Bio Science
First year
5-12-13

Chapter 10

AGRICULTURAL MICROBIAL INOCULANTS: INDUSTRIAL PRODUCTION ASPECTS

Bio-inoculant is a formulation containing one or more beneficial bacterial strains (or species) in an easy-to-use and economical carrier material, organic, inorganic, or synthesized from defined molecules. The desired effects of the inoculant on plant growth can include nitrogen fixation in legumes, biocontrol of soil-borne diseases, the enhancement of mineral uptake, weathering of soil minerals, and nutritional or hormonal effects.

A. BIOFERTILIZER

Biofertilizer - A misleading but widely used term meaning "bacterial inoculant" usually it refers to preparations of microorganism(s) that may be a partial or complete substitute for chemical fertilization (like rhizobial inoculants).

Organisms Used as Biofertilizers

Bacteria, blue green algae and a fern are used as biofertilizers

Bacteria	:	*Rhizobium, Azotobacter, Azospirillum*
Blue Green Algae	:	*Nostoc, Anabaena, Oscillatoria,*
Fern	:	*Azolla*

Role of Biofertilizers

Some of the biofertilizers convert atmospheric N_2 into nitrogenous compounds, which are used by plants e.g., *Rhizobium, Anabaena, Nostoc*, etc.

These N_2 fixers are of two types

(i) **Symbiotic nitrogen fixers:** Symbiotic nitrogen fixers live in association with other plants
e.g., *Rhizobium, Azobacter, Azospirillum, Nostoc, Anabaena* etc.

(ii) **Non-symbiotic nitrogen fixers:** Non-symbiotic N_2 fixers are free-living forms e.g., *Oscillatoria, Anabaena Nostoc, Bacillus, Clostridium*, etc.

Some biofertilizers convert inorganic phosphorus into organic compounds e.g., *Bacillus megatherium.*

Rhizobium

In the context of rhizobia, considerable change in taxonomic status has come about during the last few years. Sprigt and Jarvis outlined the current status of rhizobial taxonomy and enlisted 36 species described among seven genera (*Allorhizobium, Azorhizobium,*

Bradyrhizobium, Mesorhizobium, Methylobacterium, Rhizobium and *Sinorhizobium*) derived based on the polyphasic taxonomic approach. Although most *Rhizobium* isolates can nodulate more than one host species and also several different bacterial species are often isolated from a single legume, it is only from a few legumes that these symbionts have, so far, been investigated thoroughly. Among these, the family Fabaceae (formerly Leguminoseae) is important both ecologically and agriculturally, since it is a major source of biological nitrogen fixation.

Isolation of *Rhizobium* from nodules

Leguminous plants are carefully uprooted and the root system is washed in running water to remove the adhering soil particles. From the roots, healthy unbroken, firm and preferably pink nodules are selected and washed in water. They are immersed in 0.1 per cent acidified mercuric chloride for 4–5 min. Nodules, surface sterilized with mercuric chloride are washed repeatedly with sterile water and dipped in 70 per cent ethanol, followed by more washings with sterile water. The nodules are then crushed with the help of a glass rod. Serial dilutions are prepared from the nodule extract and appropriate dilutions are spread on yeast extract manitol agar (YEMA). The plates are incubated up to 10 days in an incubator at 26°C. Large gummy colonies of bacteria will emerge within 4–5 days. However, *Agrobacterium* a related genus may also grow on agar plates long with *Rhizobium*, which has to identified based on certain biochemical tests as given below:

Congo Red Test

This test is designed to differentiate *Rhizobium* from *Agrobacterium*. One per cent congo red dye is added to yeast extract manitol agar (YEMA). In this media, rhizobia stand out as white translucent and glitering colonies, while agrobacteria were found to have stained colonies.

Holfer's Alkaline Broth Test

In this test medium having high pH (about 11.0) is used to differentiate rhizobium from *Agrobacterium*. *Agrobacterium* will grow at high pH levels, while *Rhizobium* fails to grow.

Lactose Agar

Agrobacterium utilize lactose by the action of the enzyme keto lactase whereas *Rhizobium* cannot utilize the sugar.

Cultivation and Mass Production

Rhizobium is usually maintained by sub-culturing at frequent intervals on yeast extract mannitol (YEM) agar medium. The primary culture is designed as 'mother culture'. The selected strain is grown on YEM agar slants for 3-9 days depending on the growth rate. The cultures are then transferred from tubes to large flasks containing sterile solid or liquid medium for 4–9 days. This is known as the starter culture. Later the starter culture is transferred to seed tank fermenter at the rate of 1% volume. An oxygen partial pressure of 0.15 atmospheres is optimum and rhizobia grow best in the range of 30 to 32°C. A rhizobial count of 5×10^9 can be obtained in 96 hours with a lag phase culture of *R. japonicum* and within 24 hours for *R. meliloti* with an initial inoculum of the 5% volume of the medium. The factors influencing the output of cells are aeration, volume, initial inoculum level, bacterial strain, temperature and incubation time. Viable cell count of about $2–4 \times 10^9$ cells could be obtained by regulating these factors.

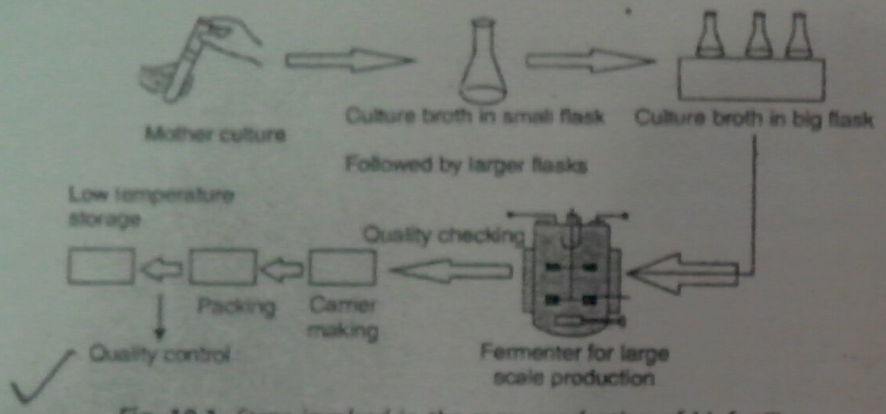

Fig. 10.1. *Steps involved in the mass production of biofertilizer.*

Checking the Broth

At the end of fermentation, the broth is checked for contamination by using the following parameters: pH test: if the broth is above eight or below six, the broth is to be suspected for contamiunation. 2) Agglutination test against 1/100 antiserum specific for the strain used 3) Gram staining for the detection of gram positive contaminants and spore formers 4) inoculation on peptone broth; if abundant growth takes place within two days, the broth could be suspected for contamination and, finally 5) Streak the broth on YEM agar for verification of the *Rhizobium* growth pattern.

