Fundaments and Concepts on Screening of Microorganisms for Biotechnological Applications. Mini Review

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Abstract

Microbial biotechnology uses microorganisms and their derivatives to generate industrial and/or environmental products that impact daily life. Modern biotechnology uses proteomics, metabolomics, quantum processors, and massive sequencing methods to yield promising results with microorganisms. However, the fundamental concepts of microbial biotechnology focus on the specific search for microorganisms from natural sources and their correct analysis to implement large-scale processes. This mini-review focuses on the methods used for the isolation and selection of microorganisms with biotechnological potential to empathize the importance of these concepts in microbial biotechnology. In this work, a review of the state of the art in recent years on the selection and characterization of microorganisms with a basic approach to understanding the importance of fundamental concepts in the field of biotechnology was carried out. The proper selection of isolation sources and the design of suitable selection criteria according to the desired activity have generated substantial changes in the development of biotechnology for more than three decades. Some examples include Taq polymerase in the PCR method and CRISPR technology. The objective of this mini review is to establish general ideas for the screening of microorganisms based on basic concepts of biotechnology that are left aside in several articles and maintain the importance of the basic concepts that this implies in the development of modern biotechnology.

Introduction

Biotechnology is an important area of research, considering its impact on everyday life. Current biotechnology research includes sustainable agriculture, vitamin and antibiotics production, COVID-19 vaccine development, and metabolite hyper-production [1–3]. Biotechnology has different origins, depending on the specific components used for its development. Plants, animals, fungi, and bacterial cells, including derivatives such as enzymes, artificial membranes, and viral particles, can all be used to apply and develop biotechnology [4–6].

For the context of this work, we focused on the concepts of microbial biotechnology which is defined as the use of microorganisms in a fermentative process for the production of metabolites of industrial interest for various applications, including industrial, medical, environmental, food, and agricultural [3, 4]. It has been observed that the evolution of biotechnology has undergone radical changes throughout history, bioinformatics tools and omics have allowed this science to acquire an unprecedented scope, however, it is contradictory to think that microbial biotechnology was an important element in the development of civilizations by the use of fermentative processes since the beginning of human history for bread and wine rudimentary production, and currently it is one of the most modern tools used to combat the current coronavirus pandemic [7, 8]. The current approach to microbial biotechnology is the use of genetic engineering to employ synthetic biology and break the physiological limits of microorganisms. Although we are not against this facet, the current research does not emphasize the potential of fermentative bases.

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and the correct selection and characterization of microorganisms isolated from natural sources that precisely allow establishing management guidelines to improve their natural biotechnological activity. Before placing a plasmid inside E. coli for bioreactor hyper-production of amylase from some bacteria, first, it is necessary to select, isolate, and know the biotechnological potential of these microorganisms, that is where the importance of the biotechnological foundations lies, especially those used in microbial screening which, according to our experience, are not defined in current works. Opportunities for the use of microbial biotechnology are endless. Every day new uses for microorganisms are discovered or scaled to an industrial level, such as new processes for wastewater treatments or improvements in bioremediation processes and only a small percentage of microorganisms have been studied [9–12].

The current scope of biotechnology has transcended interesting borders where the works focus on maximizing production, studying new therapeutic effects or modifying metabolites of interest, however, it should be noted that basic fundamentals such as establishing the significance of identifying a correct selection pressure, characterizing microorganisms based on specific selection criteria, performing an optimal screening of microorganisms from well-chosen natural sources are as important as the most modern techniques used in the field or the hyper-production of enzymes that will fight the diseases of the future. Current work in biotechnology should not ignore these elements, since they are the basis for more comprehensive and content-rich research that will allow the exponential growth of microbial biotechnology.

Advancements in genetic engineering and the implementation and use of omics in modern biotechnology have created a revolutionary biotechnology field [13, 14]. However, the techniques of microbial biotechnology are essential for taking biotechnology to the next level. Microorganisms have physiological limitations that ultimately depends on their ecological needs, so understanding the bases of these limitations will allow us to break those restrictions in the search for more and better biotechnological applications using microorganisms.

This paper aims to demonstrate the importance that basic concepts (selection pressure, selection criteria, etc.) provide in the field of biotechnology from a review of the state of the art of recent research. Few works fundamentally address the basic concepts of biotechnology since they are often ignored in order to emphasize biotechnological applications or the use of advanced technology. We focus on defining and establishing how the basic concepts in biotechnology are used for the development of this science and its presence in current research work.

