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OVERVIEW OF PLANT GROWTH PROMOTING RHIZOBACTERIA AS MICROBIAL INOCULANTS IN SUSTAINABLE FORAGE CROP PRODUCTION

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SUMMARY

There has been undue pressure on the agricultural sector to meet thefodder demand of the burgeoning global livestock population along with the augmentation in the abiotic stresses like pH,salinity, temperature etc. In addition, the natural resources are limited due to which has become a major concern for the developing nations to enhance forage yield per unit area. There has been a continuous use of chemical fertilizersto combat aforesaid moot issues. However, the deleterious effects of chemical fertilizers on the environmentand stagnation in the further improvement in yield per hectare urged to look for new promising, renewable, economical and eco-friendly technology.The rhizospheremicroorganisms play a paramount role in maintaining soil fertility and in biogeochemical cycles. The advantageous free-living rhizospheric bacteria are known as plant growth-promoting rhizobacteria (PGPR). These are increasingly available in markets as microbial inoculants and these are the potential alternative for mainteneance of soil health and enhancement of yield. Various formulations of microbial inoculants are available in the market. Therefore, in the present review, an overview of microbial inoculants has been discussed to sensitize agriculturists about the benefits of microbial inoculants.

Key words : Abiotic stress, formulation, microbial inoculants, plant growth promoting rhizobacteria, rhizospheric bacteria

India has the world's largest livestock population with nearly 14.7% of the world's cattle population and 57.3% of the buffalo population. This sector is the backbone of Indian agriculture contributing 4% to the national GDP. However, the annual forage production is about 978.7 mMT with only 4.9% area under fodder cultivation. While on the contrary, to support the existing livestock population, about 1325.7 mMT forage production is necessary. Hence, at present, India is going through deficit of 44% concentrate feeds, 35.6% green fodder and 10.95% dry roughages (IGFRI Vision 2050).

Agrochemicals have made a critical role in perpetuating the burgeoning demand for food and fodder commodities. Nevertheless, the illeffect of agrochemicals on the environment and the health of individuals cannot be overlooked. Thereupon, careful handling of these chemicals is the need of the hour. Agrochemical is a general term that includes array of inorganic chemicals used in agriculture includingfertilizers, pesticides, and other growth agents. Among these, pesticides play an essentialrole in agricultural development by eliminating detrimental pests asmore than thirty to forty percent of the food production gets wasted due to pests (Campos *et al.,* 2014).

Likewise, plants are unable touse all the nutrients applied via chemical fertilizers (Bhardwaj*et al.,* 2014), some amount of nutrients are either fixed in the soil or leached out and eventually mixed with water bodies (Mahdi *et al.,* 2010).

Thus, in order to make agriculture sustainable it is obligatory to administer a balanced and reasonable use of nutrients which are economical and environment friendly (Mahdi *et al.,* 2010); in that case microbial inoculants or bio fertilizer could be a suitable alternative (Pindi and Satyanarayana, 2012; Borkar, 2015).The use of farmyard manure, green manure, poultry manure, city waste and microbial inoculants have no detrimental impact on soil and environment (Fariha and Noreen 2014).

'Biofertilizer'or microbial inoculants also referred to as 'micro inoculants' (Arora*et al* 2010), was derived from the term 'biological fertilizer'; with biological signifying the utilisation of living organism or it can also be described as a product containing living microorganisms that colonize in the rhizosphere accompanying interior of the plant and regulates growth by increasing the availability and uptake of mineral nutrients to the host plant (Malusá and Vassilev 2014). The microorganisms in microbial inoculants re-establish natural nutrient cycle, maintain optimum nutrient level in soil and also enhance soil organic matter as a result of which healthy plants can be grown, while upholding fertility and sustainability of the soil (Shelat *et al.,* 2017).