Enumeration of *Rhizobium* Cells in the Broth

Normal plate count method is used to determine the viable count at 28±2°C incubation. Broth having viable cells higher than 10^9/ml cells may be used. It is not advisable to store the broth after fermentation periods longer than 24 hours, since the viable count of rhizobia begin to decrease. Hence the broth has to be incorporated and dispersed evenly into a carrier.

Mixing with the Carrier

In the process of mixing, the broth is sprayed to powdered peat or any other carrier material (Lignite, peat or vermiculite) and left in tubs, trays or on floor covered with polythene sheets for about ten days at 22–24°C. The product is again milled and packed in polythene bags where rhizobia increase in numbers.

Crop Response

An increase in grain yield of about 50% with *Rhizobium* inoculation was recorded in soybean, when compared with the uninoculated control. Significant yield increase was also reported in arhar (*Cajanus cajan*), chick pea (*Cicer arietinum*) and masur (*Lens culinaris*)

Azotobacter

Azotobacter (cyst forming), belong to the family Azotobacteriaceae comprising of 6 species, namely, *A. chroococcum*, *A. vinelandii*, *A. beijerinckii*, *A. nigricans*, *A. armeniacus* and *A. paspali*. *Azotobacter* is geturally regarded as a free-living aerobic nitrogen-fixer. *Azotobacter paspali*, which was first described by Dobereiner and Pedrosa, has been isolated from the rhizosphere of *Paspalum notatum*, a tetraploid subtropical grass, and is highly host specific. Application of *Azotobacter* has been reported to improve yields of both annual and perennial grasses. Saikia and Bezbaruah reported increased seed germination of *Cicer arietinum*,

Phaseolus mungo, *Vigna catjung* and *Zea mays* by *Azotobacter* inoculation. However, yield improvement is attributed more to the ability of *Azotobacter* to produce plant growth promoting substances such as phytohormone IAA and siderophore azotobactin, rather than to diazotrophic activity.

Azotobacter in Soil

The numbers of *Azotobacter chroococcum* in Indian Soils rarely exceed 10^4 to 10^5/g soil. There are two factors, which have major influence on the population of *Azotobacter* in soil. They have associative and antagonistic action of soil microflora and organic matter content of soil. Many microorganism are known to accelerate the growth of *Azotobacter* and its nitrogen fixation. Cellulolytic microorganisms, which degrade plant residues in soil, are known to encourage the proliferation of *Azotobacter* in soil. *Azotobacter* is also known to produce fungistatic substances, which inhibit the growth of fungi like *Altenaria*, *Helminthosporium*, *Fusarium* etc, when tested under laboratory conditions. *Azotobacter* cells are not usually present on the rhizoplane but are abundant in the rhizosphere.

Isolation

Azotobacter spp. can be isolated from soil by the soil dilution plating method.

1. A weighted sample of soil (10 g) is suspended in 90 ml of sterilized water and serial dilutions of the suspension are prepared by further dilutions.
2. One ml aliquots of appropriate dilutions are evenly spread over agar medium in petriplates.
3. The plates are incubated at 28°C in an incubator. After 3 days incubation, flat, soft, milky and mucoid colonies of *Azotobacter* develop on agar plates.

Production

Mother culture: A pure growth of any organism on a small scale is called as a mother culture. Mother culture is always prepared in a conical flask of 500 or 1000 ml. Capacity and then this mother culture is used for further production.

Production on a large scale: *Azotobacter* is multiplied on a large scale by two ways viz. Fermenter and Shaker. The fermenter is most automatic and accurate method of multiplication of any micro-organism. In this method, the medium is taken in a fermenter and then sterilized. After this pH of the medium is adjusted and mother culture is added. In order to get an optimum growth of the *Azotobacter* required temperature and oxygen supply is adjusted so that concentrated broth is made. This concentrated broth of the culture is then mixed with a carrier previously sterilized and bio-fertilizers is prepared.

In shake flask method; a suitable medium is prepared and transferred to conical flask of suitable capacity. These flasks are then sterilized in an autoclave at 15 lbs pressure for 15 minutes. Each flask is inoclulated with 10 ml mother culture and they are transferred to shaker for multiplication where they are kept for 72-90 hours. This broth is mixed with a suitable carrier previously sterilized. The biofertilizer is then, filled in plastic bags and stored in cool place.

Crop Response

The use of *Azotobacter* as bioinoculant started in late 1970 in India. In transplanted crops like tomato and brinjal, treatment of seedling with *Azotobacter* during transplantation resulted in a significant increase in yield from 20 to 29 per cent in tomato and about 42 per cent in brinjal has been reported

Azospirillum

Members of the genus *Azospirillum* fix nitrogen under microaerophilic conditions, and are frequently associated with root and rhizosphere of a large number of agriculturally important crops and cereals. Due to their frequent occurrence in the rhizosphere these are known as associative diazotrophs. Sen. (1929) suggested that the nitrogen nutrition of cereal crops could be met by the activity of associated nitrogen-fixing bacteria such as *Azospirillum*. This organism came into focus with the work of Dobereiner and associates from Brazil, followed closely by reports from India. After establishing in the rhizosphere, azospirilla promote the growth of plants. Despite their N_2-fixing capability (~1–10 kg N/ha), the increase in yield is mainly attributed to improved root development due to the production of growth promoting substances and consequently increased rates of water and mineral uptake. Azospirilla proliferate in the rhizosphere of numerous plant species and the genus *Azospirillum* now contains seven species—*A. brasilense*, *A. lipoferum*, *A. amazonense*, *A. halopraferens*, *A. irakense*, *A. dobereinerae* and *A. largimobile*.

Isolation

Conventional methods of isolation are not applicable for the isolation of *Azospirillum*. Enrichment procedures are required to obtain cultures of organism from plant roots as well as soil samples. The semisolid medium allows the organism to develop at low partial oxygen pressure. If ammonia is supplied to the medium, the organism grows ~~as an aerobe on agar slants~~ or in liquid media. However, it is incapable of fixing nitrogen. On ammonia containing medium, the doubling time is one hour whereas ~~on a malate containing~~ semisolid medium the doubling time is 5.5 to 7 hours (Okon *et al.*, 1977). The advantage of growing *Azospirillum* on ammonia containing medium is that a rich harvest of cells could be obtained for inoculation purposes.

