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Advances in Sustainable Bioprospecting Methods

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Advances in Sustainable Bioprospecting Methods

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Bioprospecting Marine Microorganisms

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Chapter - 3

Bioprospecting Marine Microorganisms

Dr. Ayona Jayadev

Abstract

Nature is the source of everything required for the sustenance and maintenance of life on earth. It provides organisms with space, food and all other resources. Being a system, the components work in a neatly orchestrated way providing and exchanging the conditions and materials needed by each of them. The success of life is assured by this exchange. Human beings ever since originated on earth have been using the products as well as processes provided by other forms of life first unknowingly and later knowingly. The initial examples include making wine, dough etc. where microbial processes were taking place. The wastes formed on the earth's surface were also efficiently managed by microbes in soil and water. This natural environmental remediation became a problem only when the quantity and rate formation of wastes became so much greater than the waste getting reduced by microbes. Realizing the potential of living organisms, the concept of bioprospecting emerged in which the natural products of organisms such as primary and secondary metabolites and the processes they possess were used for various purposes. After an interim bloom in chemical products which resulted from industrial revolution, the problems created by these were also understood and humans started to resort to natural products with similar capacity and potential to replace chemicals. This is seen in every walk of life and almost all products used to range from products such as cosmetics, pharmaceuticals, enzymes etc. to processes such as remediation of wastes and recalcitrant.

Keywords: Bioprospecting, marine microbes, novel microbes, relic DNA, metagenomics

Introduction

Microorganisms are one among the early originated life forms. They are cosmopolitan in their distribution ranging from normal to extreme environment and outside to inside of other living organisms. Microbes come in categories of bacteria, fungi, actinomycetes, viruses etc. These are

prokaryotic. There are eukaryotic microbes also, examples of which are yeasts, phytoplankton, zooplankton etc. Prokaryotic microbes are extremely potent organisms in various aspects due to the fact that they have a highly plastic genome which could be evolved in short duration of time. Though their morphological diversity is restricted, they have diverse activities from being pathogenic to almost all forms of life to production of bioactive products including pharmacological and pharmaceutical products, antibiotics, cosmetic products, remediation of environmental pollution such as degradation of organic wastes, recovery of heavy metals and enhancing the properties of environment such as soil fertility. They play a role in the normal environmental processes such as involving and regulating biogeochemical cycles and cycling of energy in ecosystems. Mostly microbes form the basic functional unit of most of the habitats.

The capacities of microbes are unlimited. Though we can culture so many diverse microbes from environmental samples, it is now understood that most of the organisms cannot be cultured even providing diverse growth requirements and conditions. There are some basic growth conditions needed for growth of these organisms and for the metabolic functions. The amount and type vary widely according to the type of organism. The capacity for using unique nutrients and growth conditions make microbes potent for most of the sought-after activities. Microorganisms are now increasing used for novel products and processes which are of industrial, academic and environmental importance. The metabolic capabilities vary according to the environmental conditions in which they are seen. The process of identifying and using living organisms to get useful products or processes is called bioprospecting, (Raimi and Adeleke, 2021).

Bioprospecting

Bioprospecting is the systematic exploration of metabolic products as well as unique processes that can be developed into commercially viable products and processes for the betterment of human life. This process can be used for identifying genes, compounds, replicable biological processes for betterment of human life and living conditions without disturbing nature, (Mateo *et al*., 2001). Biological diversity is an expression of chemical diversity of the organisms. From a recent past, bioprospecting has served as source of many important bioactive molecules. But there are also arguments that the chemical diversity does not scale with biological diversity, posing this as a reason for the stagnation in the discovery of new bioactive (Ramesha *et al*., 2011). This decelerated pace is also due to rediscoveries of products and increased cost of screening, optimization and characterization. The success in

bioprospecting usually require so many steps to reach a product which can be commercialized. This requires pumping of economy to reach the goal. Hence most of the companies and industries have resorted to combinatorial chemistry where some small basic chemical structures are subjected to combinatorics and it serves as source to never ending novel chemical structures which can be directly screened, bypassing the testing, screening, isolating and purification in bioprospecting process. Hence, Ramesha *et al*., (2011) opines an intelligent and non-random approach of in the future to accelerate the pace of prospecting. And combinatorial chemistry needs to be applied after bioprospecting to supplement it rather than superseding it as chemical synthesis and derivatization has high environmental implications, (Grabley *et al*., 1998).