The pivotal challenge in the coming decades will be meeting future demands of food without causing further degradation of environment. Besides enhancing the agricultural production amidst global climate change, the society is facing a challenge which threatens to minimize harvests in many areas of the world and the need to develop innovative technologies that augment agricultural yields, reduced inputs, and deter further environmental pollution also exists. Microbial inoculants can play phenomenal role in improving agricultural productivity in the era of climate change. Therefore, in present review, the importance of microbial inoculants will be scrutinized.

PLANT GROWTH PROMOTING RHIZOBACTERIA

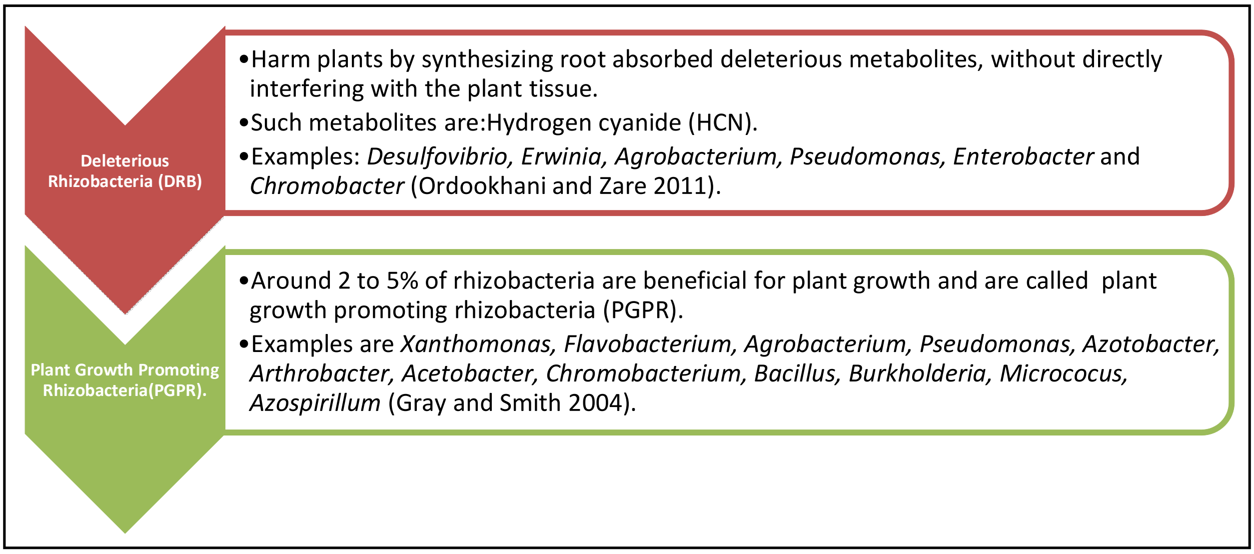
The layer of soil directly in contact with the plant root is the rhizosphere. The rhizospherehas abundant nutrients as it is affected by the root system in terms of metabolism and activity. There are numerous microorganisms in this region and the bacteria harboring in it are refereed to as the rhizobacteria. Rhizobacteria can either have no impact or favourable or damaging impact on the plants (Bhattacharyya *et al.,* 2017).

Rhizobacteria are classified into two based on the relationship with the plant (Dobbelaere*et al* 2003). The bacteria which exert negative influence are known as Deleterious Rhizobacteria whereas the bacteria exerting positive influence are termed asPlant Growth Promoting Rhizobacteria (PGPR).

CLASSIFICATION OF PLANT GROWTH PROMOTING RHIZOBACTERIA

Ahemad and Kibret (2014) classified PGPRsinto extracellular plant growth promoting rhizobacteria (e-PGPR) and intracellular plant growth promoting rhizobacteria (i-PGPR) on the basis of proximity and intimacy of association with the root system.

e-PGPRs are bacteria that resideexterior to the plant cells and do not generate nodules, but enhance growth of plant by producing signal compounds that directly stimulate plantg rowth, improveplant diseaseresistanceorboostsoilnutrient mobilization. These can occur in the rhizoshpere, or in the rhizoplane or in the spaces between the cells of root cortex. Theseaugment theyield because oftheir ability to produce phytohormones, increased mineral content, production of bacterial siderophores, enhanced phosphorus by enhanced solubilization or better resistance against pathogens and/or abiotic stresses such as frost damage(Bhattacharyya and Jha 2012).