Enumeration

Enumeration can be done by MPN method for counting *Azospirillum* from soils and roots. Up to 10 fold dilutions were made in mineral salt solution without malate. 0.1 ml of each dilution is transferred into nitrogen free brothymol blue medium (NFB) medium containing 0.025% malate and 0.025% cane sugar. Pellicle formation is a good evidence for *Azospirillum* growth. The presence of *Azospirillum* is further determined by Acetylene reduction assay (ARA).

Identification

On the semisolid malate medium, the development of white, dense and undulating white pellicle is very characteristic of *Azospirillum*. The organism is gram negative and contains poly-β-Hydroxyl butyrate granules. These organisms are characteristic curved rods of varying size with prominent fat droplets.

Mass Production

For large-scale cultivation, bottles or flasks with ammonium chloride containing liquid medium could be used and incubated on a rotatory shaker. The cells can be harvested for inoculation after three days incubation at 35°C on a rotatory shaker. The contents of the flask are incorporated into a carrier.

Crop response

Twenty years of evaluation of field experiments data showed 60–70% of all field experiments were successful with significant yield increases ranging from 5 to 30%. The effect of inoculation with *Azospirillum brasilense* on growth and yield of *Sorghum bicolor* in hydroponic systems was a significant as evident by the enhancement of dry matter content, leaf area development, and grain yield. At later stages of growth, leaf senescence was delayed in inoculated plants, thus favouring dry matter accumulation and grain filling (Sørig *et al.*, 1990). Inoculation of wheat with various strains of *Azospirillum* caused significant increases over controls in grain yield, ranging from 23 to 63 % (Caballero Mellado *et al.*, 1992). Inoculation of sunflower with *Azospirillum brasilense* Cd and *Azospirillum lipoferum* positively affected plant growth, especially under conditions of limited irrigation (Itzigsohn *et al.*, 1995).

Solubilization of Phosphates by Microorganisms

Phosphate solubilizing microorganisms (PSM) include largely bacteria and fungi, which can grow in media containing tricalcium, iron and aluminium phosphate, bonemeal, rock phosphate and similar insoluble phosphate compounds as the sole phosphate source. Such microbes not only assimilate P but a large portion of soluble phosphate is released in quantities in excess of their own requirement. The most efficient PSM belong to genera *Bacillus* and *Pseudomonas* amongst bacteria and *Aspergillus* and *Penicillium* amongst fungi. The reported bacilli include, *B. brevis*, *B. cereus*, *B. circulans*, *B. firmus*, *B. licheniformis*, *B. megaterium*, *B. mesentricus*, *B. mycoides*, *B. polymyxa*, *B. pumilis* and *B. subtilis* from the rhizosphere of legumes, cereals (rice and maize), arecanut palm, oat, jute and chilli.

Isolation of Phosphate Solubilizing Microorganisms

A known quantity of soil or rhizosphere sample (1g) is suspended in a known volume of sterile water and serial dilution of the suspension is made in sterile water blanks. Appropriate dilutions are plated on phosphate containing solid media for obtaining microorganisms capable of dissolving phosphates. The plates are incubated for 4–5 days. Transparent zones of clearing around microbial colonies indicate the extent of phosphate solubilization such cultures are isolated, identified and the extent of solubilization is determined qualitatively.

Quantitative Measurement of Phosphate Solubilizations in Culture Medium

Selected cultures are grown in 50–100 ml aliquots of Pikovskyay's liquid medium for 6–17 days at 28°C (±2). In the case of fungi, the culture is filtered using Whatman No. 42 filter paper. Due to pigments, the filtrate may often be coloured, in such case 1–2g of activated charcoal is added and shaken until the filtrate becomes colourless.

Bacterial cultures are filtered through Whatman No. 1 paper to remove insoluble phosphate and centrifuged at 10,000 rpm for 10-15 min. Filtration and centrifugation may be repeated until a clear solution is obtained which is finally made up to a known volume (50–100ml).

To 10 ml aliquot of the neat filtrate, 2.5 ml of Barton's reagent is added and the volume made up to 50 ml. After 10 min the resultant colour is read in a colorimeter using 430 Nm wavelength.

A standard curve is prepared by dissolving 0.2195g KH_2PO_4 in water and the solution made up to 1 litre. Further dilution of 10ml into 250 ml is made so as to give 1 ml = 2ppm. Aliquots of 2, 3, 4.5, 6, 8, 10, 15 and 20ml of the 2 pm stock solution are taken in 50 ml

volumetric flasks, 2.5ml of Barton's reagent added and the volume made up to 50ml mark with water. After 10 min, the colour developed is read in a colorimeter. A standard graph is then prepared from which P values for experimental sample are calculated.

Mixing with Carrier

Selected bacteria capable of high phosphate solubilization (ex. *Pseudomonas* sp., *Bacillus megaterium var phosphaticum*) are grown in Pikovskaya's broth for 7–10 days at 28°C (±2C) and mixed in suitable sterilized carrier. The mixture is cured for a week at 28°C in large trays covered with loosely fitting empty trays.

The inoculants is then packed at the rate of 300g per plastic packet and stored at 15°–20°C until use.

Normally the inoculants are used as early as possible and the seed treatment is in the same way as *Rhizobium* inoculants are used for seed inoculation.

Quality Assessment Tests for Bioinoculants

Quality of bioinoculants is one of the most important factors resulting in the success or failure and acceptance or rejection by the farmers. Quality in bioinoculant production refers to the presence of right type of microorganism in active form and required numbers. Quality has to be controlled at various stages of production, marketing and application.

Stages Requiring Quality Control

1. During mother culture stage
2. During carrier selection and sterilization
3. During broth culture stage
4. While mixing of broth in carrier
5. During packing
6. During storage

Testing of the culture is done by drawing samples from the finished product and checking it for standard specification at the time of mixing of broth in carrier.

General Test

A prescribed marking should be on the biofertilizer product like name of the product, crop for which it is applied, name and address of manufacture, date of expiry, method of application and other handling instructions.

pH of the Biofertilizer

Biofertilizer was taken and dissolved in 50 ml distilled water (1:2.5 ratios) it was sterilized well with frequent stirring with a glass rod and kept as such for an hour. The pH was measured using pH meter.

Moisture Percentage

The moisture percentage of biofertilizer was determined by weighing a known quality of biofertilizer and it was oven dried and again weighed. The loss in weight gives the moisture percentage of biofertilizer.

Sieve Size of the Carrier Material

The carrier material of the biofertilizer should pass through 106µ ISI sieve.

Quality Assessment Test

Microbial population was determined by serial dilution technique.

