ejpmr, 2018,5(5), 42-47

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Review Article

ISSN 2394-3211

EJPMR

SCREENING AND SELECTION OF INDUSTRIALLY IMPORTANT MICROORGANISMS: A REVIEW

Sikander Ali[1], Muhammad Hassan Sarfraz*[2], Ruqaiza Muhyudin[3] and Waqas Ali Awan[4]

Pakistan.

***Corresponding Author: Muhammad Hassan Sarfraz**

Pakistan.

Article Received on 25/02/2018 **Article Revised on 19/03/2018** **Article Accepted on 09/04/2018**

ABSTRACT

Screening of microbes is a valuable process for industrial biotechnology as it aids in the production of valuable products of desired quality. Microbes are preferably taken from natural sources. Enrichment culture technique is employed for their growth. For the specific selection, primary and secondary screening is carried out which employ different techniques. Selected microbes are then employed for the scale up and production processes for the industries. Although an impact has been made by advances in the instrumentation, genetics, and microbial physiology but still the screening programs are primarily based on so-called classical techniques of enrichment and mutagenesis. Although there is still room for research, knowledge and collaboration for better selection of industrially valuable strains.

KEYWORDS: Bioactive metabolites, microbial products, biocatalysts, direct assay, indirect assay, industrial screening.

INTRODUCTION

Microorganisms have been extensively utilized by human beings for their own benefit since the ancient times. Beer was the first to be brewed by ancient Egyptians while Large scale alcoholic productions started in early 1700s. People used variant strains of microbes for fermentation, without even knowing the existence of microbes, on hit and trial method for producing various products. Initially simple wooden vats, shallow bowls or barrels were used for the process of fermentation with less concentration on the quality of the product. However, with the passage of time different culture techniques came into being focusing on quality along with large scale production of industrial products. The discovery of microorganisms brought a revolution in fermentation biotechnology and it expanded the development of microbial culturing and screening techniques. Now we are living in a time where microbial products are a major part of our lives with food, textile and antibiotics being the major part of biotechnology industry. The development of the screening process for selecting the industrial important strains have advanced gradually for past 50-60 years. However, still old methods are being relied upon for enrichment, screening and mutagenesis. Although, along with the considerable use of traditional methods some modifications are also employed with the classical methods to improve the selection process. The modifications involve using membrane technology, immunological technique, coupled colorimetric reactions or chemical analogue or by employing advances in instrumentation (Steele and Stowers, 1991).

