Subject: Botany

Semester:Vth

Paper: DSE-1

Topic: Isolation of microorganism from air, soil and water.

A microorganism or microbe is a microscopic living organism, which may be single-celled or multicellular. Microorganisms are generally found in nature (air, soil and water) as mixed populations. The process of obtaining a pure culture by separating one species of microbe from a mixture of other species, is known as isolation of the organisms. The primary culture from natural source usually a mixed culture containing microbes of different kinds. But in laboratory, the various species may be isolated from one another. A culture which contains just one species of microorganism is called a pure culture. A culture is a population of microorganisms grown under well defined conditions. A mixed culture contain a very small number of particular species of microbe in comparison to the total number of microorganisms , such culture is called as mixed culture. A culture containing only one species of microbe is called pure culture.

The microbial content of the outer environment usually change with the season and atmosphere contains various pollen, spores of fungi, viruses etc. All the major groups of microbes remain suspended within the atmosphere. The microbial flora of the atmosphere is transient and variable. These air-spora or air-born particles can cause disease in living beings Among these air-borne particles, the fungal spore is the most important air-spora. The air is a good carrier for microorganisms but it is not good medium for their growth. The numbers and the types of suspended microorganisms on-air varies depending on source and location. Microorganisms occur in natural environment like soil and are mixed with several other forms of life. Many microbes are pathogenic and can cause a number of diseases with a variety of symptoms. The isolation and growth of microbe in pure culture is essential for the identification. To study the specific role played by a specific microorganism in its environmen isolation of the microorganisms in pure culture is needed.

Microbial content of the air sample can be determined by **Solid impingement devices and liquid impingement devices**. In Solid impingement devices, a solidified agar plate or sticky faced microscope slide or filter disk is used on which the microorganisms are impinged or collected directly whereas in liquid impingement devices, a broth medium or sterile water is used, through which a fine spray of air sample is passed and the organisms are trapped on them. Then the aliquots are collected from the broth or water and cultured to identify the microorganism. There are different **types of impactors such as the Casella slit samplers, the Cascada impactor, Hirstspore trap, the Andersen sample**r. Different types of **impingers can be used such as the porton impinger, pre-impinger, the multistage liquid impinger.** To study microbial content of air, two methods are generally used such as **gravity slide and settle-plate techniques. In gravity slide methods,** glycerin jelly is applied over microscopic glass slides, the petroleum jelly or silicone grease or vaseline is then exposed horizontally, adhesive surface facing upwards in a shelter, so that they are freely exposed to the air for a particular period of time. Then trapped pollen and bacteria on slide is observed using a microscope. In case of the **settle-plate method**, a petri dish containing agar

medium is horizontally exposed for 5-10 minutes. After that the plates are incubated and appeared colonies were counted over the plate. Principle behind the Isolation of microorganism from air by using the settle-plate technique. In this method a suitable medium is poured over a sterile petri dish and then allowed to solidify. After that the plate is exposed to the open air for a few minutes. Then the plates are incubated for the formation of microbial colonies on the plate. Thee colonies were observed using a microscope. Czapek-Dox agar medium with rose bengal is used for fungal cell isolation. The Czapek-Dox agar is an optimum growth medium for *Aspergillus brasiliensis, Candida albicans, Saccharomyces cerevisiae, Aspergillus niger.* The Nutrient agar medium is used to grow both spore-forming and non-spore-forming gram-negative cocci, gram-positive bacilli, and gram-negative bacilli.

There are special techniques employed to obtain pure cultures of microorganisms. In few cases it is possible to obtain pure culture by direct isolation or direct transfer. Following isolation methods are used to isolate microbes from mixed cultures

1. **Streaking**: This is the most widely used method. The technique consists of pouring a suitable sterile medium into sterile petriplate and medium is allowed to solidify. In This method the tip of a fine structure wire loop called Inoculation needle consist of a wooden or glass handle with a nichrome wire the end of which is bend to form a loop is used to transfer microbes from culture. The straight wires are similar to wire loop except they do not have loop. These are used to transfer culture in colony formed on solid culture medium. By means of a sterile loop or straight needle or a sterile bent glass-rod a small amount of microorganisms from a broth culture or bacterial suspension is streaked back and forth across the surface of agar until about one third of the diameter of the plate has been covered. The thin film in the loop is streaked in either a zig-zag manner by removing the loop backwards and forwards.