- Harm plants by synthesizing root absorbed deleterious metabolites, without directly interfering with the plant tissue.
- Such metabolites are:Hydrogen cyanide (HCN).
- Examples: *Desulfovibrio, Erwinia, Agrobacterium, Pseudomonas, Enterobacter* and *Chromobacter* (Ordookhani and Zare 2011).

- Around 2 to 5% of rhizobacteria are beneficial for plant growth and are called plant growth promoting rhizobacteria (PGPR).
- Examples are *Xanthomonas, Flavobacterium, Agrobacterium, Pseudomonas, Azotobacter, Arthrobacter, Acetobacter, Chromobacterium, Bacillus, Burkholderia, Micrococus, Azospirillum* (Gray and Smith 2004).

Fig. 1. Classification of Rhizobacteria.

TABLE 1

PGPRs have been classified into different classes by array of authors

S. No.	Types	Reference
1.	Extracellular PGPR and Intracellular PGPR	Ahemad and Kibret (2014)
2.	Biofertilisers and Biopesticides	Glick (2012)
3.	Biofertilisers, Phytostimulators, Rhizomediators and Biopesticides	Hayat *et al.* (2010)
4.	Rhizospheric PGPR and Endophytic PGPR	Thokchom *et al.* (2014)

This class in cludes members of generasuch as *Bacillus, Flavobacterium, Erwinia, Serratia, Pseudomonas, Arthrobacter, Micrococcus,* etc.

i-PGPRs are the bacteria that reside within plant cells, that form nodules and are located within those specialized structures.These i-PGPR are mainly gram-negative and rod shaped, with gram-positive rods, cocci and pleomorphic types being a lower component. N_2 fixation is the main mechanism by which rhizobia increases plant growth.Thei-PGPR also contain sendophytes as well as species of *Frankia,* both of which can symbiotically fix atmospheric nitrogen with the higher plants (Verma *et al.,* 2010). Contain members of genera such as *Sinorhizobium, Rhizobium, Allorhizobium, Azorhizobium, Mesorhizobium, Bradyrhizobium* from the Rhizobiaceae family so often collectively referred to as rhizobia.

Some PGPR have been also classified intobiofertilisersand biocontrol agents or biopesticides (Glick 2012).

Biofertilizers can be defined as the rhizobacteriawhich mainly enhance plant growth byproviding necessary nutrition under specific conditions. Beneficial bacteria in the soil will speed up certain microbial processes that increase the nutrient availability in a form that is readily assimilated by plants. The biofertiliserscan be classifiedaccording to their nature and function as nitrogen fixers, phosphate solubilisers, phosphate mobilisers and biofertilisersfor micronutrients. A few examples of various biofertilizers are represented in the Table 2.

Biopesticidesor Biocotrol Agents

Biological control can bedescribed as the utilization of beneficial organisms, their genes, and/or products, including metabolites, to lessen the detrimentalconsequences of plant pathogens and stimulate positive responses by the plant (Tranier*et al* 2014). Biocontrolhas been defined by the International Biocontrol Manufacturers Association (IBMA) as theemployment of products or agents that naturally influence pests and pathogens of the crop by restricting their proliferation. These products or agentsinclude microorganisms, macroorganisms, natural substances and chemical mediators (Lecomte *et al.,* 2016). Commonly used biocontrol agents are species of *Trichoderma*, *Bacillus*, *Pseudomonas*, *Pennicillium* etc.Rhizobacterial strains that can produce significant antibiotics can play crucial role in biocontrol activity. Thokchom*etal* (2014) gave another classification of PGPR intoendophytic PGP Randrhizospheric PGPR.

