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Role of Microbes in Industries

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ABSTRACT

Microorganisms are ubiquitous and are important area for research due to their importance in beverages, food, pharmaceuticals, and enzymes industries. Many microbial species are used in industries like- fungus, bacteria, yeast etc. They require special media to grow that should be rich in carbon, nitrogen, phosphate source, vitamins etc. Apart from this many other components are also being used to prepare media. There are enormous amount of products that microbes provide like- antibiotics, dairy products, biopolymers, cosmetics, fermented food and beverages etc. Firstly microbes are isolated from various sources and then are screened accordingly. There are primary and secondary screening methods available that can be used. Microbial cultures need to be preserved to avoid contamination so that they can be available for further uses. In this review we are looking forward to know more about microbes and their products.

***Keywords*:** Microorganisms, Products, Enzymes, Screening, Media, Preservation, Industry, Fermentation, Storage

1. INTRODUCTION

Microorganisms are present everywhere around us and have become an important topic for research as they produce industrially important products like- enzymes, beverages, food, textiles, medicines, vaccines, antibiotics etc [1]. The use of these industrially important microbes was initiated when Louis Pasteur discovered microbes that can be used in

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fermentation. This discovery led to the further advancement of industrial microbiology and many other scientists started developing culture media to grow these microbes in laboratory. This led to the development of biotechnology especially recombinant DNA technology that helps to brought many changes in microbes used in industries. The biosynthetic pathways used have now been modified by using metabolomics to extract new metabolites from microbes.

More and more microbial genomes are now being sequenced that provide us knowledge with the enzymatic and genetic makeup of them [13]. Now the microbial products have become the essential part of our life. But humans have started over exploiting the microorganisms for their own personal use [2].Technological Microbiology has started to be a new field of attention since World War I due to increased demand of glycerol to manufacture explosives and for large-scale production of penicillin [14]. Around 50% of the industrially important products are produced by using fungal species, 35% using bacterial and rest by using plant sources. Fungal species are used extensively in industries as they are easy to cultivate and can produce wide amount of extracellular enzymes and other industrially important products [3]. Due to their immense usage, they are screened by growing on solid media and obtaining pure cultures. These methods are now being modified by using chemical analogs, colorimetric tests, and immunological techniques depending on microbes being cultured. Apart from this, the use of enrichment culture further led to the isolation of microbes having special properties [2]. Actinomycetes that belong to separate group within bacterial domain are the gram positive, aerobic and spore forming bacteria that also has some fungal characters like growing like filaments and producing hyphae. They have broad range of habitats, metabolic versatility and produce huge range of industrially important compounds like- enzymes [amylases, cellulases, proteases, chitinases, xylanases and pectinase], 50% antibiotics, nutraceuticals, antitumor agents, plant growth regulators and vitamins [23,24]. In this review we will see various microbial products that are industrially important, selection and isolation of these microbes, different media used and their storage methods.

2. MICROBIAL PRODUCTS

Microorganisms are used by industries to produce tones of commercial products that generate huge revenue worldwide. They are used to produce primary metabolites like- vitamins, amino acids, fermented beverages, secondary metabolites like- antibiotics, enzymes , baking industries, biopolymers, waste management and in agriculture to produce food [4, 5]. Using these microbes does not cause any pollution problems.

Table 1. Various microbial products.

Products	Microorganisms
1. Amino Acids	
• Glutamic acid, Aspartic acid, Phenylalanine, Lysine, Tryptophan	*Corynebacterium*, and *Escherichia coli*
• Glutamate	*Brevibacterium*, *Corynebacterium*, *Micrococcus* and *Microbacterium*