It is estimated that more than 50% of products belonging to drugs and pharmaceuticals are from living organisms or inspired by them, (Zotchev *et al*., 2012). The products that are of importance to industries including pharmaceutical industries are the secondary metabolites of organisms. These products include antibiotics, anti-cancer and anti-tumor products, vitamins, organic acids, enzymes, antioxidants, neuroprotective compounds, immune suppressive agents etc., (Ab Mutalib *et al*., 2020). The property of microbes in the environment to exchange their genetic materials between them has resulted in the duplication of gene structures restricting the options to get diverse genes with diverse functionalities. This has necessitated researchers to ponder unique environments with peculiar physical and chemical properties to prospect for novel genes and genetic properties with a view that the uniqueness in the environment will change the organism's genome to suit themselves for living there. This will bring out new products and capacities which can be mined out for industrial uses. Thus, unique habitats will accelerate discovery of novel metabolites, (Lazzarini *et al*., 2000).

Most of the secondary metabolites in the microorganisms are found to be the products of extrachromosomal DNA, the plasmids. These are the genetic systems which help these organisms to withstand stress of the harsh environments. Along with having capacities to produce products useful to humans, they also impart drug resistance to the organism. This capacity of microbes has resulted in a havoc to the treatment systems. As plasmids are transferred between microbes in their natural environment through vertical and horizontal gene transfer, there is a spread in drug resistance and the development of multi drug resistance (MDR) in microbes, (Li *et al*., 2019).

Microorganisms in endophytic association is also an area of interest in bioprospecting. Endophytes in the process of their successful establishment

inside plants produces an array of products which has the potential to be used as medicines or products of industrial importance such as antibiotics, antimycotics, immunosuppressants, anthelminthics etc., (Strobel et al., 2003). The emergence of viral diseases has necessitated the pharmaceutical industry to come up with anti-viral bioactive principles which bacteria are known to possess to combat with lysis by bacteriophages. Since endophytes are seen inside plant body, there is a lot of potential in carrying out bioprospecting in them.

Marine environment

Marine environment is a complex, dynamic and multifactorial system, (Sagarin *et al*., 2016). It is also the largest ecosystem of the world and which make the planer blue. Marine systems support diverse assemblage of life. Though marine environment may look fixed as far as their geographic location is concerned, their habitats are not static in special and temporal basis. It moves with surface currents and water masses. The physical as well as chemical conditions also varies in these environments (Würsig *et al*., 2009). The life as well as other resources will be formed as per the combined effect of climate, biochemical and physical conditions. Though marine organisms represent only 13% of organisms already known, the actual composition and content will be rather huge. Compared to land or inland water ecosystems, marine ecosystems present real challenges to the organisms inhabiting due to the unique nature of it. Ocean represents dynamics of atmospheric systems, temperature differences, water currents etc in a magnitude never experienced by terrestrial systems or inland water bodies, (Steele *et al*., 2019).

Ocean, which is the largest ecosystems of the earth provide wide space for inhabitation to a large number and diversity of marine organisms. This includes microbial life too. Sponga *et al*., (1999) opines that the system contains different enzymes and other biochemical compounds due to the activities of marine biota in the detritus formed. But as a matter of fact, marine systems are less exploited. Due to the physical and chemical stress to which the marine biota is exposed to, they show many unique properties of survival. Of the most important group of organisms which can be prospected from marine environment is the marine microbes. The potentials of marine microbes in chemical, cosmetic and pharmaceutical industries have been recognized in last decades. Researchers around the world have isolated diverse marine microbes in which more than half constituted actinomycetes. (Sponga *et al*., 1999). Prospecting marine organisms started in the early 1990's itself. Bioactive molecules with unique capacities were identified and commercialized. So many private and public instates started deep water bioprospecting with objectives of detecting peculiar properties in organisms.

Marine microbes produce enzymes which have novel chemical as well as stereochemical properties. This is due to the conditions that they are exposed to. These organisms have to withstand high fluctuations in pressure, temperature, salinity etc. Because of this variation of enzymatic properties also the genetic makeup, scientists are much interested in looking for novel chemicals in them. Microbial prospecting started with culture-based technique in which microbes in marine samples were cultured giving all possible media and culture conditions, (Leclerc *et al*., 2002). But now high-throughput and cutting-edge techniques such as culture independent study, metagenomics and genome sequencing methods are also incorporated in bioprospecting. This will not only help to prospect bioactive and industrially important molecules but also identifies new enzymes and novel gene sequences without a barrier on the basis of organism type (Abida *et al*., 2013).