Endophytic PGPR are the bacteria that inhabit plant tissuessuch as root, leaf, stem and seeds. Crop endophytes appear as essential founders of theseedling bacterial population when seeds germinate(Hardoim*etal* 2012).Such microorganisms trigger the growth of non-leguminous crops. These express the capability of solubilization of mineral $PO_4^{-3,}$ N_2 fixation, production of siderophores, phytohormone production, and control of soil pathogens (Egamberdieva *et al.,* 2017). In addition, they also produce phytostimulators (phytohormones), cofactor pyrroloquinolinequinone(PQQ) and volatile acetoin (Tomer*et al* 2016). Examples include *Azospirillum, Gluconacetobacter, Methylobacterium, Burkholderia, Klebsiella, Pseudomonas, Arthrobacter, Mycobacterium, Streptomyces, Paenibacillus*etc. Endophytic microbes are also of great significance as biotransformers of various chemicals and aid in recycling of nutrients. (Yadav *et al.,* 2019).

RhizosphericPGPR : These bacteria are either tightly or loosely-adhered to the rhizosphere. As a bioinoculant, rhizobacteria can survive and proliferate in the plant rhizosphere upon inoculation and can even induce changes in its host species' microbial rhizosphere population (Philippot*et al* 2013). Microorganisms in the rhizosphere gain from 20 to 50 percent of photosynthetic carbon (C) (Haichar*et al*2014) transmitteddirectly by plant roots or released by mycorrhizal hyphalnetworks.

Phytostimulators : Microorganisms that have the innateability to control the production of various growth regulator enzymesarecalledas plant growth regulators or phytostimulators. Several PGPR can alter the level of phytohormones in plants, the reuponimpacting the hormonal balance of the plant and its response to stress. As a consequence of such regulation of the plant hormonal balance by the PGPR, the processes namely cell division, enlargement, and extension in symbiotic and nonsymbiotic roots are modulated (Mahanty *et al.,* 2017). These phytohormones are present in traces but affect diverse dimensions in growth of plant like physiological, morphological and biochemical processes of the plant.Vitalphytohormones are gibberellin, abscisic acid (ABA), auxin, ethylene and cytokinin(Maheshwari*et al* 2015).

Rhizomediators : The microorganisms which degrade the soil contaminants are referred to as rhizomedators.Bioremediation can be defined as a process or technique which utilises living organisms or their products either naturally or artificially to remediate/destroy or immobilize the pollutants in the environment (Uqab *et al.,* 2016). Several PGPR have the capability to degrade the toxic compounds including pesticides,solvents, herbicides, organic compounds and might provide a reasonable and effective means of destroying toxic compounds (Murali and Mehar 2014).

TYPES OF MICROBIAL INOCULANT FORMULATIONS

Solid Carrier Based Formulations of Microbial Inoculants

In solid formulation is a preparation in which inoculum is blended in sufficient proportion to a solid carrier where carrier is an inert material that acts as a vehicle to transfer microbes from laboratory to land. Materialssuch ascharcoal, lignite powder, rock phosphate pellet, peat, rice bran, paddy straw compost, vermiculite, clay, seed, soil, wheat bran, talc or a combination of these are being used as carriers. These carrier materials are chosen for better shelf-life of microbial inoculant based on the viability of the inoculated microorganisms.

Carriers can be broadly classified into four main categories:

Liquid Microbial Inoculants

Liquid inoculants are not the normal broth culturefrom a fermentor or water suspension of thecarrier-based microbial inoculant, as is sometimes known. These comprise of medium containingcarbon, nitrogen and vitaminstogrow microorganisms and certain compoundsthat serve as cell protectant. Such defensive cells andadditives are applied to the broth to enhance quality of inoculants such as:

- Prevent osmolysis
- Greater adhesion to seed surface
- Product stabilization
- Inactivation of soluble seed coattoxins
- Enhancement of survival during storage
- Protect inoculumfromextreme environmental conditions upon inoculationto seed and planting (Sahu and Brahmaprakash 2016).