2. Vitamins • Riboflavin • Vitamins B12 • Vitamin A (β-Carotene)	*Eremothecium ashbyii* and *Ashbya gossypii* (Ascomycetes) and genetically engineered *Bacillus subtilis* and *Corynebacterium ammoniagenes* *Propionibacterium shermanii* and *Pseudomonas denitrificans* *Mucor circinelloides*, *Rhodotorula*
3. Organic Acids • Citric acid • Itaconic acid • Lactic acid • Acetic acid	*Aspergillus* sp *Aspergillus terreus*, *Ustilago*, *Candida*, and *Rhodotorula* *Lactobacillus* sp, *Rhizopus oryzae* *Acetobacter*, *Gluconacetobacter*, and *Gluconobacter*
4. Enzymes • Lipase • Protease • Cellulase • Amylase	*Aspergillus niger*, *A. oryzae*, *Candida* *Aspergillus niger*, *B. subtilis*, *Pseudomonas* sp., *Streptomyces* sp *Aspergillus niger*, *Penicillin funiculosum*, *Bacillus* sp. *Aspergillus* sp., *Bacillus subtilis*, *B. licheniformi*, *B. amyloilquifaciens*, *Streptomyces* sp.,
5. Alcohol • Ethanol • Glycerol	*E. coli*, *Klebsiella oxytoca* and *Clostridium thermocellum* *Candida glycerinogenes*
6. Antibiotics	*Penicillin notatum*, actinomycetes, streptomycetes
7. Alkaloids	*Claviceps sclerotium*, *Aspergillus*, *Penicillium*, *and Rhizopus*
8. Bio pesticides	*Bacillus thuringiensis*, *Bacillus subtilis*, *Pseudomonas fluorescens*
9. Oil Spills	*Pseudomonas putida*
10. Food • Bakery • Probiotics • Flavouring agents • Single cell protein	*Saccharomyces cerevisiae* *Lactobacillus* and *Bifidobacterium* *A. niger* and *Pycnoporus cinnabarinus* *Bacillus*, *Hydrogenomonas*, *Methanomonas*, *Methylomonas*, *and Pseudomonas* (bacterial strain), *Saccharomyces*, *Candida*, and *Rhodotorula* (yeast strain), *Fusarium*, *Aspergillus*, and *Penicillium* (fungal strain) and algal strain of genus *Spirulina*

Figure 1. Microbial products

The various products used by industries along with the type of microbe used are mentioned in the **Table 1** and **Figure 1** [4, 14].

2. 1. Microbial enzymes

They are the most important area of interest for the industries as they are stable, can be easily produced and optimized. They are used by food, agriculture, chemical, pharmaceuticals industries etc to reduce their time, cost and nontoxic compounds. They can degrade the waste into something valuable. Uses of microbial enzymes in various industries are-

i. <u>Pharmaceutical industries-</u> Microbes are used in pharmaceutical industries for-biocontrol of diseases, production of vaccines, production of antibiotics and production of biotherapeutics. Majority of diseases are caused by mosquitoes like of genera *Aedes* and *Anopheles*. Recently it has been discovered that bacterium *Wolbachia* an endosymbiont of *Aedes aegypti* mosquito that spreads dengue, yellow fever and chikungunya, can be used to reduce life span of this mosquito. Many vaccines are been developed recombinant, live and attenuated dengue virus vaccine, HPV (human papilloma virus) vaccine for cervical cancer, DNA vaccines containing genes encoding for immunogenic antigens of interest [15]. Hormones (calcitonin, parathyroid hormone, human growth hormone, glucagon, somatropin, insulin and

glucagon), anticoagulants, antibodies or antigens are produced in industries using recombinant microbial cells like- bacterium *E. coli* and yeast *S. cerevisiae* [14].
Enzymes like

- L-tyrosinase, galactosidase are produced by *E.coli*, *Pseudomonas acidovorans* and *Acinetobacter* has anti tumor activity.
- Streptokinase, urokinase produced by *Streptococci* sp., *Bacillus subtilis* are used as anti-coagulants.
- Enzymes are also used to determine diabetes and other health disorders like- glucose oxidase that detects glucose, urease detects urea, lipase, carboxyl esterase, and glycerol kinase detects triglycerides [5].

ii. <u>Food industry-</u> In food industries, microbes are used in two different roles. First, used as starters in fermentation and second for production of various foods. In bakery, microbes like yeast are used that help to improve and soften the dough, increase the shelf life, and improve texture, colour and freshness of bakery products. Various genetic engineering methods are used to modify yeast properties so that its performance in fermentation can be improved [14]. Various microbial enzymes used in bakery, brewing, dairy, juice production etc are

- Amylase, lipase and xylanase are used in bread making.
- Enzymes like proteases, lipases, esterases, lactase, catalase, etc are used in dairy industry to enhance flavour, aroma, colour and yield of products. Rennet enzyme is used to coagulate milk into curd for producing cheese.
- Cellulases, amylases, and pectinases are used in processing fruit juice. Microbial amylases are used to hydrolyse starch to sugar in fermentation of alcoholic beverages [5].
- β-lyase produced by yeasts improves the sensory properties of wines by the release of aromatic thiol [14].