Organisms, originated in oceans evolved through time and started expanding to the terrestrial environment. Being unique in diversity and ancestral roles, marine organisms are excellent and potent sources for prospecting and extracting molecules with bioactivity as well as industrial applications, (Boeuf, 2011). While the most important objective of bioprospecting is the isolation of chemicals of bioactivity and industrial importance, the technique also can be applied for remediation of environmental pollution. Marine systems are the sinks of almost all kinds of water and sediment pollution of the land areas and inland water bodies. Thus, there are a lot of pollutants in the oceans. Depending on the toxicity it poses to the marine life, there could be a decline in the diversity and number of organisms. But in the process, some organisms, mostly microbes will develop tolerance and resistance against these pollutants. The genetics of microbes being plastic, they adjust and adapt to the presence of the chemical and develop capacity to use the pollutant as either carbon or nitrogen or energy source.

Microbes of marine environment

As any other ecosystem, marine microbes may be bacteria, fungi, actinomycetes, viruses, phyto- and zoo planktons. Because of the unlimited capacities of marine bacteria, they are often considered as chemical gold, (Williams, 2009). As per Williams, (2009), if properly studied, marine bacteria will provide pharmaceuticals for the coming 100 years. The bio actives produced by these microbes can be used as excellent drugs against cancer and infectious diseases. Carbon, nitrogen and energy acquisition by marine bacteria is also diverse. Due to the complexity of the environment there such as oxygenic, suboxic or anoxic conditions persisting, they can be obligate

or facultative aerobes or anaerobes. As the micro conditions of growth in marine systems fluctuates greatly, marine bacteria show several adaptations. In periods of starvation, they divide and shrink to one-third of their normal size. These are called ultramicrobacteria (UMB). They will resume their normal form after the period of starvation. Even in this state of their life, they do invaluable services such as degradation of moving organic pollutants as these ultra-sized bacteria can move along with flow paths of these pollutants, (Ginn *et al*., 2006).

Relic DNA and bioprospecting

Deep layers of marine systems may not be having enough nutrients and growth supplements for the life of microbes. But studies of Lennon et al., (2018) shows that marine microbes can use the relic DNA (extracellular DNA or DNA inside cells which are no longer intact) as phosphate source for their life. The presence of these relic DNA can also contribute to genetic variations because bacteria are capable of uptake of extraneous DNA into their cells. Though inactive outside a living cell, relic DNA on incorporation in the genome can become active contributing to the expression of genes of varied constitution. This will also result in the getting peculiar activities.

Most of the marine ecosystem may also harness microbes those were present since geological past. This is possible because of the vastness of ocean, depth, as well as conditions which preserve genetic wealth of geologic past. As conditions of the geologic past was not so much conducive to the growth of microbes, there is possibility that these possess genetic capacities to sustain in those harsh conditions. These abilities will help them to thrive in highly polluted environment by utilizing the pollutants and otherwise by degrading them, this promising potential for biodegradation, (Stan-Lotter, 2019).

Prospecting marine microbes

Because of the unique nature of marine ecosystems and the diverse harshness that the organisms inhabiting this system is exposed to, they produce numerous highly potent bioactive principles. The natural products they produce is not completely known to us. Thus, there are enough chances to isolate novel compounds with novel activities. So many drugs have been discovered and isolated from marine microbes from the past onwards. Of these, the greatest contributors are actinomycetes, which form the source of 45% of the drugs. Rest 38% is from fungi and Only 17% is from marine bacteria, (Liu *et al*., 2010). As per Zhang *et al*., (2007), most of these compounds commercialized are not chemically modified to reach markets showing the effectiveness of these natural compounds. It is understood that as

habitat diversity increases, biodiversity increases, providing genomic diversity in biota resulting in biochemical diversity and production of diverse bioactive molecules. Hence drug discovery aims at identifying and isolating novel strains of microbes.

The compounds isolated from marine microbes can either be used directly after fermentation as already mentioned, modified after fermentation chemically or can be used as a lead molecule in which subsequent chemical modifications can be done to make it more potent in its action. But the process is highly time consuming as it requires culturing, optimization of growth conditions, isolation of the active principle, purification and ultra-purification, characterization, structure elucidation, testing and releasing. The fact that almost 99% of microbes in any environment stand unculturable also need to be acknowledged which means that most of the potencies of microbes cannot be harnessed in the bioprospecting journey. Hence the researches incorporated metagenomic approaches in drug discovery with the advances in molecular biological techniques.