Historical background

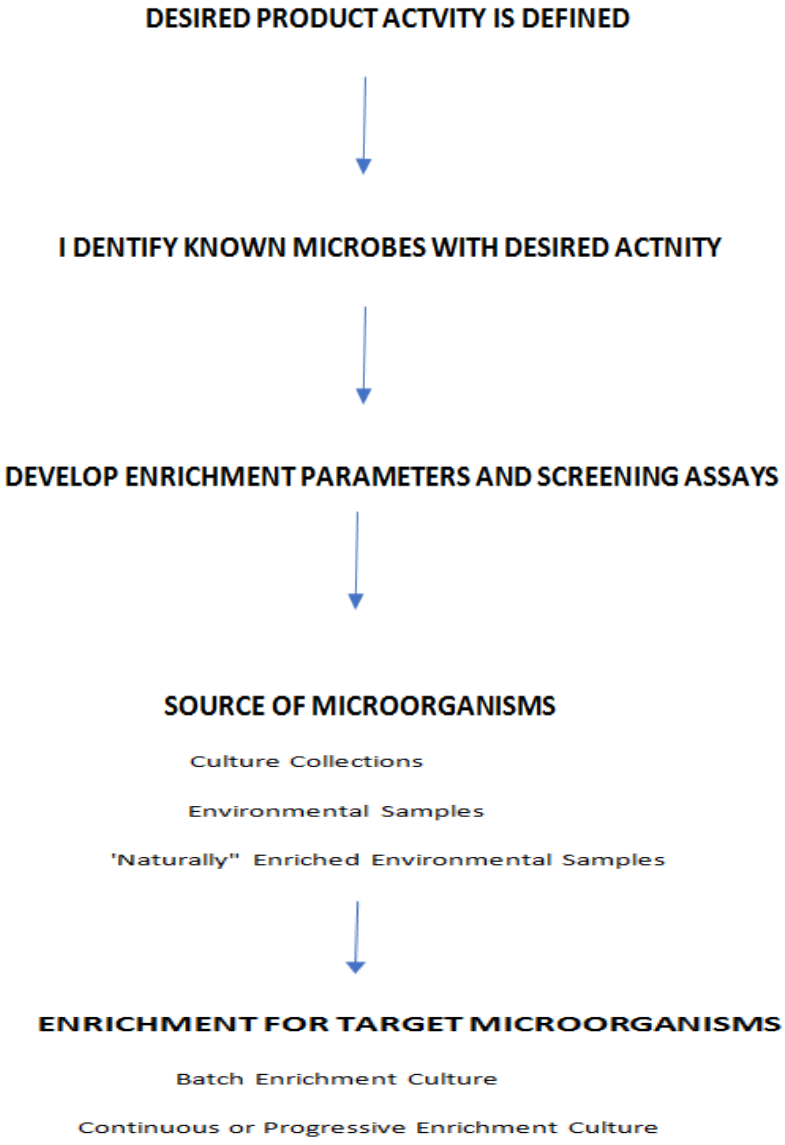
Various scientists and researchers have made their contributions in the advancement of the microbiology, some providing the ideas while giving solid evidences, forming the base of the subject field. It all starts with the Antoni Van leeuwenhoek who, with his own designed single lensed microscopes, observed and studied the microbes and marked their existence. However, the roots of industrial microbiology were laid down by Pastuer as he was the one to discover that it's the microbes causing the fermentation process. With this discovery he put an end to the spontaneous generation theory (Wainwright et al., 1992). He further made contributions in beverage production and discovered the heat killing of microbes.

Pastuer's contemporary, Robert Koch, gave the method for isolating pure cultures of microorganisms with the aid of solid culture media. Further he provided with the germ theory of disease and proved that the causative agent of diseases are microorganisms. Following Koch, Paul Ehrlich performed experiments in search of antimicrobial substances and obtained noble prize in 1908 (Wainwright et al., 1992). Martinis W. Beijernick introduces the concept of enrichment culture which lead to our understanding of the role of microorganisms in nature. It allowed for the easier isolation of microorganisms on basis of their specific metabolic properties. Kulyver was the first one to perform true

studies on physiology of microbes. It leads to the understanding of similarities and differences among different living beings. Sergie Winogradsky developed the winogradsky column where microbes bring about changes in their surroundings to develop the environment compatible for their growth resulting in different regions in the column occupying varying microbes. The idea of using the enrichment culture technique for the isolation of microbes is still being used for the screening process of microbes. It has become a major milestone in microbiological screening programs. It has proven out to be quite a reliable technique eliminating the non-target microbes.

Selection strategy and techniques

A strategy is being developed for the screening of industrial important microorganism. The strategy to be develop for the screening process must be systematic isolating the target microbe while removing the non-target ones. For the screening process, first it is important to define the desired activity of the product required in the industry. After the desired product is identified, different microbes are being selected which are suspected to give the desired activity. To ensure the best selection enrichment assay and selection parameter is developed. The source of microorganism is identified and using culture techniques the enrichment of target microbes is carried out (Steele and Stowers, 1991). First primary screening is carried out which is followed by secondary screening. Secondary screening eliminates the false negative ones and also evaluate the product activity as well. The product is being developed at pilot scale which is then brought up to the industrial level and production at industrial level is carried keeping in mind the control parameters. The scheme below represents the general screening process of microbes to be used at the production level at industries:



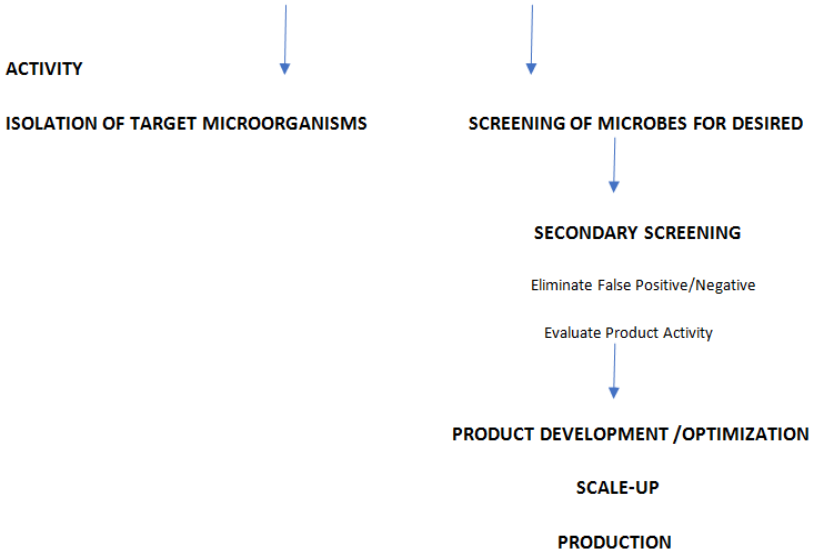


Figure 1: General scheme of Screening.

Sources of microorganisms

Most auspicious tendency in the enrichment technique is to look for the innovative surrounding environment for propagation and selection of the desired microbe. The researchers are more interested in looking for sort of natural environment for enrichment, rather than enriching the microorganisms on anonymous soil or water samples. Examples of such natural enrichments include thermal springs, glacier ice or industrial effluent facilities. The natural environment allows for the selection and enrichment of microbes as the conditions are more suited for their growth resulting in their quick adaptation to the environment ensuring preferable growth over non-target microbes. Considering that many experts estimate that probably < 1% of the world's microorganisms have been properly examined and the dramatic discoveries that can be made, often by accident, this lack of effort in screening is surprising (Cheetam, 1987). Different localities are being observed looking for natural sources of microorganisms. One of them is yellow Stone National Park. Different labs and other groups have previously explored this park in search of microorganisms (Brock, 1967). As a result of which different useful microbes have been isolated which includes thermophiles, alkalophiles and acidophiles. Many different enzymes and useful products have been isolated from these microbes (Doemel and Brock, 1970).

Furthermore, bat-inhabited caves were also examined as natural sources, looking for microbes. The search was performed in order to look for the microbes having industrial potential. The Mexican free tailed bat has many economic benefits. Historically, the mining of bat guano provided the raw material for gunpowder and fertilizer industries. Bat guano contains rare micro bacteria that offer unknown potential uses in medications and dissolving industrial waste by-products. The elemental analysis of guano bat was performed to check the types of microbiota specie. Analysis proves the similarity with the medias which are being used throughout the world (Steele D.B., 1989). Furthermore, High Andes of Peru and Bolivia were also exploited. These regions have extreme environmental conditions resulting in freeze-thaw conditions. Such environmental conditions harbor microbes which have developed mechanisms to withstand the freeze thaw conditions. The study resulted in the isolating microbes having antifreeze activity and ice nucleating abilities (Steele and Stowers, 1989).

Further researches were also carried out in which industrial wastes were used. The wastes were taken from industries and were brought to the treatment facilities where heat treatment of the wastes was carried out. This resulted in successful isolation of the target organism. Heat treated aerobic sewage sludge contains a variety of thermophilic Bacilli which have been isolated and characterized biochemically. Extremely thermophilic Bacilli isolated from heat-treated sewage sludge are shown to be a source for enzymes stable and active at high temperatures (Grueninger *et al.*, 1984). Wastes were also taken from the fruit processing and potato facilities and were processed. It resulted in the isolation of microbes having the ability to produce amylase enzyme having industrial potential (Gee *et al.*, 1980).

