a power and useful working tool.

Index terms: alga, bacteria, relationships, interactions, metagenomics.

I.   INTRODUCTION

Bacteria are a huge group of ubiquitous organisms with a variety of metabolic pathways and diverse behavioral and reproductive adaptations for surviving in different environments[1]. On the other hand, unicellular or pluricellular algae are a complex group of photosynthetic organisms that have exhibited several applications on diverse fields for their efficiency as bioindicators, remediation of natural resources and producers of substances for energetic purposes[2,3]. These organisms can establish many kinds of interactions that have been proved to be useful and beneficial for many aspects of human life.

Nowadays, the understanding of different ecological relationships between algal and bacterial organisms are included in a wide study field, because of the benefits that can be applied from the deep knowledge that can be reached. While algae there are well known, bacteria are so far from this reality because of their high genetic diversity and mutation rates, that determines their speciation rates and quick adaptation to adverse environments and conditions [4]. In this sense, we are far from have good estimations of the number of species of bacterial organisms. Nevertheless, several molecular techniques have been developed to accomplish this matter.

Metagenomics has been proved to be a powerful and useful technique for identifying groups of bacterial taxa and their abundance as necessary tools for richness and diversity estimations of these organisms in experimental or natural communities. This method could be used as a tool; not only for biodiversity estimations purposes or community ecology studies, as well for detecting interactions between species of bacteria helping to identify what taxa of bacterial could enhance and bring more efficiency to optimize processes as wastewater management [5].

To conduct this review, we started from basic concepts of ecological relationships between organisms to aboard the aspects of the algal-bacterial interactions. Then, we define metagenomics and mentioned examples of the use of the technique in problems of algae-bacteria relations reported on specialized literature in order to compile and analyze the trends in the use of this technique for resolution on several problems in diverse environments and organisms as models of study and different scales.

II.   ECOLOGICAL RELATIONS

Species are not living in isolation. The different ecological relations between them are the base of ecosystems. Ecological relationships describe the interactions between and among organisms within their environment. These interactions may have positive, negative, or neutral effects on species and their fitnesses[6] Different types of interspecific interactions have different effects on the

organism involved, which may be neutral, positive, or negative. Sorting this, derived six major types of ecological interactions: mutualism, competition, parasitism, commensalism, amensalism and neutralism. All these relations are present in bacteria species [7].

One of the remarkable examples of of symbiosis in which bacteria are involved are legumes, who perform symbiosis that most other plants do not, including *Arabidopsis* [8]. Legumes are considered as important source of protein for animals and for human populations. Nitrogen-fixing soil bacteria from genus *Rhizobium* colonized their roots and with mycorrhizal fungi that contribute to the acquisition of phosphorus. The development of the root nodule meristem is unique, as it is possible to define your site, the time of initiation, the type of target cell and ontogeny [8].

   a.   Ecological relations between algae and bacteria

Bacteria are crucial to ecosystem functioning, and play vital roles in carbon, nitrogen, and sulfur cycles. The interaction between two microbes is the fundamental unit of microbial interactions, in combination with ecological interactions previously mentioned, traditionally considered by the investigation efforts on microbial interactions understanding [7]., and this interactions networks between species are the result influence of abiotic and biotic factors [8].

In different ecosystems, microorganisms form heterogeneous communities, different proportions and various entities of microorganisms (Archaea, Eukarya domains and kingdom Protista), collectively known as "microbiome" [9]. Microorganisms in a microbiome do not live in isolation, but actively interact with other members within their community. Taken together, these interactions are a description of how microbial community works generally, and what role each individual play within the community. As such, the characterization of microbial interactions is a key step towards understanding community organization, which may be using metagenomic and the engineering of microbial communities for biomedical and industrial applications[7].

III.   USE OF METAGENOMICS IN EXPLORATION OF ALGAE-BACTERIA INTERACTIONS

   a.   Metagenomics

The objective of metagenomic studies consist doing an evaluation of the coding potential of environmental organisms, quantifying the relative abundances of (known) species, and estimating the amount of unknown sequence information (environmental sequences) for which no species, or only distant relatives, have yet been described [6]. . Metagenomics can be defined as the genomic analysis of microbial DNA from environment communities. This hypothesis is proposed by many authors as: *"Genomic DNA sequence, and even complete genomes in some cases, has been generated from organisms that exist only in tight association with other organisms, including various obligate symbionts and pathogens, members of natural microbial consortia and an extinct cave bear."* [7].