iii. <u>Polymer industry-</u> Biodegradable polymers like- poly (beta-hydroxybutyrate; PHB), poly (beta-hydroxyvalerate; PHV) and poly (hydroxybutyrate-co-valerate; PHB-V) can be made using microbial enzymes as they are eco-friendly and degrade biologically. Example- Lipases used to produce polyesters or polycarbonates [5, 14].

iv. <u>Paper and pulp industry-</u> To remove lignin and hemicelluloses from the pulp, Xylanases and ligninases are used. Amylase, cellulase and lipase are used in starch coating, deinking and improving softness.

v. <u>Leather industry-</u> Alkaline proteases, neutral proteases, and lipases are used in leather industry to remove non fibrillar proteins, dehairing and degreasing to remove fats respectively.

vi. <u>Textile industry-</u> *Bacillus* sp. produces amylase, laccase, protease that helps in desizing, fabric dyeing and degumming of silk. *Aspergillus niger* produces cellulase, catalase that helps in cotton softening, denim finishing and bleach termination [5].

vii. <u>Waste treatment-</u> Many microbial enzymes are being used to degrade toxic products like- phenols, aromatic amines, nitriles, etc in the waste that could be hazardous. Industrial effluents contain chlorinated phenolic compounds that could be degraded by using various enzymes mentioned in **Table 2** [14].

Table 2. Enzymes used for degradation of various Pollutants.

Pollutant	Enzyme used for degradation
1. Herbicides, insecticides, fungicides	Monooxygenases and dioxygenase
2. Triglycerides	Lipase
3. Phenols, biphenols, and chlorophenols	Peroxidases, polyphenol oxidases, and tyrosinase
4. Benzopyrene	Laccase
5. Lignin	Manganese peroxidases

Now day's researchers are focusing on the use of microbial fuel cells (MFC) to treat solid waste. In this microbial cells use electrons produced by organic components of waste and generates energy. In this mixed MFC cultures can also be used against wide variety of substrates [14].

viii. Agriculture- Microbes used in agricultural industries should have pesticidal, herbicidal, insecticidal or nematicidal activities. For example

- Chlamydospores of *Phytophthora palmivora* are mycoherbicide that is used to control *Morrenia odorata*.
- *Puccinia canaliculata* is used to control yellow nutsedge weeds.
- The best pesticides used are the Cry and Cyt endotoxins produced by the *B. thuringiensis* (Bt) bacteria that help to control pests in cabbage, potato, and grains. Now a days many transgenic plants such as tomato, tobacco, and corn having these endotoxins have been produced that are effective against spread of caterpillar especially *Lepidoptera*.
- Metabolites known as avermectins produced by bacterium *Streptomyces avermitilis* are toxic against nematodes but non-toxic to mammals.
- *Trichoderma* spp. is effective against many phytopathogenic fungal species such as *Sclerotinia*, *Fusarium*, *Verticillium* and *Macrophomina* [14].

ix. Biosensors and Biomaterials- Biosensors use the microbes that can produce measurable signals depending on concentration of analytes and are used in medicine, environmental monitoring, food processing etc. Immobilized recombinant E. coli cells expressing organophosphorus hydrolase (OPH), *S. cerevisiae*, for Cu2++ and *P. putidaas* used as a biosensor for catechol, nitrophenol, benzene, toluene, are some of the examples of biosensor. Biomaterials are produced by microbes naturally or artificially like- bioplastics, Polysaccharides (chitosan, alginate, xanthan gum, and cellulose) are used in medicine industries [14].

The main source of producing these industrially important enzymes like- amylases, cellulases, proteases, chitinases, xylanases and pectinase is the Actinomycetes by using proteomics and metaproteomics [24]. Some examples include-

a) **Lignocellulolytic enzymes** are used to degrade the lignocellulose present in plants biomass. It includes cellulase, hemicellulase and lignolytic enzymes.