In addition to maintaining high microbial numbers, liquid cultures containing cell protectants also foster the formation of resting cellssuch as spores and cysts thatprovide higher resistance against the abiotic stresses, thereby increasing thebacterial survivability.Some commonly used polymers are polyethylene glycol, polyvinylpyrrolidone (PVP),trehalose, methyl cellulose, gum arabica, Fe-EDTA, sodium alginate, glycerol, tapioca flour, etc.

Nanobiofertilizers

Nanotechnology can be expounded as the engineering of functional systems throughthe administration ofatoms and molecules in the nanoscale to create nanomaterials or nanoparticles that manifest functional and definite physical and chemical properties. Nano-biofertilizerscan be defined as a conjugate nanomaterial and bio-inoculant applicationtechnology that could ensure timely and targeted delivery of the nutrient to the testcrop, in addition to increasing the functional benefits imparted by the bio-fertilizer component of the formulation (Gouda *et al* 2018).

Nano-biofertilizers can be investigated as a prospective substitute to overcome the inadequacies in current nutrient management scenarios since these combine thebenefits of nano-fertilizers and bio-fertilizers. The benefits can be dichotomized toimprove nutrient efficiency through reduced application rates, increased bioavailability, diminished environmental losses, and improved PGP properties and conditions such as increased shelflife, minimize cell sedimentation percentage in the formulation, improvedcell viability, bioinoculant protection from drying and increasedplant growth promoting substances and secondary metabolite synthesis (Timmusk *et al.,* 2018).

TABLE 2
Some examples of microbial inoculants

S. No	Types of Microbial inoculants	Examples
Nitrogen Fixing Microbial inoculants		
(a)	Free-living	*Azotobacter, Derxia, Clostridium, Anabaena, Klebsiella, Desulfovibrio, Klebsiella, Rhodopseudomonas, Nostoc, Beijerinkia*
(b)	Symbiotic	*Trichodesmium, Rhizobium, Anabaenaazollae, Frankia*
(c)	Associative Symbiotic	*Azosprillum* sp., *Acetobacterdiazotrophicus, Alcaligenes, Bacillus, Enterobacter*
Phosphorous Solubilising Microbial inoculants		
(a)	Bacteria	*Bacillusmegaterium, B. subtilis, B. circulans, Pseudomonas striata, Burkholderia, Micrococcus, Erwinia*
(b)	Fungi	*Penicillium* sp., *Trichoderma, Aspergillus* sp.
Phosphorous Mobilizing Microbial inoculants		
(a)	Arbuscular mycorrhiza	*Scutellospora* sp., *Acaulospora* sp., *Glomus* sp., *Sclerocystis* sp., *Gigaspora* sp.
(b)	Ectomycorrhiza	*Pisolithus* sp., *Laccaria* sp., *Amanita* sp., *Boletus* sp.
(c)	Ericoid Mycorrhizae	*Pezizellaericae*
(d)	Orchid Mycorrhiza	*Rhizoctonia solani*
Microbial inoculants for Micronutrients		
(a)	Silicate and Zinc Solbilisers	*Bacillus subtilis*, *Thiobacillus thiooxidans*, *Saccharomyces* sp.

Microencapsulated Microbial Inoculants (Polymer Entrapment)

Encapsulation can be defined as the process in which a coating or matrix is employed to surround or embed the active chemical or ingredient (Gutiérrez and Álvarez 2017; Gutiérrez 2018).

The encapsulation of cells is of two types: macroencapsulationand microencapsulation. Macroencapsulation canbe characterised as encapsulation with the help of surface coating materials,such as polymeric organics (e.g., resins and plastics)or inert inorganic materials to substantially minimisesurface exposure to possible leaching media. Rounded beads, cubes or even sheaths can be used as the encapsulated material. Microencapsulationmay be defined as the method of enveloping or surrounding one substancewithin another substance on a very small scale,producing capsules ranging from less than 1µ toseveral 100µ. The key materials would either be released gradually by diffusion through the capsule walls or when the capsule walls are triggered to melt, dissolve or burst, by external conditions.