This has been considered a specific, sensitive, quantitative, and high-throughput tool for microbial detection, identification, and characterization in natural environments. Metagenomics is an emerging technology to study microbial communities, as well is a powerful tool for determinate thousands of genes simultaneously in a single experiment. Sequencing of the entire genome (metagenomic) using the *"shotgun"* technique, approach that employs cloning and paired-end sequencing of plasmid libraries. Until now many projects based on these methodologies include data sets from an acid mine biofilm, seawater samples, deep-sea sediment, or soil and whale falls [6].

Microbial communities have been characterized in natural and engineered ecosystems using a variety of molecular methods, the results obtained with these methods can be incomplete as they do not capture the whole complexity of microbial communities [8]. This methods were the following, ribosomal spacer analysis (RISA), terminal restriction fragment length polymorphism (t-RFLP), denaturing gradient gel electrophoresis (DGGE), 16S rRNA clone libraries, and fluorescence in situ hybridization (FISH) were applied to evaluate bacterial community structure not only in biological reactors from wastewater treatment plants but also in other ecosystems such as edaphic, marine and atmospheric microbial systems. [8].

Gophna *et al.* (2016) worked with *Oscilospira* an under-studied anaerobic bacterial genus of Clostridial, that cannot isolate in cultures, they signalized that in recent years used of study with 16S rRNA gene was used for interesting traits about these bacteria. For understand the metabolism and physiology, they used nearly complete genomes derived from shot-gun metagenomic data from their natural environment, the human gut, to analyze *Oscilospira* and related bacteria [9]

Recent metagenomic studies exposed that Antibiotic Resistance Genes (ARGs) predominantly cluster by ecology. Implying that the resistome in soils, and wastewater treatment plant differ significantly from that of human pathogens [10,11] that is one of the important points of metagenomics studies. ARGs are in many causes associated with conjugative elements such as plasmids or transposons. While the transfers of these elements may also occur through transformation or transduction, conjugation is often considerate as the

most likely responsible mechanism [12]. With all the increase in environmental levels of antibiotics, driven by medical and agricultural demand, is unprecedented and has disrupted the natural balance between microbes and antimicrobials [12]. That can include bacterial community in reactors.

Xia *et al.* (2010) explored the microbial community composition of five biological wastewater treatment reactors in China and the United States applying high-density microarrays targeting universal 16S rRNA genes. This study got as a result a consistent composition of microbial community structure among all five reactors. They took in account interactions between the organisms in the microbiome as a description of the overall function of the microbial community. As such, the characterization of microbial interactions is a key step towards the understanding of the community organization and the engineering of microbial communities for biomedical and industrial applications [13].

Exists alternative approach for doing a taxonomic profiling of complex communities of bacterial using a set of protein-coding marker genes, extracted from large-scale environmental shotgun sequencing data, to provide a more direct, quantitative, and accurate picture of community composition than that provided by traditional ribosomal RNA–based approaches depending on the polymerase chain reaction [14]. This is an alternative for the commonly microarrays targeting universal 16S rRNA genes.

Lima-Mendez *et al.* (2015), presented part of the "Tara Oceans" project. In this work, they used environmental factors and organism abundancy profiles for study the photic zone interactome. They found that environmental factors could predict the community structure but, in an incomplete way. Also, they found that local and global patterns had an influence on the non-randomly distribution of the functional types and phylogenetical groups present at as compounds of plankton. Finally, they identified interactions among grazers, primary producers, viruses, and (mainly parasitic) symbionts and validated network generated hypotheses using microscopy to confirm symbiotic relationships, providing a resource to support further research on ocean food webs and integrating biological components into ocean models [3].