- Cellulase are produced from *Cellulomonas fimi*, *Microbispora bispora*, and *Thermobifida fusca* used in production of bioethanol, in ligand binding studies, textile industry, pulp and paper making.
- Hemicellulase consists of xylan and mannan components used in biobleaching, deinking of paper waste, clarification of fruit juices, upgradation of feed, fodder and fibres, and saccharification of hemicelluloses. Xylanase produced from *T. bifida*
- Lignolytic enzymes including laccase, manganese peroxidases and lignin peroxidases are used in biofuel production, paper, textile, food industry, wastewater treatment, and bioremediation, and organic synthesis, cosmetic and pharmaceutical industries [23].

b) **Protease** from *Streptomyces pactum* and *S. thermoviolaceus* used in pharmaceutical, leather, food, brewing and detergent industry.
c) **Keratinase** from *Actinomadura keratinilytica* used in leather industry.
d) **Amylase** from *Streptomyces erumpens* and *Thermobifida fusca* used in detergent, baking, textile, paper and pulp.
e) **Lipase** from *Streptomyces exfoliates* and *Nocardiopsis* alba used in cosmetics, detergent, paper and pulp industry.
f) **Chitinase** from *Streptomyces thermoviolaceus* and *Nocardiopsis prasina* used in textile and leather industry.
g) **Pectinase** from *Streptomyces lydicus* used in beverage and textile.
h) **Dextrinase** from *Streptomyces* spp used in degradation of dextran and in production of sugar.
i) **Peroxidases** and **tyrosinase** from *Nocardia* spp used in textile dyes and in wastes treatment plant.
j) **Nitrile hydratase** from *Pseudonocardia* used in the biotransformation.
k) **Cutinase** from *Streptomyces* spp. and *Thermobifida fusca* [24].

2. 2. Antibiotics

Actinomycetes are used for production of wide amount of antibiotics that are used to improve human health by acting against the pathogens. The first antibiotic used for human treatment was the Penicillin and since then there are numerous antibiotics that have been discovered and being used. The genus Streptomyces has been used for numerous valuable chemicals and is used in production of streptomycin (*Streptomyces griseus*), chloramphenicol (*S. venezuelae*), tetracycline (*S. rimosus*), erythromycin (*Saccharopolyspora erythraea*), leucomycin (*S. kitasatoensis*), vancomycin (*S. orientales*), gentamicin (*M. purpurea*) actinomycin and streptothricin [25].

3. SELECTION AND ISOLATION METHODS FOR MICROBES

To select our microbe of interest, there are various strategies which we have to follow like- decide your activity of interest, choose the microbe having your activity of interest, isolation of that microbe, and develop enrichment culture and other screening methods.

- Primary screening is done to isolate microbe from large population using simple methods. Spread plate method is used in which the sample is spread on the plates containing agar. Other than this, pour plate and streaking is also done. It is used for microbial testing and for isolation purposes [18]. In indirect assays, an agar-plate medium along with colorimetric reaction is used to grow the organism producing protease, cellulase etc. Here the parameters like- pH, temperature etc can easily be manipulated. Bioassays have also been developed to select organisms with antibiotic or antitumor activity, insecticidal agents and surfactant producing microbes are detected using blood agar.

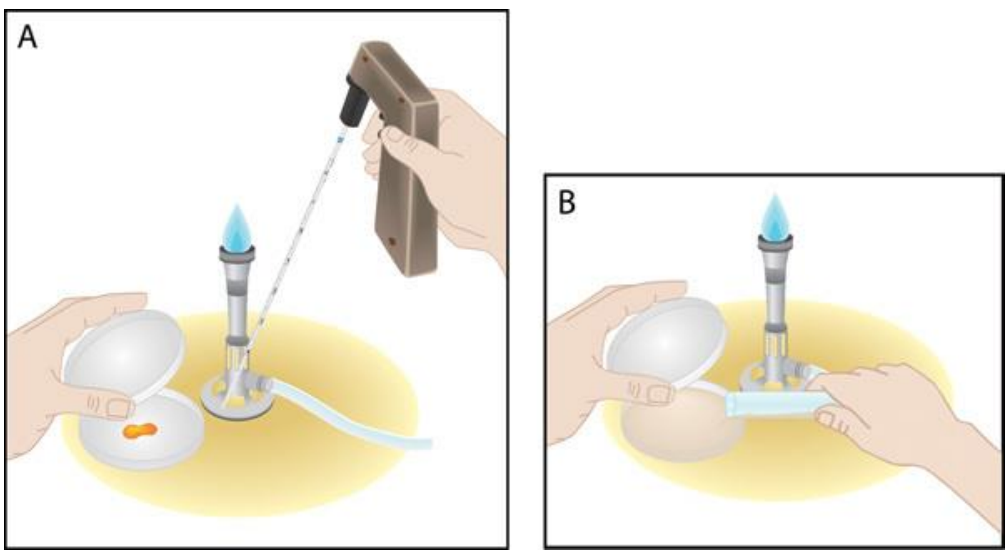

Figure 2. Pour plate method [18]