Microbial population = Average no population × dilution factor. The standard values regarding different parameters are given below

Table 10.1. Standard Values of Biofertilizers Used for Commercial Purposes

Parameters	Rhizobium	Azatobacter	Azospirillum	PSM
Total Viable count during entire period of shelf life	$>1 \times 10^7$	$>1 \times 10^6$	$>1 \times 10^6$	$>1 \times 10^6$
Contamination	Nil at 10^{-7} dilute	Nil at 10^{-5} dilute	Nil at 10^{-5} dilute	Nil at 10^{-5} dilute
pH Value	6.0–7.5	6.0–7.5	6.0–7.5	6.0–7.5
Strain Efficiency	Capable of fixing atleast population. The dry weight of treated plants should be 50% compared to control	Capable of fixing 10 mg of N_2 gm of sucrose used	Should promote root development and the dry weight of treated plants should be 10% more compared to control	Capable of solubilizing 30% insoluble P in test medium of 10 mm zone of P-solubilization

Source: *Regional Biofertilizer Development Centre, Nagpur*

Certain parameters regarding the quality of inoculants are given below

1. Inoculant should be a carrier based one
2. Inoculant should contain a minimum of 10^8 cells/g of carrier with 15 days from date of manufacture and 10^7 cells/g dw at the time of expiry.
3. The inoculant should have maximum expiry period of 6 months from the date of manufacture.
4. Inoculant should not contain any contamination at the desired dilution.
5. The pH of the inoculant should be 6.5 to 7.5.
6. Carrier material should be able to pass through 75–106 µ sieve and it is neutralized by $CaCO_3$ and sterilized
7. Inoculant should be packed in 50–75 µ in low-density polythene bag.
8. Each packet should give the following information: (*a*) name of the product (*b*) Crop to which it is applied (*c*) Name and address of the manufacture (*d*) Type of carrier (*e*) Batch and code number (*f*) date of manufacture (*g*) date of expiry (*h*) net quantity (*i*) storage instructions.

Sampling and Quality Test for Final Products in Lots

In order to supply a quality based product to the consumer; it is essential to test the quality of the product batch wise. The BIS has recommended the sample size/ lot depending upon the total size of the lot as given below

Azospirillum

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Strain Efficacy	Capable of fixing 40 mg of N/gm of sucrose used after 96 hrs incubation. The dry weight of treated plants should be 20% compared to control	Capable of fixing 10 mg of N/gm of sucrose used	Shade promotes root development and 4 to 6 dry weight of treated plants should be 10% more compared to	Capable of solubilizing 30% insoluble P in test medium of 10 mm zone of P solubilization

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1. Inoculant should be a carrier based one
2. Inoculant should contain a minimum of 10^8 cells/g of carrier with 15 days from date of manufacture and 10^7 cells/g dw at the time of expiry.
3. The inoculant should have maximum expiry period of 6 months from the date of manufacture
4. Inoculant should not contain any contamination at the desired dilution.
5. The pH of the inoculant should be 6.5 to 7.5.
6. Carrier material should be able to pass through 75–106 μ sieve and it is neutralized by $CaCO_3$ and sterilized
7. Inoculant should be packed in 50–75 μ in low-density polythene bag.
8. Each packet should give the following information: (*a*) name of the product (*b*) Crop to which it is applied (*c*) Name and address of the manufacture (*d*) Type of carrier (*e*) Batch and code number (*f*) date of manufacture (*g*) date of expiry (*h*) net quantity (*i*) storage instructions.

Sampling and Quality Test for Final Products in Lots

In order to supply a quality based product to the consumer, it is essential to test the quality of the product batch wise. The BIS has recommended the sample size/ lot depending upon the total size of the lot as given below

Table 10.2

No of packet in the lot	Sample size
Up to 100	7
100–1000	11
1001–10000	15
10001 and above	19

Procedure to Pass the Batch

Collect seven packets from each batch (100nos) and mark them with serial numbers one to seven. Use five packets out of seven for the quality test and store the remaining two packets for further use.

1. Take out the required quantity of material from each of the five packets.
2. Test the material of each packet with the following tests (*a*) Viable cell count (*b*) Gram staining test (*c*) pH (*d*) Moisture content.

If two of the five packets fail the batch fails. The other packets held in storage with same batch number are tested. If this packet fails again the additional two packets, which are kept unused is tested. If they all fail the particular batch is rejected.

Blue'green Algal Inoculants

Rice (*Oryza sativa*) is largely grown in wetland conditions with a layer of standing water, which encourages the growth of blue-green algae. These blue-green algae possess the ability of both photosynthesis as well as biological nitrogen fixation. Analysis of the blue-green algae flora from rice fields has revealed the occurrence of species of *Anahaena, Anahaenopsis, Aulosira, Cylindrospermum, Nostoc* and *Calotheix* as some of the dominant nitrogen fixers. Besides fixing nitrogen, these algae excrete vitamin B_{12}, auxins and ascorbic acid, which may also contribute to the growth of rice plants.

Isolation of Blue-green Algae

(*i*) Blue-green algae are generally covered with mucilage and it is easy to locate them as colonies floating on flooded rice fields.

(*ii*) The mucilage contains bacteria and therefore for practical purposes, no attempt is made to isolate bacteria free algal cultures.

Prepare of Unialgal Cultures

Several conical flasks or bottles containing a nitrogen free blue-green algal liquid medium are prepared and autoclaved at 121°C for 20 min. Sterile water blanks are prepared and serial dilutions of soil or of algae sample from a field location are prepared with the help of sterilized pipettes. Aliquots of appropriate dilutions are the inoculated into liquid media in flasks and incubated for several weeks in an illuminated growth room at 28°–32°C. Individual colonies are picked up either for enrichment on fresh aliquots of liquid media or on agar slants for growth, identification and preservation.

Preparation of Blue-green Algae in Open-air Shallow Culture

(i) It has been recommended that trays of galvanized iron sheet or brick and mortar or pits lined with polythene are prepared.

(*ii*) A mixture of 10 kg soil and 200 g super phosphate is added to trays and water filled in the trays to a level of 2–6 ft.

(*iii*) It is necessary to keep the pH of soil to neutral and liming is recommended for acidic soils.

(*iv*) When the soil settles, sawdust is sprinkled and a soil based starter culture consisting of *Tolypothrix, Nostoc, Anabaena* is sprinkled on the water in the tray.

(*v*) Within a week's time, a thick algal scum is formed on the surface.

(*vi*) The water is allowed to dry at this stage, the dried algal flask are collected from the trays and stored in polythene bags.

(*vii*) It is recommended that such an algal preparation be applied at the rate of 10kg/hectare in the field, one week after rice transplantation.