For the evaluation of predicted interactions, they realized a co-occurrence technique have heretofore mainly been applied to bacteria (Figure 1). They detected eukaryotic interactions based on analysis of sequences at the V9 hypervariable region of the 18S ribosomal RNA (rRNA) gene. They built a literature curated collection of 574 known symbiotic interactions (including both parasitism and mutualism) in marine eukaryotic plankton. Prokaryotic 16S rDNA metagenomic reads were identified, annotated, and quantified (Figure 2) [3]. With Spearman and Kullback-Leibler dissimilarity measures, they constructed co-ocurrence networks, from inferred networks trough taxon-taxon.
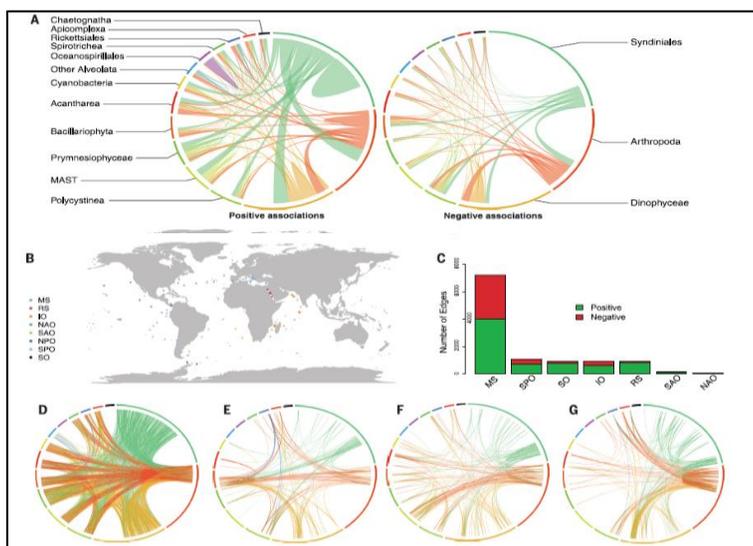

Figure 1: Taxonomic and geographic patterns within the co-occurrence network presented by: [3]. (A) Top 15 interacting taxon groups depicted as colored segments in a CIRCOS plot (B) Tara Oceans sampling stations grouped by oceanic provinces. (C) Frequency of local co-occurrence patterns across the oceanic provinces, showing that most local patterns are located in MS. (D to G) Taxonomic patterns of cooccurrences across MS (D), SPO (E), IO (F), and RS (G). Deeper descriptions of these figures are contained in [3].
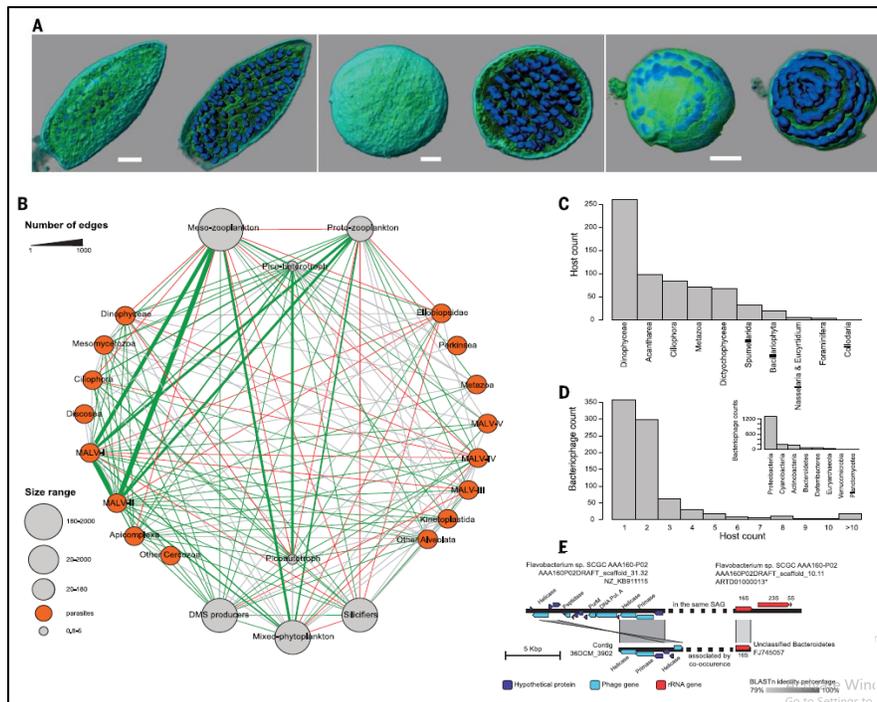
Figure 2: Top-down interactions in plankton obtained and described by [3]. . (A) Three different dinoflagellate specimens from Tara samples display an advanced infectious stage by syndiniales parasites. (B) Subnetwork of metanodes that encapsulate barcodes affiliated to parasites or PFTs. (C) Parasite connections within micro- and zooplankton groups. (D) Number of hosts per phage. (Inset) Phage associations to bacterial (target) phyla. (E) Putative Bacteroidetes viruses detected with co-occurence and detection in a single-cell genome (SAG). More detailed explanations of the charts are included in [3]