Enrichment and isolation

The process of enrichment involves providing a locality to the microorganisms which is suitable for them and supports their growth. It allows the growth of the specific microbe while inhibiting the non-target microbe. The concept of the enrichment has still not changed. It is similar to the days when used by Winogradsky and Beijernick though some variations have been brought for isolating the industrially valuable microbes. The traits of the target microbe are kept in mind and the growth medias are designed using that information, which then supports the growth of that microbe. Specific substances like sole carbon source is used for the screening of the degradation of the compound. Different inhibitors are

also employed which have the ability to block a specific metabolic pathway of the non-target microbe. Furthermore, pH and temperature are also adjusted, favoring the desired microbes, facilitating the screening. Another approach is utilizing specific energy source which only the specific microbe will be able to utilize while the rest won't be able to utilize it. The substance used as an energy source will not be any common substance used abundantly. The source needs to be unique which cannot be utilized by majority of microbes other than the specific one. This approach resulted in the isolation of microbes having the ability to consume unique compounds. Examples include aniline (Aoki *et al.,* 1984), chlorinated hydrocarbons (Scholtz *et al.,* 1987), benzene (Shirai, 1986). In another study, utilizing the similar culture technique a novel strain of agrobacterium sp. was isolated. The bacterium had the ability to degrade EDTA with concentrations as high as 100mM (Lauf *et al.,* 1990).

In another study samples were taken from the fields and enrichment culture technique was used for screening benzene assimilating organisms. The sole energy source used for the purpose was benzene. 150 samples were collected from rice fields, vegetable gardens, and forests. As a result, ninety-five strains utilizing benzene were isolated (Shirai, 1986).

For some organism's batch culture technique is carried out. However, for others there is sometimes a requirement of another selection method. Sometimes our desired microbe, in a large stack of varying microbes, is in very small number or that particular organism produces the desired product in very small amount or it is inhibited by the compound we seek to degrade. In the above-mentioned situations an adjustment is made by using continuous culture.it used for enriching slowly growing microbe. The specific target microbe is selected and progressive enrichment is carried out. For continuous enrichment, amount of fresh media is added to the reaction in the exact amount the medium which though the continuous culture method is long, requiring months, and tedious but it is quite effective in some cases. Using the continuous culture technique, researchers successfully screened and selected specific microbe among a mixed population. The isolated microbes have the ability to grow on halogenated alkanes (Keuning *et al.,* 1985) and herbicides (Killbane *et al.,* 1982).

Primary screening

In primary selection microbes are selected on the basis of a particular activity. Assays are being performed which helps in the selection of the particular microbe on basis of a particular microbial activity. Assay for the screening process must be simple, must be cost effective and must be specific. This is the major step pf the whole screening process and this step will decide if the screening is successful or not. Assay methods can be divided into two categories. These can either be direct or indirect. Direct assay identifies the target product specifically while indirect assay detects the product through the enzymatic reaction involving color change or producing fluorescence. With the advancement both direct and indirect assay have been improved. Advances in instrumentation and development of enzymes new orogenic and chromogenic enzymes along with the better understanding of the physiology of the microbes have led to the development of direct and indirect assays.

1. Indirect assay

In this type of assay a specific compound is added to the nutrient medium which is used for the growth of the microorganism. The compound has the ability to either react with a metabolic product of the microbe or affect its growth. This will result in color change in the medium or produce clear zones respectively. Examples of the indirect reactions include the hydrolysis of casein (clear zones are formed) and the reaction between starch and iodine (purple color is developed). The color change or zone formation will aid in determining the activity quality. Indirect assays are carried out for the screening of microbes which produce industrially important enzymes. In different studies enzymes, cellulases (Cresswell, 1988) and uricases (Lehejčková *et al.,* 1986), were screened by indirect assay. In both cases hydrolytic zones were produced. Furthermore, parameters like temperature or pH are also been exploited along with which further facilitates the screening purpose. As a result, the screening is accomplished quickly.