Multiplication of blue-green Algae in field and inoculation effect on the yield of rice

1. Five kg material of the algal preparations for each cent is spread in the field and the field flooded with water to a level of 2.5 cm followed by the addition of 2 kg of superphosphate.
2. The application of phosphate promotes algal multiplication in 2 weeks on clay soils and in 3–4 weeks in sandy soils.
3. Application of pesticides may be necessary to prevent pests, snails and mosquitoes.

The average yield of algae ranges from 16–35 kg for each cent area of land.

Azolla

Azolla is a floating fresh water fern and is ubiquitous in distribution.

There are six species of *Azolla*

1. *A. caroliniana*
2. *A. microphylla*
3. *A. nilotica*
4. *A. filiculoides*
5. *A. Mexicana* and
6. *A. pinnata*

The common species of the fern in India is *A. pinnata*. The importance of *Azolla* as an organic input in rice cultivation was first demonstrated in North Vietnam in 1957.

Cultivation

Small nurseries are prepared and *Azolla* is initially grown on them for four weeks. The nurseries are covered with plastic sheeting's when temperature is low. The fields are prepared for rice cultivation and flooded with water followed by seeding with *Azolla* at the rate of 7.5t/ha. After 5–10 days, the water in the field is drained off and the *Azolla* mat is ploughed into soil by using a tractor.

Azolla meets the nitrogen requirement of rice to the extent of 50%.Work done at the Central Rice Research Institute; Cuttack (Singh, 1977) has revealed the following strategy for *Azolla* cultivation:

(*i*) Standing water of 5–10cm deep and the application of superphosphate at the rate of 4–8 kg P_2O_2/ha are essential for rapid growth of *Azolla*.

(*ii*) *Azolla* nurseries in small plots (50-100sqm) are preferable to large plots to avoid wind erosion.

(*iii*) *Azolla* inoculum at the rate of 0.1 to 0.4 kg per sq m is desirable for the rapid multiplication of the fern in nurseries for production of 8–10t/ha green matter in 20 days.

(*iv*) A pH of 8.0 is ideal and acidic soils (below pH 4.6) are not suitable unless lime is used to correct pH.

(*v*) It can grow in a wide range of temperature 14–35°C. However, the optimum range is 20–30°C.

(*vi*) Use of carbofuran at 1–2kg/ha prevents the rapid spread of insect parasites and the consequent destruction of *Azolla* nurseries.

(*vii*) Harvested *Azolla* accumulated as heaps decomposes rapidly in 7–10 days.

(*viii*) The composition of *Azolla* is approximately 94% water, 1% of P, K, Ca, Mn and Fe and 5% N.

(*ix*) It is necessary to plan a series of *Azolla* nurseries several weeks ahead of rice plantings.

Limitations

The limitations are the use of *Azolla* in areas where water is not available or in areas where establishment is difficult due to other factors such as adverse temperature and pests. Transportations of *Azolla* from distant parts are risky since it is quickly perishable after removal from water.

Method of Application

Two methods of *Azolla* application have been recommended in India: as a green manure either by incorporating into fields prior to rice planting or secondly by dual cropping with rice when the fern grows side by side with the main crop for sometime.

VAM Fungi Inoculation Technology

VAM fungal inoculation technology is principally limited by the lack of production. The source of VAM inoculum is defined by its biology. All infecting structures of the fungi can be used as inoculum such as spores, mycelium, and internal and external infected roots.

Spores of VAM Fungi as Inoculum

Using spores of VAM fungi is found to be a suitable method for experiments and for special cases (e.g., plants growing in *vitro*). Spores are one of the important source inoculum for the establishment of pure culture of VAM fungi in previously sterilized substrate on host plants. A single spore can initiate their mycorrhiazal symbiosis. About 100 spores/g of inoculum/seedling plant could be produced by this method.

Infected Roots as Source of Inoculum

Large-scale production of infected root is possible in aerophonic culture. Infected roots contain internal and external mycelium and spores. Infected roots can colonize the root after 1–2 days of inoculation. Root inoculation with spores should be used within one week. The efficiency of infested root is found to be higher than that of spores. The production process is difficult and expensive. The other problems are: (*a*) infected roots introduced as inoculum act as an attractive source for several saprophytic and parasitic microorganisms, (*b*) Short survival time, (*c*) Requirement of a large quantity of inoculum.

Soil Based Inoculum

Soil inoculum produced using traditional pot culture techniques contains VAM fungal structures and are highly infective. The success of good soil inoculum production depends on the selection of host plant and ambient condition under which a defined VAM fungus can be mass multiplied. Soil inoculum is most frequently used in green house and field experiments.

B. BIO-CONTROL AGENTS

Biological control of plant diseases through host plant resistance and cultural practices received considerable attention for the past several decades and may continue to receive importance for years to come. Although the biological control through introduced antagonistic microorganisms has been studied for the past 65 years, the progress in this direction has been slow. However, continued interest has been shown by scientists all over the world during the last 20 years due to the necessity of finding out the natural control of plant diseases which may not leave toxic residues in the soil water and plant ecosystems.

Bacteria as Biocontrol Agents

The following bacteria have been reported to be antagonistic to several plant pathogenic organisms. *Agrobacterium, Alcaligens, Arthorabacter, Bacillus, Azotobacter, Pseudomonas, Rhizobium, Streptomyces and Xanthomonas.* However, very few of the above listed bacterial was effective under controlled green house conditions.

Characteristics of an Ideal Biocontrol Agent

An ideal biological control organism should have the following characteristics:

(*a*) Ability to survive for an extended period in rhizosphere or plant roots in an active form.

(*b*) Ability to produce inhibitory substance for killing the pathogen directly or indirectly.

(*c*) Multiplication should be made by simple and inexpensive method in the laboratory.

(*d*) Amenable to efficient and cheap process of packaging, distribution and application.

(*e*) Should be active under the appropriate environmental conditions.

Psuedomonas Fluorescens

The biocontrol abilities of these strains depend essentially on aggressive root colonization, induction of systemic resistance in the plant and the production of diffusible or volatile antifungal antibiotics. Evidence that these compounds are produced in situ is based on their chemical extraction from the rhizosphere and on the expression of antibiotic biosynthetic genes in the producer strains colonizing plant roots.

Biocontrol Properties

Well-characterized antibiotics with biocontrol properties in *P. fluorescens* include:

- Phenazines
- 2,4-diacetylphloroglucinol

- Pyoluteorin
- Pyrrolnitrin, lipopeptides and hydrogen cyanide.

Pseudomonas fluorescens encompasses a group of common, nonpathogenic, saprophytes that colonize soil, water and plant surface environments. As its name implies, it produces a soluble, greenish florescent pigment, particularly under conditions of low iron availability. It is an obligate aerobe, except for some strains that can utilize NO_3 as an electron acceptor in place of O_2. *P. flouorecens* has simple nutrition requirements and grows well in mineral salts media.