2. **Plating**: It includes diluting of a mixture of microorganisms until only a few hundred bacteria are left in each millilitre of the suspension. A very small amount of the dilution is then placed in a sterile petriplate with a sterile loop or pipette. The melted agar medium is cooled to about 45°C and is poured into plate. The microorganism and agar are well mixed. When the agar is solidified and microorganisms grow to a visible colony.

3. **Dilution**: It is a method used for the microorganisms which cannot be easily isolated by streaking or plating method. Sometimes when several organisms are present in a mixture, with one organism predominating, the predominating form may be isolated by this method.

4. **Enrichment Procedure:** The procedure involves the use of media and conditions of cultivation which favour the growth of the desired species. It is used to isolate those microorganisms, which are present in relatively small numbers or that have slow growth rates compared to the other species present in the mixed culture. This provide a specially designed cultural environment using a specific nutrient in the medium and by modifications in the physical conditions of the incubation. Malachite green and crystal violet, are used to inhibit the growth of bacteria and yeast. Sodium azide inhibits the growth of anaerobic bacteria.

5. **Single Technique:** This is one of the most ideal and difficult method of securing pure culture. Here a suspension of the pure culture is placed on the under-side of a sterile cover-glass mounted over a moist chamber on the stage of the microscope. A single cell is removed with the help of sterile micropipette under the microscope and transferred to a small drop of sterile medium on a sterile cover-glass and is mounted on a sterile hanging drop slide, which is then incubated at suitable temperature.

6. **Serial dilution method:** It is one of the most old and usable method which is used for the isolation of bacterial colony. In this method samples (from soil water, milk, food) are collected and its dilution in the test tube are made. It is used for those microorganisms that have not yet been successfully cultivated on solid media and grow only in liquid media. The inoculum is subjected to serial dilution in a sterile liquid medium, and a large number of tubes of sterile liquid medium are inoculated with aliquots of each successive dilution. The sample from the diluted test tubes is inoculated in the prepared Nutrient Agar plates by using Pure Plat Method and then incubated the cultured plates at 37 degree Centigrade for 24 hours. After 24 hours the cultured plates were observed. The aim of this dilution is to inoculate a series of tubes with a microbial suspension so dilute that there are some tubes showing growth of only one individual microbe.

Micromanipulator have been built, which permit one to pick out a single cell from a mixed culture. This instrument is used in conjunction with a microscope to pick a single cell (particularly bacterial cell) from a hanging drop preparation. The disadvantages are that the equipment is expensive and it requires a skilled operator.

Preservation and maintenance of pure culture

Once a microorganism has been isolated and grown in pure culture, it becomes necessary to maintain the viability and purity of the microorganism by keeping the pure cultures free from contamination. Normally in laboratories, the pure cultures are transferred periodically onto or into a fresh medium (subculturing) to allow continuous growth and viability of microorganisms. The transfer is always subject to aseptic conditions. Pure cultures may be stored by refrigeration, paraffin method, cryopreservation, and lyophilization.

A. REFRIGERATION- Pure cultures can be successfully stored at 0-4°C either in refrigerators or in cold-rooms. This method is applied for short duration (2-3 weeks for bacteria and 3-4 months for fungi). Their growth continues slowly, nutrients are utilized and waste products released in medium. This results in, finally, the death of the microbes after sometime.

B. Cryopreservation- It (i.e., freezing in liquid nitrogen at -196°C) helps survival of pure cultures for long storage times. In this method, the microorganisms of culture are rapidly frozen in liquid nitrogen at -196°C in the presence of stabilizing agents such as glycerol that prevent the formation of ice crystals and promote cell survival.

C. Lyophilization- (Freeze-Drying)- In this method, the culture is rapidly frozen at a very low temperature (-70°C) and then dehydrated by vacuum. Under these conditions, the microbial cells are dehydrated and their metabolic activities are stopped. Lyophilized or freeze-dried pure cultures and then sealed and stored in the dark at 4°C in refrigerators.