- Apart from this, secondary screening is also used that allows to further isolate industrially important microbe having our activity of interest from primary screening by using more precise and specified analytical techniques like- mass spectrometry, HPLC and gas chromatography. It is highly efficient and productive as large number of samples can be screened [2]
- Microbe of interest upon isolation is characterised based upon growth intensity, pattern, colony colour and soluble pigments formed by streaking them on medium like- oat meal agar, inorganic salt starch agar, glycerol asparagine agar, peptone yeast extract iron agar and tyrosine agar. Various physiological and biochemical tests, enzymatic assays and hierarchical cluster analysis are performed.
- After these desired metabolites are produced, extracted, purified and characterised [16]. The majority of industrially important microorganisms are isolated from soil or water samples but can also be found in thermal vents, glaciers, waste water etc [2]. Different genera of

actinomycetes have been isolated using novel methods like- pre-treatment techniques along with enrichment methods. Serial dilution, pour plate, streaking and centrifugation techniques are used to isolate them but for selective isolation 6 approaches are being used:

a. Nutritional selection- In this media component is used that are essential only for actinomycetes. The carbon and nitrogen source used include- starch (or glycerol), chitin, casein, nitrate and L arginine that only favours actinomycetes.
b. Selective inhibition- It includes the use of antifungal like- Actidione, nystatin and pimaricin and antibacterial agents' like- Nalidixic acid, trimethoprim, tunicamycin, leucomycin, faridomycin, kanamycin, chlortetracycline etc.
c. Pre-treatment of sample- It includes the use of physical methods like- moist incubation using radiation, glycerol, air dry, dry heat, centrifugation, cellulose infiltration, pollen baiting followed by drying etc. and chemical methods like- use of calcium carbonate and chitin treatment, calcium chloride, Phenol, SDS, yeast extract, Germicide, Chemotactic agents, and Chloramine-T etc. to remove contaminants.
d. Enrichment method- It includes the use of additional supplements in nutritional media that only favours actinomycetes for example- coal-vitamin medium, Rehydration and centrifugation (RC) method etc.
e. Membrane filter method- 0.22-0.4545µm pore size cellulose ester membrane filter is overlaid on nutrient agar medium that allow actinomycetes to penetrate the pores of membrane as it possess highly branched hyphae.
f. Integrated method- There is use of combinations of treatments and is most preferred method. In this physical chemical method along with antibiotics, pollen-baiting and drying method and $CaCO_3$, rehydration and centrifugation method are used in combination.

Apart from these methods, high throughput cultivation (HTC) technology that uses agarose microcapsules having single cells trapped in it and fluorescence-activated cell sorting (FACS) that distinguish between fast and slow growing microbes are also being used recently [26].

Figure 3. Spread plate method [18]

Figure 4. Streaking [19]

3. CULTIVATION

Fermentation broth must be used for producing majority of biologically active ingredients in actinomycetes strains. Solid and liquid fermentation are mainly used for cultivation of actinobacteria. Fermentation strategies including broth composition, concentration and pH as well as aeration, time and temperature varies for different strains.

a) Liquid fermentation- Fermentation via 4 to 8 broths must be done for every strain for approx. 4-7 days depending on the optimum conditions. For analysing active ingredients produced by actinomycetes various fermentation broths can be utilised such as-

- <u>Seed culture-</u> 30-60 hours are the fermentation time for seed culture on shaker. YIM 38 broth and YIM 306 broth can be used for this purpose.
- <u>Fermentation culture-</u> YIM 301 broth, YIM 302 broth, YIM 305 broth, YIM 307 broth, YIM 308 broth, YIM 310 broth, YIM 61 broth and YIM 312 broth can be used for this method.

b) Solid fermentation- This method can also be utilised in cultivating actinomycetes in some research stages. As compared to the liquid fermentation process, actinomycetes produce more amounts of active ingredients via this process. The optimum components of medium vary according to the actinobacteria thus it's not necessary that all actinomycetes can grow in solid fermentation [27].