Mass Multiplication of *Pseudomonas fluorescens*

Preparation of P. fluorescens

Transfer one loopful of *P. fluorescens* culture to 100ml of Kings B broth in a 250 ml conical flask under aseptic condition. Incubate at room temperature for 48h and quantify the population of *P. fluorescens* in the prepared broth. (minimum of 9×10^8 cfu/ml is necessary)

Use this as a mother culture (1 lit) to inoculate 100 lit of Kings B broth in a fermenter for 72 hrs. Count the population ($\times 10^9$ cells/ml) and harvest the culture. Adjust pH of the substrate (Peat soil/talc powder) to 7 by addition of 150 g calcium carbonate/kg of peat soil. Sterilize in an autoclave at 1.1 kg/cm^2 for 30 min. Transfer 1000 g of the sterilized substrate (Peat soil/talc powder) and 5.0g of CMC into a polythene bag or any sterile container under aseptic condition and add 400 ml of *P. fluorescens* suspension.

Mix thoroughly the formulation and stored it in polythene bags. The products should contain a minimum of 2.5×10^8 cfu/g.

Seed Treatment and Soil Application

Soak 50 kg paddy seeds in 600 g peat based product in 65-litre water for 12h. Drain-off bacterial solution and incubated under darkness for 24 h and sow, for other crops talc based product is used at 4g/kg of seed.

Peat based product can be applied to soil at the rate of 2.5kg/ha after mixing with 50 kg organic marine (or) sand, 30 days after sowing

Mode of Action

It is not clear exactly the mode of biocontrol action of *P. fluorescens* is achieved, some theories includes:

- The bacteria might out compete other (pathogenic) soil microbes e.g., by siderophores giving a competitive advantage at scavenging for iron.
- The bacteria might produce compounds antagonistic to other soil microbes, such as phenazine-type antibiotics of hydrogen cyanide.

Disease Control

P. fluorescens are well adapted to soil; therefore different strains are being investigated extensively for use in applications that require release and survival of bacteria in the soil. Chiefly among these are bioremediation of various compounds and biocontrol of pathogens in agriculture.

P. fluorescens has been found effective in controlling fungal pathogens such as wilt/root rot fungi, *Fusarium oxysporum sp, Pythium spp. R. solani, R.* and *Oryzae* and bacterial pathogens like *Xanthomanas citri* and *P. solanacearum* in field tests. Bacterial preparations are widely being used in organic spice cultivation of Southern India.

Trichoderma

Trichoderma viride is a filamentous soil fungus known to be an effective biocontrol agent of a range of important airborne and soil borne pathogens. *Trichoderma* spp is the most widely studied biocontrol agents (BCAs) against plant pathogens. It was observed that they could excrete extracellular compound, which was named gliotoxin. Many antibiotics and extracellular enzymes were isolated and characterized later and their biocontrol mechanisms has now became clear.

Liquid Fermentation Method

Mix 30g molasses and 5 g brewers yeast in the ratio of 1:1 in water. Distribute 60 ml in each conical flask. Autoclave the medium at 12°C for 30 min. inoculate 8mm mycellial disc of *Trichoderma* in the medium. Incubate for 10 days at room temperature. This inoculum is used for multiplication in the fermenter. Prepare 50 litre of molasses + yeast medium; and sterilize for 30 min in the fermenter. Transfer aseptically one litre of *Trichoderma* already grown in flasks. Incubate for 10 days. Assess the population of spores using a haemocytometer. (A population of 10^8 spores/ml is required).

Preparation of Formulation

Mix 500 ml of fungal biomass of *Trichoderma* grown in fermenter along with one kg talc powder. Air-dry and add carboxymethyl cellulose (CMC) as a sticker at 5g/kg of the product. The final product is stored in polythene bags.

Quality Control

Draw samples from the product and estimate the population of *Trichoderma* as follows: Take one gram of product and make it up to 10 ml with sterile water and shake well (1:10). Make serial dilutions and transfer one ml of the desired suspension to sterilized petriplates. Add 15 ml of melted and cooled *Trichoderma* selective medium in the same petriplate. Rotate the plates gently and allow solidifying. Incubate at room temperature 5–7 days. Observe for the development of colonies. Count the number of colonies and calculate the colony forming units present in 1gram of product (minimum 2×10^7 cfu/per g).

Seed Treatment with Antagonistic

Talc based *Trichoderma* formulation is used as seed treatment agent for the control of root rot diseases of pulses, oil seeds, cotton etc. This product can be treated with the seeds as dry seed treatment at the rate of 4g/kg. The treated seeds can be sown immediately. The antagonistic was found to colonize the rhizosphere region and protect the crop against root rot pathogen.

Testing Efficiency of Antagonist in Seed

Prepare suspension of test pathogen *Macrophomina phaseolina* in sterile distilled water and mix with melted and cooled PDA.

Distribute 15 ml of the medium in sterile petridishes and allow it to solidify. Transfer the treated seeds to the center of the medium. Incubate at room temperature for 5–6 days. Observe and measure the inhibition zone.

C. BIOPESTICIDES

Conventional pesticides are generally synthetic materials that directly kill or inactivate the pest. Being single chemical entity, chemical pesticides have resulted in increased

resistance in pests. Biopesticides also known, as Biological pesticides are pesticides derived from natural materials as animals, plants, bacteria, and certain minerals. Biopesticides are less toxic and also reduce the pollution problems caused by conventional pesticides.

Microbial pesticides consist of a microorganism (e.g., a bacterium, fungus, virus or protozoan) as the active ingredient. For example the Biopesticides based on *Bacillus thuringiensis*, Nuclear polyhedrosis virus etc.

Total biopesticide sales were estimated to occupy a market share of 3% in future out of which 10% are bacterial based. *Bacillus thuringiensis* is an insecticidal bacterium marketed worldwide for the control of many important plant pests. It occurs naturally in soil and on plants. It represents about 1% of the total agrochemical market across the world.

The commercial by products are powders containing mixtures of dried spores and toxin crystals. They are applied to leaves or other environment where the insect larvae feed. The toxin genes are also been genetical engineered into several crop plants.

Bacillus Thuringiensis (BT)

A Japanese scientist Ishiwata first discovered Bt in 1902 from a disease silk larvae and he named it as *Bacillus satto*. It was a German microbiologist who isolated the bacterium from flourmill and named it as *Bacillus thuringiensis* after province of Thuringia, Germany. It was used commercially as biopesticide in USA in 1960.