Dagan *et al.* (2013) reported the genome sequences for the morphologically most complex true-branching Cyanobacteria, and for *Scytonema hofmanni* PCC 7110, which with 12,356 proteins is the most gene-rich prokaryote currently known. They investigated components of cyanobacterial evolution that have been vertically inherited, horizontally transferred, and donated to eukaryotes at plastid origin. The vertical component showed a freshwater origin for water-splitting photosynthesis. Networks of the horizontal component reveal that 60% of cyanobacterial gene families have been affected by lateral gene transfer. Plant nuclear genes acquired from cyanobacteria define a lower bound frequency of 611 multigene families that, in turn, specify diazotrophic cyanobacterial lineages as having a gene collection most like that possessed by the plastid ancestor. To identify plant nuclear genes of cyanobacterial origin, they reconstructed 35,862 phylogenetic trees containing both eukaryotic and prokaryotic homologs and looked for trees in which plants and cyanobacteria branch together. Also, they focus they attention to the larger set of nuclear genes of cyanobacterial origin whose homologs are not universally distributed among cyanobacteria. For 611 plant nuclear gene families identified as plastid acquisitions, scored gene presence and absence, and protein sequence identity among cyanobacterial genomes (Figure 3).

Today plastids supply fixed carbon to plant cells, but they also have a myriad of other functions in amino acid, lipid, and cofactor biosynthesis as well as nitrogen metabolism. They think that the crucial reasoning on the selective advantage, was to the establishment of the plastid has it that the production of carbohydrates by the cyanobacterial endosymbiont was the key. An alternative suggestion is that the initial advantage of plastids may have simply been their uniquely useful metabolic product, O_2, as a boost to respiration in early mitochondria [15].
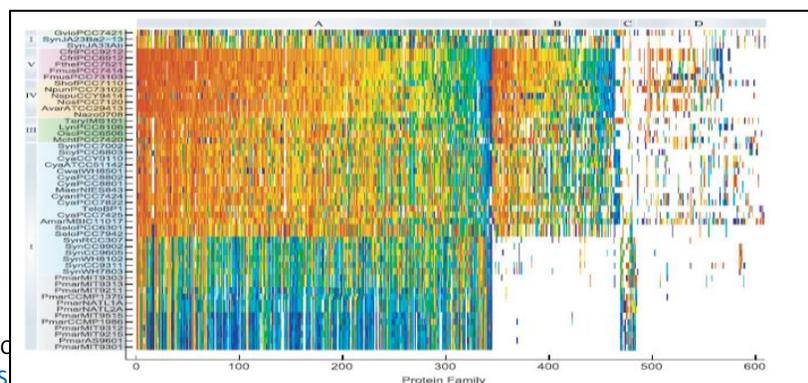
Figure 3: Presence/absence and sequence similarity patterns of cyanobacterial protein families by comparison with their homologs of endosymbiotic origin in six photosynthetic eukaryotes by [15] "Amino acid sequence similarity between the cyanobacterial proteins (x axis) and their counterparts in the eukaryotic plastid-derived set of protein families (y axis), as deduced for the genomes in the data set. Cell shades in the matrix correspond to the similarity ranking for each protein family (i.e., line) according to a color gradient from red (high similarity) to blue (low similarity). White cells correspond to genes lacking in the respective genomes. Protein families are ordered according to their distribution pattern into (A) nearly universal, (B) sparse representation or (C) highly frequent in the oceanic species, and (D) generally sparse representation.

IV.   CONCLUSION

The microbial world is a fascinating world, so far, we need to go deeper and get to know the microbiomes in depth. Considerable progress has been made in recent years, the studies of the different ecological relationships, those relationships that are the basis of ecosystems. Great advances have been made, one of the great key points for the study of communities of microorganism and their relationships has been the metagenomic, this technological advance has allowed to deepen the knowledge about the ecological relationship between species of the microbial world. Much work remains to be realized, there is still a long way to go until the moment to know what the role of each species is and how these are related to each other, in addition to their role within the community and the niche to which they belong to in addition to their ecological role in nature.

Also, the metagenomic studies can be the basis for other studies such as proteomics, based on the genetic sequences, we can continue to advance to depth and discover the behavior and the different interactions between different organisms.

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