Isolation and Selection of Microorganisms with Biotechnological Potential

Natural Sources of Isolation of Fermentative Biotechnological Microorganisms

Classical microbial biotechnology relies on finding microorganisms with interesting activities isolated from natural sources. Usually, for the isolation of microorganisms with biotechnological interest, environments with particular conditions are preferred, for example, locations where selection pressures are constantly present, places where the microorganisms are in contact with certain substrates, that have extreme conditions of temperature or pH, little explored lands or with interesting microbiome due to its geographical conditions, or soils contaminated with some recalcitrant compound, etc. [15–19].

The possibilities are endless given the great variability of conditions in which microorganisms can be found naturally. In the literature, we can find many examples of natural sources of isolation from hypersaline lakes, ponds contaminated with hydrocarbons, desert lands in Mexico, arctic seas, and forest floors [17, 20–22].

Metabolites generated by microorganisms isolated from extreme environments may provide attractive biotechnological and economic advantages. Enzymes that resist extreme pH or temperatures, new antibiotics, and more potent drugs can be developed from this type of microorganism. For example, the DNA polymerase obtained from the archaea Thermophilus aquaticus isolated from a thermophilic environment made recombinant DNA technology possible. This had a substantial economic and logistical impact on industry and research [23, 24].

It is important to emphasize that when selecting an isolation source, the natural conditions for the development of microbial life must be considered. We often focus on industrial interest and ignore microbial physiology, looking for sources of isolation that are too demanding for life to develop. Complex nutrient sources, high concentrations of pollutants, absence of carbon or nitrogen sources, and low water activity are some examples of the mistakes made when selecting natural isolation sources. Although microorganisms adapt to various conditions, the probability of success for the isolation of microorganisms is reduced if we do not consider the minimum requirements necessary for growth [25].

Selection Pressure

From the point of view of this work, selection pressure can be defined as any external factor, biotic or abiotic, that
affects the growth of a particular microbial population. These selection pressures can be used when choosing the isolation source or used in the laboratory under the scheme of progressive enrichment cultures to favor the growth of populations that have the activity of interest (Table 1).

The selection pressure depends on the desired activity of interest, the geographic conditions of the isolation, the availability of resources, and the physiology of the microorganism itself. Thanks to advances in genomic sciences, modern biotechnology can simplify the process by detecting the desired activity in a specific species of microorganisms and only isolating that specific microorganism. However, natural sources of isolation with their specific selection pressures continue to be a field of interest for biotechnology as new and better variants of microbial biotechnological activity are being discovered.

With the pressure of selection, it is also possible to enrich microbial communities that possess the desired activity. Natural samples for the isolation of microorganisms are conditioned under selection pressure. Over time, only those capable of withstanding the selection pressure proliferate. For example, a soil sample obtained from agricultural soils of rice fields can be placed in the presence of starch and hypersaline solution to isolate halophilic microorganisms that produce amylases.

Selection Criteria

The selection criteria are the quantitative and/or qualitative characteristics that reveal the activity or product of interest in a microbial community and serve as the basis for selecting a microorganism of biotechnological interest [40]. The selection criteria vary and depend on the biotechnological activity being sought. Colonial morphology, enzymatic activities, inhibition, or hydrolysis halos are some examples of selection criteria. Table 2 shows examples of selection criteria reported in the literature.

It is important to point out that the selection criteria must be specific according to the desired biotechnological activity, they must be easy to visualize and identify and, as far as possible, applicable to a large number of samples to facilitate the work. The most commonly used selection criteria include changes in the color of the solid medium, changes in the pH indicator, halos of hydrolysis or solubilization, among others. Figure 1 shows the selection criteria for microorganisms with biotechnological potential for the production of siderophores, enzymes, organic acids, and bioinsecticides.

Primary and Secondary Screening

Based on the selection criteria, there are two types of selection: primary and secondary screening. Primary screening is designed to isolate potentially interesting microorganisms. It reveals the activity or desired product based on qualitative and usually indirect selection criteria without going into detail about the desired activity. It is especially useful when there are many samples to process. In contrast, secondary screening is based on qualitative and quantitative criteria to determine the best producers of the activity of interest. It explores the activity in more depth by describing the qualitative criteria of primary screening.

While the primary screening tends to use rapid tests adapted to a large number of samples (usually in solid medium), the secondary screening must be more sensitive and specific. Therefore, it uses more precise and direct quantitative chromatographic or spectrophotometric methods (Fig. 2). The success of the primary selection depends on the source of isolation, the type of culture, and the isolation technique, among others. However, secondary screening bases the success of isolation on primary screening, so the primary selection criteria must be specific and sensitive to avoid false positives.