2. Direct assay

The progressive progression of the techniques and instrumentations have aided in the direct detection of the microbial compounds resulting in microbial screening. Though the review is more concerned about the primary screening but the secondary screening also has an important role in the whole process. It is an important process in the screening of the microbes. It involves removal of false positive and negative microbes. It is very important keeping in mind the industrial level production. If a false positive microbe is selected for production at industrial level then it will result in huge loss economically. So secondary screening should also be carried out before moving to large scale up and optimization processes.

Different techniques are used for carrying out both primary and secondary screening. Micro instrumentation can be used both in primary and secondary screening. It is employed when simpler techniques are not preferred. In addition to this other technique are also employed. These includes HPLC (High performance liquid chromatography), GC (Gas chromatography), MS (mass spectromectry) and NMR (nuclear magnetic resonance spectrometry). The techniques allow for the quick, specific and highly sensitive detection. In one study high performance liquid chromatography technique was used for the screening purpose. The technique was coupled

with detector (photodiode array detector). It aided in the screening of new microbial metabolites (Fiedler, 1984).

Future Potential and Needs

The future of the screening processes for the selection of the best inoculum is promising. This is due to the development of knowledge and expertise in the fields of protein research and genetic engineering which will have a significant impact. The field of Industrial microbiology have been significantly impacted by protein and genetic engineering specifically at the point of maintaining the optimization of the product. Optimization of the product in maintained by bringing about significant changes in the protein i.e. increasing the stability of the protein. Different studies proved the increased stability of the subtilisin protein (alkaline protease) after some alterations were brought about as a result of genetically engineered (Bryan *et al.*, 1986). In another study, a site directed mutation was made in the protein subtilisin. As a result of this mutation the activity of the subtilisin protein was enhanced (Takagi, 1988). In order to surge the product formation, the target is to enhance the gene expression. In order to achieve this many firms are aiming to develop enhanced genetic promoters which would increase the expression of the gene and thus the product formation. Sayler used colony hybridization technique to identify the exact DNA sequence which was specific for the catabolism of hydrocarbons. The gene expression of the identified DNA sequence was enhanced to increase the catabolic activity (Sayler *et al.*, 1985).

Later techniques aiming at the unique DNA sequences of organisms can be employed for the purpose of screening. A common example is dot blot. It involves the hybridization of the sequence of the microbes with the probes. So, one can look for microbes with unique sequence using this technique involving hybridization of DNA from environmental samples with the designed probes (Holben *et al.*, 1988). The screening potential of the microbes is greatly dependent upon the diversity of the microbes present in the environment. There are a number of microbes yet to be discovered and they are living around us. We just need to develop appropriate techniques to isolate them and then select the best ones for industrial purposes. However, major issue is the deficiency of research programs and lack of collaboration because this is multidisciplinary task and thus it needs cooperation from different disciplines i.e. chemists, engineers and microbiologists.