Marine microbes come under the categories of free living which can be isolated from environmental samples such as water and sediments and surfaces of marine structures and also, they may be in association such as endophytes, on the surface of and inside sponges, inside inveterate and vertebrates etc. Microbes associated with marine sponges have shown to possess many activities. They produce bioactive as a chemical defence mechanism against predators as well as pathogens.

Diseases such as Alzheimer's Disease (AD) is difficult to be dealt with. When the fact that it cannot be prevented but theoretical possibilities are there to restrict progression of this disease by preventing deposition of amyloid plaques in neural system, require the discovery of novel lead molecules which can be engineered to effective drugs. There are a class of compounds called Non-Ribosomal Peptides (NRPs). These are secondary metabolic products which have functions similar to siderophores, pigments, toxins, antibiotics etc. These have many modules and are referred as multi-modular enzymes (Martínez-Núñez *et al*., 2016). These classes of compounds are used as human medicines, as products for crop protection as well as for environmental remediation applications. The main producers of this class of non-ribosome dependant products are bacteria. So many NRPs are reported from marine microbes (Agrawal *et al*., 2017).

Leishmaniasis is a vector-borne disease which is seen in tropics, sub-tropics and southern parts of Europe. It causes skin lesions and ulceration in

exposed body parts. These areas leave long lasting scars. Another type damages internal organs such as liver and spleen. This infection is called visceral leishmaniasis or 'kala azar'. The infection was treated with chemicals such as drugs called antimonials. Apart from the fact that these chemicals are costly and have adverse toxic effect to humans, there are also reports of development of resistance against this class of drugs (Sundar *et al*., 2010). Recently prospecting of marine microbes by Ortega *et al*., (2021) have shown that some compounds isolated from marine actinomycetes have anti-protozoal activity which can be used against leishmaniasis.

Dyes are used to increase the marketability of many products including textiles, cosmetics, utensils, food and even drugs. But most of these are synthetic and are toxic causing carcinogenesis. There also have environmental implications in being non-biodegradable. The problems associated with synthetic dyes are now widely known. Hence demand for a natural alternative is very high. There are so many natural pigments produced by plants, animals as well as microorganisms. Microbial pigments are stable, biodegradable and non-toxic (Priya *et al*., 2020). Marine microbes are recognized as excellent sources of various shades of pigments. Though may be related to their terrestrial counterparts, marine microbes have a very distinctly different genomic sequence.

Microbial products are of diverse kinds. They produce several biomolecules. The marine counterparts also produce same kind of molecules, but with an enhanced capacity due to the stress they are constantly exposed to. Microbial prospecting studies have been carried out in marine system near an underwater volcano Tagoro. The team could isolate 182 microbial isolates. This system experienced so many perturbations such as thermal changes, water acidification, mineral surge etc which changed the microenvironment significantly (García-Davis *et al*., 2021). These kinds of unique environments will be hotspots of microbial diversity and hence bioprospecting. Microbial communities surviving these harsh changes will be having genetic system making them capable existences in these conditions. With this potential, they are expected to be reservoirs of bioactive products and produce special chemicals of diverse kind which can be sourced for industrial and pharmaceutical products as well as processes.

But it has also been observed that theoretically the drug discovery should be directly related to microbial diversity, in practice, there are always overlapping in the products produced by various kinds of microbes. Structural analysis also has revealed the same which causes stagnation in marine bioprospecting as discussed in the start. Marine drug discovery has been

supported by computer assisted techniques. But even now there are unsolved problems such as treatment against cancer, AIDS and the need of a potent drug against evolving and spreading viral diseases.

By applying the cutting edge molecular biological techniques such as sequencing and analysis of genomes, the relevant genes and gene clusters will become understood and bioprospecting bottle necks can be overcome.

Conclusion

Microbes from marine systems are invaluable as far as their genetics as well as metabolites are concerned. Most of the problems experienced by mankind in aspects related to health and industry can be solved from the products obtained from marine microbes. Bioprospecting and the associated techniques for that is a great tool for mining the potential of microbes. The changes in environment will be reflected by the microbial inhabitants because of quorum sensing. The screening techniques can be either culture dependant or culture independent. A couple of decade ago there was a hype in bioprospecting. Though these are highly useful in bringing out novel products and processes, the isolation of products from microbes have been decreasing and reaching a bottle neck. This is due to the time taken from the initial step to till commercialization, the property of microbes to share the genetic makeup, similarity in the products produced by seemingly diverse organisms etc. Hence research has to find new directions to properly mine the properties of microbes, especially seen in unique habitats such as mangroves and marine systems.

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