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MIXED BACTERIAL INOCULANTS AND MICRO - ENCAPSULATED SYNTHETIC INOCULANTS: PRESENT STATUS AND FUTURE PROSPECTS

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The immediate outcome of plant inoculation with beneficial bacteria is that shortly after the bacteria are introduced in the soil, the bacterial numbers usually decline progressively [1]. Thus, the major role of the inoculant is to provide a temporary and dependable environment to help the bacteria avoid destruction in the soil [2].

OPTIMAL CHARACTERISTICS OF A CARRIER FOR INOCULANTS

Ideally, a good carrier should: 1. deliver the right number of viable cells which are in good physiological shape at the appropriate time, 2. have a high water-holding capacity (for wet carriers), 3. be nearly sterile or easily sterilized, 4. be as chemically and physically uniform as possible, 5. be non-toxic, biodegradable and non-polluting, 6. the pH be easy to adjust, 7. be consistent in quality, yet allows the addition of nutrients, 8. provide rapid and/or controlled release of bacteria into the soil, 9. be easily mixable both in the factory and on the farm, 10. be easily manufactured by the existing biotechnology industry, 11. be suitable for as many bacterial species and/or strains as possible, 12. be made of a raw material that is in adequate supply and reasonably priced, 13. have a sufficient shelf-life, 14. be easy to use and apply with standard agrotechnical machinery, 15 and finally, a good carrier should not pose any environmental risks such as dispersal of cells to the atmosphere or to the ground water [3,4,5]. Naturally, no carrier has all these qualities, but it should have as many as possible. A "super-inoculant" such as the one described above is theoretically possible. However, to date, no known effort has been made to synthesize a carrier with superior characteristics, probably because of the cost involved. Most raw materials for commercial carriers are cheap and abundant (peat and vermiculite).

Although peat formulations are the carrier of choice, new trends in formulations using unconventional synthetic materials are being slowly introduced, using polymers (like alginate) which offer substantial advantages over peat. The encapsulation of microorganisms is only experimental in the field of inoculation technology. To the best of our knowledge, there is no commercial product that uses this technology. These formulations encapsulate the living cells, protect the microorganisms against many environmental stresses and release them to the soil gradually when the polymers are degraded by soil microorganisms. They can be stored dried at ambient temperatures for prolonged periods, offer a consistent batch quality and a better defined environment for the bacteria, and can be manipulated easily according to the needs of the specific bacteria. These inoculants can be amended with nutrients to improve the short-term survival of the bacteria upon inoculation. However, they are rather expensive relative to peat-based inoculants and require more bio-technical handling by the industry [2].

ENCAPSULATED FORMULATIONS: MACRO AND MICRO FORMULATIONS OF ALGINATE

Alginate is the most common material for encapsulation. The preparation of macro-beads (1-3 mm in diameter) containing bacteria is fairly easy and involves a multi-step procedure [3]. The main advantages of alginate preparations are their non-toxic nature, degradability in the soil, and their slow release of microorganisms into the soil. This technology was used to encapsulate the Plant Growth-Promoting Rhizobacteria (PGPR) Azospirillum brasilense and Pseudomonas fluorescens [3] which were later successfully used to inoculate wheat plants under field conditions. The bacteria survived long enough in the field and their populations were comparable to the survival of bacteria originating from peat-based inoculants [6].

It appears that alginate preparations have solved many of the disadvantages of traditional peat inoculants. However, the use of macro alginate beads has one major disadvantage, the need for an additional treatment during sowing which is not always feasible.

To overcome these problems, the micro-bead concept was conceived. If the beads are small enough, yet still able to encapsulate a sufficient number of bacteria, it would be possible to produce an almost powder-like formulation. This "bead-dust" would be coated on the seeds at the factory and sold to the farmer as "improved seeds". The production of micro-beads can be achieved by several simple procedures. This technology produced alginate beads in sizes of 100-200 μm which entrapped a significant number of *A. brasilense* (approx. 10^8-10^9 cfu/g), similar to the level contained in alginate macro-beads [3]. Application of this novel formulation to the soil is still pending.

MIXED BACTERIAL INOCULANTS

Several PGPR like *Azospirillum*, have an erratic and unpredictable behavior under field conditions making them unlikely candidates for commercialization [7]. However, the potential of these beneficial bacteria can be economically substantial. To better exploit their potential, we are proposing a new applicative concept: mixed inoculation of more than one microorganism in a single carrier, inoculated simultaneously. The beneficial effect on plants is the synergistic effect of both (or several) microorganisms. In this inoculation type *Azospirillum* may take the role of a "helper" bacteria or be "helped" by other bacteria to overcome its own unpredictability. In this regard, several synergistic combinations of *Azospirillum* with other microorganisms are known. Cellulose breakdown by *Cellulomonas* [8] and pectin degradation by *Bacillus* [9] were enhanced when co-cultured with *Azospirillum*. Synergism in nitrogen fixation occurred when cultures of *Azospirillum* were mixed with *Arthrobacter* [10] or *Staphyloccocus* [11]. Nodulation of legume [12,13] or synergistic effects with VAN fungi were increased [14,] when mixed or co-inoculated with *Azospirillum*. However, to date, mixed inoculation is at the far end of contemporary inoculation research and development.

In sum, it is clear that future research should focus on the development of better and agronomically feasible, synthetic inoculant carriers as well as further developing of the concept of mixed inoculation.

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