Most of the strains of Bt have the same basic toxic structure, but they differ in insect host range, because of different degrees of binding affinity to the toxin receptors in the gut.

Bacillus thuringiensis types and host range

- var *tenebrionis*—Colorado potato beetle and elm leaf beetle larvae.
- var *Kurstaki*—Caterpillars.
- var *Israelensis*—mosquito, black fly and fungus gnat larvae.
- var *Aizawai*—wax moth larvae and various caterpillars, especially the diamond back molar caterpillar.

Bt Toxins and their Classification

B. thuringiensis strains produce two types of toxin. The main types are the cry (crystal) toxins, encoded by different crygenes; genes and on which different types of Bt are classified. The second types are the Cyt (Cytolytic) toxins, which can augment the crytoxins; enhance the effectiveness of insect control.

The toxins are as assigned to more than 15 groups on the basis of sequence similarities and more than 50 of the genes, which encode the different toxins, have been sequenced.

Gene	Crystal shape	Protein size (kDa)	Insect activity
Cry I Several subgroups A(a), A(b), A(c), B, C, D, E, F, G	Bipyramidal	130–138	*Lepitoptera* larvae
Cry II Subgroups A,B,C	Cuboidal	69–71	*Lepitoptera* and *Diptera*
Cry III A,B,C	Flat/irregular	73–74	*Coleoptera*
Cry IV subgroups A, B, C, D	Bipyramidal	73–134	*Diptera*
Cry V-IX	Various	35–129	various

Mode of Action

The crystals are aggregates of a large protein (about 130–140 kDa), which is actually a protoxin and must be activated before it has any effect. The crystal protein is highly insoluble in normal conditions. So it is entirely safe to humans, higher animals and most insects.

It is solubilised in reducing conditions of high pH (above 9.5) these conditions are commonly found in the midgut of *Lepidopteran* larvae and so it is a highly specific insecticidal agent.

Once it has been solubilised in the insect gut, the protoxin is cleaved by a gut protease to produce an active toxin of about 60 kD termed as delta-endotoxin. It binds to the mid gut epithelial cells, creating pores in the cell membranes and leading to the equilibrium of ions. The osmotic equilibrium of cell is disturbed and the cell swell and burst. The protein toxin damages the gut lining leading to the gut paralysis. Affected insect stop feeding (and die from the combined effects of starvation and tissue damage).

The gut pH is lowered by equilibrium with blood pH. This lower pH enables the bacterial spores to germinate, and the bacterium can then invade the host causing lethal septicemia.

Recent Approaches to Strain Development

In a single Bt strain the different plasmids can encode different toxin genes. The plasmids can be exchanged between Bt strains by a communication like process, so there are a potentially wide variety of strains with different combinations of cry toxins and they also contain transposons.

These properties increase the variety of toxins produced naturally by Bt strains and provide the basis for commercial companies to create genetically engineered strains with novel toxin combinations e.g., Raven ® for enhanced control of colarado potato beetle as well as for caterpillars that attack *Solanaceae* crop.

This Bt strains contains two different beetle active cry III proteins (binding affinity for midgut cell membranes of Colorado beetle) as well as two caterpillar active cry I proteins and these approach is often termed as gene pyramiding is designed to delay the development of resistance in target pests as resistance has to be develop simultaneously to different toxins since resistance can develop quite rapidly when one type of toxin is involved. It happened within 1 or 2 years when the mosquito active Bt strains when widely used in tropical countries. The basis of resistance seems to be complex, involving several factors. But one encourage finding is that at least in some insects, the receptor for Bt toxin is an essential gut enzyme, aminopeptidase-N, and so only change in the receptor cause a loss of binding to the toxin which could be detrimental to the insect potentially reducer the fitness, of the resistant insects.

Mass Multiplication

The mass production in submerged fermentation on relatively cheap media (Faust, 1974) in which organism is slowly bubbled as a steam of air through a culture solution of 15 cm depth (Ignoffo and Anderson, 1979).

Composition of B_t Medium for Bt Production

Ingredients	Amount in g/lit
Cooked cotton seed flour	10
Dextrose	35
Yeast extract	2
Bactopeptone	2
$MgSO_4.7H_2O$	0.3
$FeSO_4.7H_2O$	0.02
$CaCO_3$	1.0

Problems with Commercial Available Bt Biopesticides

- Corn earworm, squash vine borer larvae they rapidly bore into and or protected by the plant tissue.
- Bt formulations may be deactivation by in sunlight and be effective for only one to three days.
- Rain or overhead irregular can also reduce effectiveness by washing Bt from crop foliage.
- Diamond back moth, a major worldwide pest of uole crop has developed resistance Bt var *Kurstaki* toxins.

Nuclear Polyhedrosis Virus

Nuclear polyhedrosis virus is rod shaped 20–50µm broad and 200–400 µm long. They are enclosed in two proteinaceous membranes, which together with the virus particle constitute the polyhedral body. There could be one or more (up to 100) virus particles in a polyhedron. A polyhedron is usually hexagonal, but other shapes such as tetrahedron and irregular shapes are also reported.

Mechanism of Infection

On ingestion, the membrane of the polyhedra is dissolved by alkaline gut juices, setting the virus particles free. The virus particles then penetrate the gut epithelium and come to infect the blood cells (haemocytes), tracheal matrix or epidermal cells. Inside the cells, they enter the nuclei, attach themselves to chromatin and multiply. As the multiplication goes on, chromatin is consumed. Eventually, the nuclear and cell membranes rupture, releasing the polyhedra into the body cavity to invade other cells. In advanced stage of infection, tissue like silk glands, buds etc may also get infected.

Symptoms

Symptoms of NPV infection appear only at a late stage when the insect is nearing death. The incubation period is 1–3 weeks. The insect stops feeding and becomes sluggish and pale in colour. It may swell slightly, and then become limp and flaccid.

Host Range

Nuclear polyhedrosis is primarily a *Leptopterian* disease, occurring also in some *Diphtheria* and *Hyenoptera*. Among the different NPV the best known include: silkworm. *Bombyx mori, Lymantria monacha , Portheria dispa, Helicoverpa armigera* and *Spodoptera litura.*

Mass Multiplication of *H. Armigera*

For multiplication of natural enemies like parasitoides or NPV, it is important to mass muliply *H. armigera* in large numbers.