4. MEDIA

The media used to grow microbes consists of carbon source, nitrogen source, solidifying agents, trace elements, vitamins etc. Various supplements are used like peptone, yeast or beef extract, growth supplements like- tomato juice for *Lactobacillus*, hay extract etc [6]. pH of media is also maintained by adding buffers. Media can be liquid called nutrient broth in which the nutrients are dissolved in water or solid gel with agar or gelatin that is used for bulk growth of mycorrhizal fungi. Microbes need various nutrients to grow which include

i. Water- It helps in solubilising, transporting of nutrients and their hydrolysis [17].

ii. Carbon source- It is most important component as it provides energy to the microbes and helps them to grow and produce various products. Various carbon sources are used for various products like- Galactose used for antibiotic production, Glucose is specifically used for penicillin production, Glycerol used for Actinomycin D, Cephalosporin, Fructose and Maltose for Gentamycin, Sucrose for Erythromycins and Starch for Kanamycin.

iii. Nitrogen source- It is also important for the microbes to produce desired metabolite. Microbes can use both organic and inorganic sources. Examples of nitrogen sources are- NH_4^+, Urea, Nitrate, various amino acids, meat extract, and peptones can also be used.

iv. Phosphates- It is used by the microbes to make their cell membranes or nucleic acids. But it is added to media in low concentrations as it has inhibitory effect on secondary metabolite production [7].

v. Solidifying agents- Agar is most commonly used solidifying agent extracted from seaweed. Apart from this gelatin, silica gel etc can also be used.

vi. Growth factors- It helps to increase bacterial multiplication. Various growth factors includes-Amino acids like- glutamic acid and valine, Vitamins as they are precursors of cofactors like- biotin, folic acid, riboflavin or vitamin B12 and Antioxidants in case of strict anaerobes [17].

Selective culture media are used to isolate specific microbe only and to delete the unwanted microbe by adding certain inhibitors like-

i. Antibiotics- Many antibacterial and antifungal antibiotics can be added in the culture medium example- penicillin G, bacitracin or vancomycin for gram positive bacterial species, colistin or polymixin B for Gram-negative bacteria and Amphotericin B, cycloheximide or nystatin for fungal and yeast species.
ii. Sodium salts- They are added to isolate halophilic bacteria.
iii. Other chemicals- Chemicals like- potassium tellurite and bile salts inhibits Gram-positive and lithium chloride inhibits Gram-negative bacteria.
iv. Dyes- They are used as colour indicators like- Crystal violet is used to select *Streptococci* [17].

In order to grow fungi used for amylase production starch agar is used that contain peptone, yeast extract, KH_2PO_4, agar as solidifying agent, starch and chloramphenicol as antibiotic. If we have to isolate fungi that produce cellulase, we use cellulose media and for protease production use Casein agar [3]. These media used routinely are very costly so scientists are trying to find alternative and cheap source to grow these microbes. One such approach is the use of organic waste i.e. fruit peel waste to grow microorganisms as they contain complex and simple sugars and they can be used to produce bioethanol, biogas, animal feeds and it also solve the problem of pollution caused by dumping them. It is a unique approach in which microbes convert these fruit waste into valuable products. Usually fungus are grown on expensive and chemical media like- Potato dextrose agar but it has been reported that some fungus like- cellulase producing *Aspergillus niger* has been grown on pineapple waste, lipase producing *A. fumigatus* grown on sugarcane bagasse and *Penicillium chrysogenum* was grown on pine apple, mango, sweet lime and pomegranate. [8].

Figure 5. Microbes grown on media [19]

5. CULTURE STORAGE AND PRESERVATION

Microbial culture needs to be stored in stable conditions to avoid risk of contamination, loss of viability or mutations. The most important methods include storage in soil culture, agar slants, mineral oil overlay, refrigeration at 4 °C, lyophilisation [9] and cryogenic storage.

A. Soil culture- Fungi species like- Penicillium chrysogenum, Fusarium sp. and Aspergillus sp. are being preserved on sterilized soils and then it is refrigerated [10].

