Enrichment, Isolation, Purification and Characterisation of Nitrogen Fixing Organisms

by

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Pure cultures of microorganisms are not usually found in natural habitats. They live in close associations with other organisms. However, much of the present knowledge in microbiology is based on studies with pure cultures. Hence, it is important to learn how to isolate, purify and characterise microorganisms. Nitrogen fixing organisms constitute a very minor porportion of all the microorganisms reported so far. Therefore it would be appropriate to study the general criteria used in these procedures and then look at the details involved in the case of nitrogen fixing microorganisms.

a. Enrichment

For successful isolation of a given organism into pure culture, the organism must generally comprise a sufficiently high proportion of the mixed population. Isolation is easily achieved when the organism in question is the numerically dominant member of the population. Enrichment methods are designed to get an increase in the percentage numbers of a selected organism by accelerating its growth, survival or its spatial separation from other members of a natural, mixed population.

The methods of enrichment could be categorized as follows,

a.1 Physical Methods of Enrichment

Physical criteria like growth temperature, heat treatment and ultra violet irradiation could be used to kill or inhibit all types of organisms other than the organism of interest. Some physical criteria such as cell size and motility could be utilized to concentrate the desired organisms by preferential separation from the rest of the population.

As an illustration let us consider the criterion motility.

a.1.1 Enrichment Method for Cyanobacteria

There are certain blue green algae, also called Cyanobacteria, which need light for growth. Consequently, they are phototactic, meaning they move towards sources of light. If a drop of a mixed culture of Cyanobacteria is placed in a single spot on the periphery of a solid medium in a petri dish and a source of light is kept on the opposite side, the phototactic bacterial cells will move towards the light. The cells which move away from the original place of inoculation could be collected as a pure culture.

a.2 Chemical Methods of Enrichment

These methods could employ toxic agents to kill or inhibit the rest of the population without affecting the desired organism. Alternatively, we could use a nutrient source that can be used preferentially by a particular component of the mixed population.

As an example we will consider the method of isolation used for Azospirillum and Azotobacter.

a.2.1. An Enrichment Method for Azospirillum and Azotobacter.

Almost all microorganisms need a fixed nitrogen source for growth in addition to a source of carbon, energy and several nutrients. But Azospirillum and Azotobacter can fix atmospheric nitrogen gas in laboratory culture.

If we inoculate a medium having all other requirements for growth of bacteria other than nitrogen with a speck of soil having Azospirillum and Azotobacter and incubate for sometime we expect to enrich for organisms which can fix atmospheric nitrogen.

a.3 Biological Methods of Enrichment

These methods make use of specific hosts for selective multiplication of a parasitic or a symbiotic bacterium or else the ability of a pathogenic microorganism to invade a host.

The nodule forming ability of rhizobia on a specific legume host could be considered as an example.

a.3.1 Enrichment for Rhizobia

Soils naturally contain different kinds of rhizobia which can form nitrogen fixing nodules on legume roots. If we want to selectively enrich the type of Rhizobium which nodulates a particular legume, for example, Soy bean, Soy bean seedlings could be grown under sterile conditions and a small volume of soil can be added to the medium in which the root is grown. After sometime the nodules will

grow on the roots of the Soy bean seedlings. If the nodules are collected under sterile conditions and spread on an artificial nutrient medium under laboratory conditions, then we can enrich for the Rhizobium, specific for Soy Bean.

b. Isolation

Isolation could be done on solid or liquid media according to the most suitable method of growth for a particular organism. For isolation of nitrogen fixing organisms media without a nitrogen source can be used.

b.1 Isolation on Solid Media

b.1.1. Spread Plate Method

For this method a solid growth medium should be prepared in a petri dish and a drop of the enriched culture is spread on the surface of the solid medium under sterile conditions. The cultures are allowed to grow for a few days and when isolated colonies appear, they are spread again and again to make sure there are no contaminating organisms.

b.1.2. Streak Plate Method

A solid medium is prepared as in b.1.1. and a drop of the enriched medium is streaked on the solid medium.

Incubation of the medium could be carried out as in b.1.11.

b.1.3 Pour Plate Method

The only difference here is that the cell culture is mixed with the medium before the plates are been poured.

b.2. Isolation on Liquid Media

This method is chosen when the organism of choice cannot be grown on solid media. Prepare a dilution of the enriched culture so that an aliquot of 1 ml contain less than 0.05 organisms. Prepare a large number of tubes containing growth medium. Add a 1 ml aliquot of the dilute culture to each tube. Inoculate the tubes till a growth is visible. These tubes would have most probably received a single bacterium. Therefore the culture would be most probably pure.

c. Purification

Purification is usually achieved by growing an isolated organism on a nutrient rich solid medium, incubating till growth is visible in the form of colonies. A pure culture should yield colonies that appear similar to one another, and microscopic observation of the culture should reveal cells that are resonably similar to each other in appearance.

d. Characterization

In microbiology all organismswhich are similar in certain characteristics are grouped together. This helps in studying and communicating among microbilogists. To make this effective, we have a set of international rules and recommendations.

First of all, we have to study the characteristics of known microorganisms as much as possible. Then according to the similarities and variabilities shown in the different types of organisms we have to work out a system of classification. The basic unit in microbiological classification is the SPECIES. For each species we have a TYPE STRAIN In a permanent collection. Examples of permanent microbiological collections are the American Type Culture Collection in Rockville, Madison, USA or the National Collection of Type Cultures in London, England. When you find a microorganism, which has to be characterised, we check the properties of the new organism with the ones reported before, find the catogory to which it should belong and as a final check send it to and International Collection for comparison with the type strain. This confirms the final characterization of the new organism.

There is no official, internationally accepted classification of microorganisms. However, the scheme for most widely used classification is given in Bergey's Manual of Determinative Bacteriology.

d.1. Specific Methods of Characterization for Nitrogen Fixing Organisms

There are two well known methods used for detecting nitrogen fixing organisms, namely,

(a) 15 N Incorporation and

(b) Acetylene Reduction Assay

The principle of the acetylene reduction assay is based on the presence of the nitrogenase enzyme complex in all organisms capable of nitrogen fixation. This enzyme is capable of reducing acetylene (instead of its normal substrate, nitrogen) to ethylene. The ethylene production can be quantitatively determined by gas chromatography.

The organism to be tested is cultured under conditions conducive to nitrogenase formation. Acetylene gas is then added to the culture vessel, and the formation of ethylene is determined after a period of incubation.