REFERENCES

1. Aoki, K., Ohtsuka, K., Shinke, R. and Nishira, H., (1984). Rapid biodegradation of aniline by Frateuria species ANA-18 and its aniline metabolism. *Agricultural and biological chemistry*, *48*(4): 865-872.
2. Brayan, P.N., Rollence, M.L., Pantoliano, M.W., Wood, J., Finzel, B.C., Gilliland, G.L., Howard, A.J. and Poulos, T.L., (1986). Proteases of enhanced stability: characteization of a thermostable variant of subtilisin. *Proteins: Structure, Function and Bioinformatics*, *1*(4): 326-334.
3. Brock, T.D., (1967). Life at High Temperatures: Evolutionary, ecological, and biochemical significance of organisms living in hot springs is discussed. *Science*, *158*(3804): 1012-1019.
4. Cheetham, P.S., (1987). Screening for novel biocatalysts. *Enzyme and microbial technology*, *9*(4): 194-213.
5. Cresswell, M.A., Attwell, R.W. and Dempsey, M.J., (1988). Detection of cellulolytic actinomycetes using cellulose-azure. *Journal of microbiological methods*, *8*(5): 299-302.
6. Doemel, W.N. and Brock, T.D., (1970). The upper temperature limit of Cyanidium caldarium. *Archiv für Mikrobiologie*, *72*(4): 326-332.
7. Fiedler, H.P., (1984). Screening for new microbial products by high-performance liquid chromatography using a photodiode array detector. *Journal of Chromatography A*, *316*: 487-494.
8. Gee, J.M., Lund, B.M., Metcalf, G. and Peel, J.L., (1980). Properties of a new group of alkalophilic bacteria. *Microbiology*, *117*(1): 9-17.
9. Grueninger, H., Sonnleitner, B. and Fiechter, A., (1984). Bacterial diversity in thermophilic aerobic sewage sludge. *Applied microbiology and biotechnology*, *19*(6): 414-421.
10. Holben, W.E., Jansson, J.K., Chelm, B.K. and Tiedje, J.M., (1988). DNA probe method for the detection of specific microorganisms in the soil bacterial community. *Applied and Environmental Microbiology*, *54*(3): 703-711.
11. Keuning, S., Janssen, D.B. and Witholt, B., (1985). Purification and characterization of hydrolytic haloalkane dehalogenase from Xanthobacter autotrophicus GJ10. *Journal of bacteriology*, *163*(2): 635-639.
12. Kilbane, J.J., Chatterjee, D.K., Karns, J.S., Kellogg, S.T. and Chakrabarty, A.M., (1982). Biodegradation of 2, 4, 5-trichlorophenoxyacetic acid by a pure culture of Pseudomonas cepacia. *Applied and environmental microbiology*, *44*(1): 72-78.
13. Lauff, J.J., Steele, D.B., Coogan, L.A. and Breitfeller, J.M., (1990). Degradation of the ferric chelate of EDTA by a pure culture of an Agrobacterium sp. *Applied and environmental microbiology*, *56*(11): 3346-3353.
14. Lehejčková, R., Demnerova, K. and Kralova, B., (1986). Screening of microorganisms with uricase activity. *Biotechnology letters*, *8*(5): 341-342.
15. Sayler, G.S., Shields, M.S., Tedford, E.T., Breen, A., Hooper, S.W., Sirotkin, K. and Davis, J.W., (1985). Application of DNA-DNA colony hybridization to the detection of catabolic genotypes in environmental samples. *Applied and environmental microbiology*, *49*(5): 1295-1303.
16. Scholtz, R., Schmuckle, A., Cook, A.M. and Leisinger, T., (1987). Degradation of eighteen 1-

monohaloalkanes by Arthrobacter sp. strain HA1. *Microbiology*, *133*(2): 267-274.
17. Shirai, K., (1986). Screening of microorganisms for catechol production from benzene. *Agricultural and biological chemistry*, *50*(11): 2875-2880.
18. Steele, D.B. and Stowers, M.D., (1991). Techniques for selection of industrially important microorganisms. *Annual Reviews in Microbiology*, *45*(1): 89-106.
19. Steele, D.B., (1989). Bats bacteria and biotechnology. *Spring*, Vol 7(9).
20. Takagi, H., Morinaga, Y., Ikemura, H. and Inouye, M., (1988). Mutant subtilisin E with enhanced protease activity obtained by site-directed mutagenesis. *Journal of Biological Chemistry*, *263*(36): 19592-19596.
21. Wainwright, M., Lederberg, J. and Lederberg, J., (1992). History of microbiology. *Encyclopedia of microbiology*, *2:* 419-437.