Some examples of primary screening criteria are hydrolysis halos, inhibition halos, precipitation or emulsification halos, pH indicator changes, growth in solid medium, and colonial or microscopic morphology. Primary screening criteria focus on simply evaluating the presence or absence of the desired activity. In the case of secondary selection criteria, quantitative techniques are used to determine the activity of interest, for example, the enzymatic activities, the

| Selection pressure | Examples | Natural sources | References |
|--------------------|----------|----------------|------------|
| Carbon sources     |          |                |            |
| Complex polymers   | Starch, chitin, chitosan, lignin | Agricultural waste, fishing waste, soil, Tree bark | [16, 26, 27] |
| Xenobiotic         | PHAs, dyes, herbicides | Contaminated water and soil | [17, 27, 28] |
| pH                 | Extremophiles | Soil, food products, industrial waste | [29–31] |
| Temperature        | Extremophiles | Arctic sea, deserts | [15, 21, 32, 33] |
| Osmolarity         | Extremophiles | Saline lakes, saline soils, solar salttern, industrial waste | [22, 34, 35] |
| Others             | Pressure, oxygen, nitrogen sources | Various sources | [36–39] |
| Biotechnological activity                                     | Microorganism                                           | Isolation source                                      | Selection criteria                                                                                     | Reference |
|---------------------------------------------------------------|---------------------------------------------------------|-------------------------------------------------------|--------------------------------------------------------------------------------------------------------|-----------|
| Probiotic                                                     | *Lactobacillus* and *Enterococcus* strains              | Food, plants, and human                                | Resistance to gastric acidity and resistance to bile salt. Adherence to mucus and/or human epithelial cells and cell lines. Antimicrobial and antagonism activity against potentially pathogenic bacteria | [41, 42] |
| Wine production                                               | Non-*Saccharomyces* Yeasts                              | Grapes, musts, or wines                                | Fermentative power, aroma profile modulation, acidity regulation                                       | [40, 43] |
| Phytoxic secondary metabolites with herbicidal activity       | *Fusarium fujikuroi*                                    | Brazilian Pampa biome                                  | Germination in pre-emergence, phytotoxicity, plant height and root length in post-emergence, and lesions in detached leaf-punctured assay | [44]     |
| Sourdough-based fermentation and bread production             | Bacteria belonging to the family *Lactobacillaceae*     | Italian sourdoughs                                     | Tolerance to acid, salt, sucrose, and ethanol stresses, urease, amylase, and proteolytic activities   | [45]     |
| Microbial antagonists fungi plant pathogens                   | Yeast, bacteria, and fungi                              | Varied isolation sources                               | Inhibition of spore germination and radial growth                                                   | [46, 47] |
| Exopolysaccharide production                                  | Bacteria strains. *Bacillus velezensis*                 | Soil samples from Al-Bahariya Oasis                    | EPS precipitation using 70% ethanol and the bacteria colony ropy strand formation                   | [48]     |
| Polyhydroxybutyrate [PHB] production from methane             | Methanotrophs                                           | Recycled activated sludge [RAS] from the Humber wastewater treatment plant situated in Toronto, Canada | Gas consumption and PHB accumulation after enrichment culture technique                               | [49]     |
| Protease activity                                             | Coagulase-negative *staphylococci* [CNS]                | Traditional Chinese fermented sausage                  | Hydrolysis halos revealed with coomassie blue and enzyme activity                                 | [50]     |
| Siderophores production                                       | *Azotobacter vinelandii*, *Bacillus megaterium*, *Bacillus subtilis*, *Pantoea allii*, and *Rhizobium radiobacter* | Collection                                             | Level of siderophore production determined by chrome azurol S, CAS method                           | [51]     |
| Bacteriocin production                                        | Bacteriocinogenic lactic acid bacteria and *Bifidobacterium* spp | Honeycomb filled with oregano honey                    | Surrounding clear zone in the MRS agar, biochemical and morphological characterization                | [52]     |
amount of the metabolite of interest is measured directly by HPLC or spectrophotometry, the amount of gas generated or carbon source is quantified, among others [32, 33, 42]. Usually, the primary and secondary screening can be carried out simultaneously. In some cases, the primary screening can be omitted. This depends on the activity that is being
sought, the number of samples to analyze, and the availability of resources, among others [53, 54].

**Conclusion**

Microbial biotechnology has enormous scope and impact on current life. The development and innovation of biotechnology are increasing every day. As described in this work, the industrial potential of microorganisms is still unexplored and can generate enormous benefits for humanity. Although modern biotechnology uses quantum tools for its development, the bases and foundations of the search for microbial activities of interest as well as the isolation and selection of potentially biotechnological microorganisms cannot be left aside since nature continues to be a source to resources that could bring the next biotechnology revolution. The predictions of this work focus on being able to establish a starting point for future research in biotechnology and emphasize the importance that the basic concepts of microbial selection have, the technological revolution reached us and it seems that modern microbial biotechnology focuses more on the use of more advanced techniques underestimating the biochemical and physiological bases.

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**Declarations**

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