For encapsulation, different forms of polymers can be used: natural (protein material, polysaccharides) or synthetic (polyurethane, polyacrylamide) andhetero-, homo- or co-polymers. More than 1,350 potential poylmer combinations exist whichcan be used for encapsulation. The most widely usedpolymers for encapsulation are polyacrylamide and alginate.

APPRAISAL OF MICROBIAL INOCULANTS IN IMPROVING YIELD AND QUALITY OF FORAGE CROPS

In order to study the effect of *Azotobacter chroococcum*, *Azospirillum brasilense*, *Azospirillum lipoferum* and *Pseudomonas fluorescens* application on fodder maize (*Zea mays* L.), a field experiment was carried out by Hamidi*et al* (2006) and various parameters were recorded such as plant height, plant fresh weight, dry weight (biomass) per hectare and per plant, leaf number and stem diameter. It was reported that the yield attributes were improved with the inoculation of the aforementioned bacteria with respect to the control.

Effect of different nitrogen fertiliser levels and biofertilisers on forage sorghum indicated that the application of 75 kg/ha N (urea), 25kg/ha N (castor cake) and inoculation by *Azospirillum* increased the crude protein and quality of the forage (Yadav*et al* 2007). Similarly, it was indicated in a study conducted by Ibrahim *et al* (2009) that amalgamation of plant growth promoting rhizobacteria (PGPRs) and nitrogen fixing bacteria results in enhanced growth, forage yield and quality traits of fodder pearl millet along with saving of about 50% of nitrogen fertiliser without any environmental effects causedby inorganic nitrogen fertiliser.

Golada*et al* (2012) studied influence of FYM, nitrogen levels and *Azospirillum* on the productivity and economics of forage pearl millet. It was revealed that FYM at 10 t/ha, nitrogen at 100 kg/ha and inoculation with *Azospirillum* recorded significantly higher green forage yield net return and benefit to cost ratio as compared to rest of treatment combinations.

A study was carried out by Ghanbari Zarhmehri *et al,* (2013) to study the influence of ACC-deaminase containing PGPR (*Pseudomonas fluorescens* and *P. putida*) either alone or in combinations with zinc fertilizers on forage maize under water deficit conditions. It was revealed that inoculation with *Pseudomonas fluorescens* and *P. putida*resulted in the enhanced forage and grain yield under normal as well as water deficit stress conditions. Siahmarghue *et al,* (2014) employed three microbial inoculants namely, Nitroxin, Barvar 2, Super Nitroplus to study their effects on quality characters of forage pearl millet and reported an increase in the crude protein content while a decrease in the fiber with respect to the control.

The effect of Nitrobine containing *Azospirillum* species (biofertilizer) on yield and dry matter content of forage pearl millet cultivars was investigated by Eissa*et al* (2015) and indicated that the application of biofertiliser along with mineral nitrogen resulted in highest significant fresh and dry yields.

Kushwaha *et al,* (2018) in a study on fodder pearl millet observed the influence of liquid microbial inoculants on the yield and quality characteristics and reported increased in the ether extract, ash content and crude protein as compared to the control and an elevation in the green fodder yield was also recorded with the application of liquid microbial inoculants.

A study was conducted to evaluate the potential of *Rhizobium* to enhance the growth of fodder Berseem (*Trifolium alexandrinum* L.) in the presence and absence of tryptamine by Ijaz *et al,* (2019). The results revealed the highest plant height (103.45 cm), number of seeds per head, thousand seeds weight were recorded with co-inoculation and tryptamine @10^{-5} M. In addition, crude protein (30.23%), ADF (26.56%), and NDF (33.45%) also gave significant results as compared to control.