Mass Production of Virus

When the larvae reach nearing early fifth instar; 90% of the larvae are used for NPV production, whereas remaining 10% is retained for continuing the healthy culture. Natural diet of bhendi fruit is fed to the larvae. 10µl virus suspension (5×10^7 POB/ml for *H. armigera*) is dispensed and spread into the natural diet. Early fifth instar larvae are released individually into these vials. All the vials are incubated at 25°C. After 6–7 days the larval mortality is observed. All the dead larvae are collected, macerated; semipurified and enumerated using double ruled neubaur Haemocytometer and the virus thus collected are preserved in refrigerator for further use.

Quality Control

1. Should contain a minimum of 1×10^9 POB/ml or g.
2. Free from contaminants, such as *Salmonella, Shigella* or *Vibrio*. Other microbial contaminats should not exceed 1×10^4 count/ml or g.

D. BIO-HERBICIDES

Agricultural fields, gardens, ponds, lakes are invaded by many unwanted plants. The unwanted plants are called weeds. They compete with the main crop for space, water, light and nutrients and thus prevent the growth of the main crops.

Destruction of weeds using biological agents is called biological control of weeds. The biological agents that destroy weeds are called bioherbicides. The bioherbicides include both pathogenic insects and pathogenic fungi, which selectively cause diseases in weeds. The preparation containing spores of such fungi is often called microbial herbicide or mycoherbicide. e.g. *Puccinia chondrillina.*

The use of bioherbicides is advantageous over chemical herbicides. The advantages are:

1. Bioherbicides are very cheap
2. They do not give residual effect in plant products
3. They are usually more specific in action
4. They do not persist for a long time without their proper hosts.

Mycoherbicides

Many fungi of native origin are pathogenic to specific species of weeds. Such fungi are used to kill the weeds selectively and eliminate them from the area. These weed-killing fungi are called mycoherbicides.

e.g., *Phytopthora palmivora* is used to control the weed *Morrenia odorata.*

Production of Mycoherbicides

The phytopathogenic fungus that attacks the target weed in a large proportion is isolated from the infected weed species. It is cultured in a suitable nutrient medium. Fungal spores are isolated and sprayed on a wide variety of plant species including weeds and main crops to assess the host range of the fungus. If the fungus attacks the target weed species alone, it is elected as a mycoherbicide for the weed.

The successful mycoherbicides must produce abundant spores in culture medium; it must be efficient in a broad-range of climatic conditions.

Methods of Applications

Some mycoherbicides can multiply on the target weeds and spread rapidly. In addition, they persist in the killed weeds for a long time in the form of spores till new weeds appear. These mycoherbicides are introduced here and there in the field for weed control. This method is called inoculation or introduction e.g., *Puccinia chondrillina* and *Cercospora rodmanii*.

Some mycoherbicides kill weeds and get destroyed. So a known volume of a mycoherbicide is diluted and sprayed directly over the plants. However, the fungus cannot persist for the forth-coming seasons e.g., *Phytopthora palmivora*

Important Mycoherbicides

1. *Puccinia chondrillina* causes rust in *Chondrilla juncea* and kills the weed. It is applied by inoculation method.
2. *Cercospora rodmanii* is pathogenic to water hyacinth, *Eichhornia crassipes*. Therefore, it is inoculated in ponds and lakes to control overgrowth of water hyacinth. In the USA, it is available in the name ABG5003.
3. The spore suspension of *Phytopthora palmivora* is sprayed on milkweed vine (*Morrenia odorata*) in citrus orchards to control the weed. It is available in the name De Vine.
4. Spores of *Alternaria sp* and sprayed on sickle weed *Cassia obtusifolia* among soyabean and groundnut to kill the weed. It is available in the name CASST.
5. The preparation containing spores of *Colletotrichum coccodes* is used to control *Abutilon theophasoti*. It is available in the trade name VELGO.
6. COLLEGO is the trade name of fungal preparation containing spores of *Collectorichum gloeosporioides*. It is use to control *Aeschynomens virginica*, a common weed in paddy fields.

E. INOCULANT FORMULATIONS

Formulation is the crucial issue for inoculants containing an effective bacterial strain and can determine the success or failure of a biological agent. Formulation is the industrial "art" of converting a promising laboratory -proven bacterium into a commercial field product. The two major problems of microbial formulations are (*i*) loss of viability during short storage in the grower's warehouse and (*ii*) long shelf life and stability over the range of -5° to 30°C within the marketing distribution systems.

Optimal Characters of a Carrier for Inoculant Formulation

The carrier is the delivery vehicle of live microorganisms from the factory to the field; however, no universal carrier or formulation is presently available for the release of

microorganisms into soil. The materials of which the carrier is composed and the type of formulation vary and the carrier can be slurry or a powder. A good carrier should have one essential characteristic: the capacity to deliver the right number of viable cells in good physiological condition at the right time. Additional desirable characteristics for a good inoculant should be as follows:

Chemical and Physical Characteristics

The inoculants should be nearly sterile or easily sterilized, and as chemically and physically uniform as possible. They should also be of consistent quality, high water-holding capacity (for wet carriers) and suitable for as many bacterial species and strains as possible.

Manufacturing Qualities

The inoculant should be easily manufactured and mixed easily; it should allow for the addition of nutrients, have an easily adjustable pH, and be made of a reasonably priced raw material in adequate supply.

Farm Handling Qualities

A good inoculant allows for ease of handling, provides rapid and controlled release of bacteria into the soil, and can be applied with standard agrotechnical machinery.

Environmental Characteristics and Storage Qualities

The inoculant should be nontoxic, biodegradable and nonpolluting, and should minimize environmental risks such as the dispersal of cells to the atmosphere or to the ground water. The inoculant should have sufficient shelf life.

Naturally, no single carrier can have all these qualities, but a good one should have as many as possible. A "super-inoculant" such as the one described above is not practically possible.

Types of Existing Carriers for Inoculants

Carriers can be divided into four basic categories:

(*i*) **Soils:** peat, coal, clays, and inorganic soil

(*ii*) **Plant waste materials:** composts, farmyard manure, soybean meal soybean and peanut oil, wheat bran, "press-mud" (a by-product from the sugar industry, agricultural waste material, spent mushroom compost and plant debris

(*iii*) **Inert malerials:** vermiculite, perlite, ground rock phosphate, calcium sulfate, Polyacrylamide gels and alginate.

(*iv*) Plain lyophilized microbial cultures and oil-dried bacteria.

These preparations can later be incorporated into a solid carrier or used as they are. To produce an inoculant, the target microorganism can be introduced into a sterile or non-sterile carrier.

Inoculants come in four basic dispersal forms:

Powders

This form is used as a seed coating before planting. The smaller the particle size, the better the inoculant will adhere to the seeds. Standard sizes vary from 0.075 to 0.25 mm, and the amount of inoculant used is around 200 to 300 g/ha. These inoculants are the most common both in developed and developing countries.