B. Agar slants- They are being prepared by pouring media in test tubes and keeping it in slant position. Microbes can be preserved here but media need to be periodically changed. But it is for short term storage only.

Figure 6. Agar slant culture [20]

C. Mineral oil overlay- Many bacterial cultures can be stored under sterile paraffin oil. It provides many advantages like- less contamination, no preliminary treatment required, organisms live longer, can be used for single or mass colonies, no seals like- rubber caps required, no special apparatus needed etc [11].

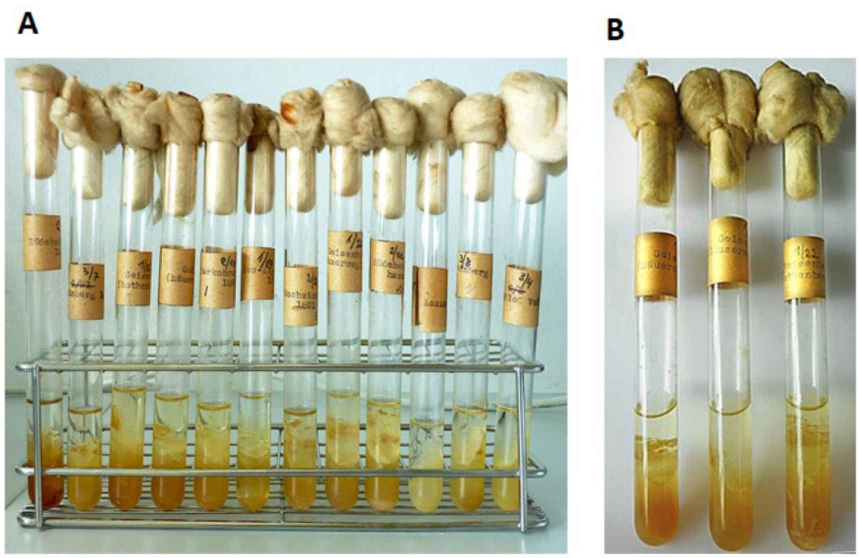

Figure 7. Oil overlay [28]

D. Refrigeration- The cultures that are needed daily grown on agar slants or agar plates can be stored at refrigerator at 4˚C to avoid contamination.

Figure 8. Refrigeration [21]

E. Lyophilisation- It is used for long term storage of microbial cultures like- bacteria, yeast and sporulating fungi etc. In this cells are freezed at low temperature and then water is removed from the cells using vacuum. A lyoprotectant is generally used such as sucrose [12].

F.

Figure 9. Lyophilisation [22]

G. Cryopreservation- In this method, the microbial samples are stored at −80 °C i.e. dry ice or at −196 °C in liquid nitrogen. Such a low temperature protects the cell, its DNA and proteins from damage and halts the cellular activities for long term. Cryovials are used along with cryoprotectants like- 10–15% glycerol or 5–10% DMSO which protects the cells from cryo-injuries and formation of ice crystals in the cells [12].

Figure 10. Cryopreservation [29]

6. CONCLUSIONS / FUTURE POTENTIAL

Our earth contains unique environments like- high altitude deserts, thermal springs, volcanic eruptions etc. that are still unexplored and can contain large number of microbes that can be isolated and evaluated for new products [2]. Still a lot of microbial strains including that of actinomycetes and their products are unknown to us which need proper methods to be isolated and being used by mankind. Many new methods have been developed or are developing to use media for selective isolation of microbial strain. Media optimization is another field that need to be enhanced so that various carbon and nitrogen source can be screened to improve microbial growth, viability and yield. Expensive components of media should be replaced by cheaper components. In order to increase microbial productivity, genetic manipulation can also be used. To reuse microbe for long term fluidized bed bioreactor should be designed [7].

In future better screening programs could be developed by making progress in genetic and protein engineering areas. For now, these methods are being used to increase the stability and specificity of microbial proteins like- alkaline protease subtilisin. In order to increase the gene expression and product formation, genetic promoters are being developed. In order to find microbes with specific DNA sequence, colony hybridization method or PCR based methods can be developed. Screening programs are still based on classical techniques of enrichment and mutagenesis although advance instrumentation, genetics and microbial physiology are developing. [2]

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