In a field experiment on forage cowpea, Ramya (2019) reported elevation in the green fodder and dry matter yield and yield attributing characters (vine length, stem girth, leaf length, leaf stem ratio etc.) and quality parameters (crude protein, in vitro dry matter digestibility, total sugars etc.) with the application of *Burkholderia seminalis*, *Burkholderia* sp. and *Bradyrhizobium* sp. with different combinations of RDF. It was concluded that liquid microbial inoculants could play an essential part in integrated nutrient management for increasing the productivity and improving the quality.

Allahdadi *et al,* (2020) conducted an experiment to evaluate the yield and quality parameters of artichoke fodder inoculated with chemical fertilisers alone and in combinations with different microbial inoculants. The application of chemical fertilisers and bioinoculants was recorded as the most superior as it exhibited the highest dry fodder yield, digestibility, crude protein and ash content and minimum amount of ADF content.

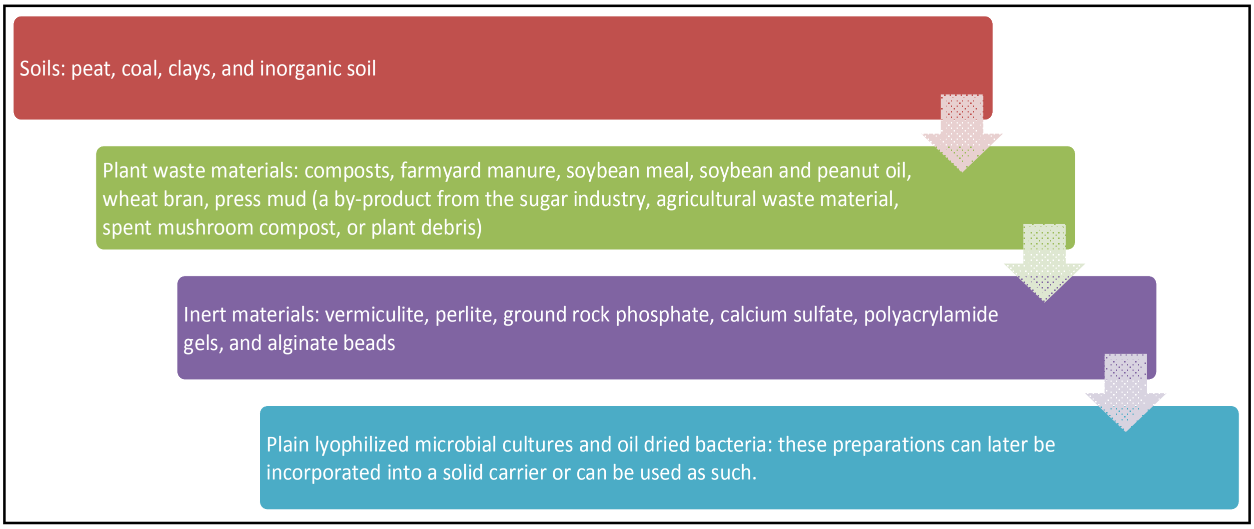


Fig. 2. Classification of carriers.

CONCLUSION

Environmental stresses have become a matter of concern all over the world and as a consequence of it; the productivity of crops especially the fodder crops has been continuously decreasing continuously as the area under fodder crop is also decreasing due to the progressive importance of cereals and cash crops. It is well known fact that excessive usage of chemical fertilisers has detrimental influence on ecosystem. Under these conditions, microbial inoculants have proved to be a potent alternative which not only could meet the demand of fodder for the increasing population of livestock but are also beneficial for the environment. However, this technology is still nascent and evolving and there is lot of scope to expand its horizon under different environmental conditions. Microbial inoculants can be used in integrated manner along with chemical fertilisers. Application of inoculants not only improves growth, productivity and yield but quality also. In addition, long term application of microbial inoculants can improve soil fertility and sustainability. Microbial inoculants can act as a probiotic for soil health.

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