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Advancement in Research of Plant Beneficial Microorganisms: From Fermentation to Formulation Strategies and Applications in Agriculture

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Abstract

In the last years, different biotechnological approaches were developed to reduce the indiscriminate use of chemical fertilizers and pesticides and enhance plant growth and health. The most attractive, safe, and environmentally friendly alternatives include those based on plant beneficial microorganisms. After a long period of studies on isolation, selection, and characterisation of plant-beneficial microorganisms, the main lines of research have been oriented to optimising the fermentation processes to produce high-quality and large volumes of biomass/spores and their further formulation. However, further well-structured schemes for improvement of all main steps of the production of formulations should be developed following the ideas based on "healthy soil-healthy plants-healthy humans". This brief review highlights the strengths and weaknesses of the techniques applied in the biotechnological production of plant beneficial microorganisms.

Keywords: Plant beneficial microorganisms; Fermentation; *Formulation*; Optimization; Future tendencies

Introduction

Plant beneficial microorganisms, both biofertilizers and biocontrol agents, such as Archaea, Bacteria, and Fungi, are increasingly recognized as a sustainable alternative to chemical fertilizers due to their numerous benefits for agriculture, environment, and human health. Microbes are now recognize to play an integral role in crop development, with microorganisms capable of causing great harm or benefits to final yields [1]. Therefore, changes in plant microbiomes will affect the host, such as yield or resilience to stresses. Particularly in soil-plant systems, the microorganisms present in formulations have the ability to carry out various important processes such as fixing atmospheric nitrogen, solubilizing insoluble phosphates, and secreting growth-promoting metabolites [2].A biofertilizer could be defined as a formulated product containing one or more microorganisms that enhance the nutrient status, the growth and yield of plants by either replacing soil nutrients and/or by making nutrients more available to plants and/or by increasing plant access to nutrients [3]. It should be noted that this definition differentiates biofertilization from biological control. While the emphasis of biofertilization is on the effects of plant beneficial microorganisms that improve plant growth, stress tolerance and quality, biocontrol agents reduce/suppress plant pathogens partially or completely by producing phytopathogenic inhibitory substances or, indirectly, by increasing the natural resistance of the plant. Both terms are not specific and do not differentiate between organisms that have plant growth promoting or biological control capacity [4]. The multifunctional properties of microorganisms should be mentioned, which can exert plant growth-promoting and biocontrol activity simultaneously , including production of different metabolites (organic acids, siderophores, indol-acetic acid, etc. [5]. From both scientific and practical point of view, the production and widespread adoption of biofertilizers face several bottlenecks that need to be addressed to fully realize their potential benefits. After a long period of studies oriented to isolation, selection, and characterization of plant beneficial microorganisms, during the last years the main lines of research are focused on the optimization of the fermentation processes for production of high-quality and large volumes of biomass/spores and their further formulation [6]. It is also important to study and analyze the whole biotechnological chain for biofertilizer/biocontrol production as all their parts are interdependent what is particularly true for fermentation-formulation and even storage-application processes and procedures [7]. The production and widespread adoption of bioformulations face several bottlenecks ranging from efficient

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production stage to effective formulation and successful application in field conditions [6]. Here, we will try to clearly define and address the most critical process stages to fully realize their potential benefits. Some of the main challenges at the production level are the selection and scale-up of the laboratory fermentation technologies, cost of production (including the growth media), and quality and safety control. Comparing to chemical fertilizers, biofertilizers need special equipment; selected, cheap and available substrates; and controlled optimized conditions for the microbial growth [7,8]. Further scaling-up of the process should maintain the viability and safety of the selected microorganism and its efficacy while avoiding contamination and variability of the strain bearing in mind the dual nature (both plant beneficial characteristics and pathogenicity for humans) of the majority of the soil microorganisms [9-12]. More research is needed to identify, develop, and characterize functional microbial strains, with a stable effective interaction with plant/soil systems and optimize their production (Figure 1) and formulation.

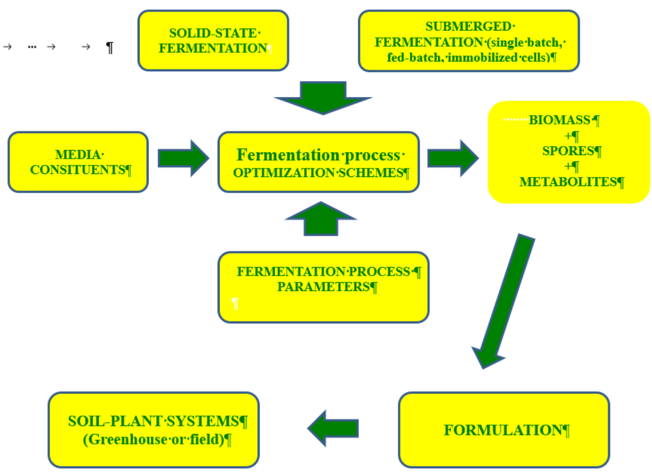

Figure 1: General scheme of submerged and solid-state-fermentation-based production of bioformulates.

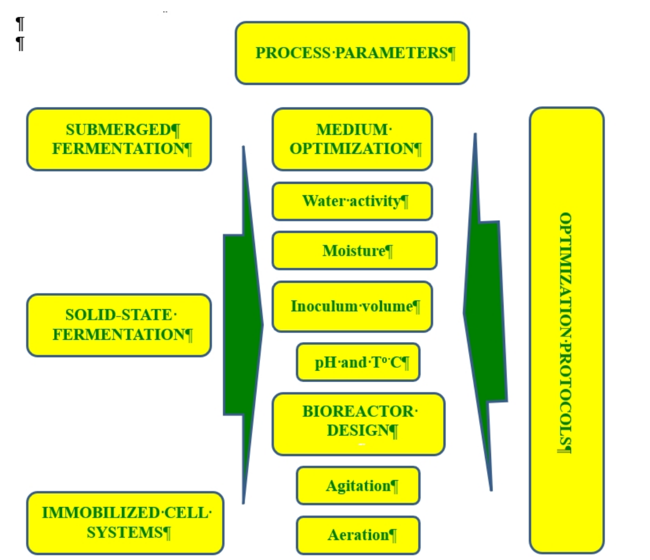

Figure 2: Main points of optimization options for different fermentation profiles always bearing in mind further formulation procedures.

Production Details

In general, there are limited number of studies on techniques and related bioreactors for the production of inoculants based on plant beneficial microorganisms, while the increasing volume of bioformulations application in field conditions is highly controversial [12]. The most applied at laboratory conditions and small soil-plant trials are cells, mycelium, and/or spores. These are produced on plate dishes/shake flasks, with widely used specific media depending on the type of the microorganism (Figure 2). In many cases, they are introduced directly without (or limited) further formulation into soil-plant system [14,15]. What we know is that in general the production process could be carried out in solid-state or submerged conditions. Many research groups work on just one of these production options. However, it would be of great utility for the biotechnological companies producing plant beneficial microorganisms to have the possibility to select the production mode. Therefore, if the strain is able to grow and develop sufficient amount of biomass/spores in solid-state and submerged conditions, it could be better to offer two production schemes (Figure 2). Both fermentation processes offer specific advantages and disadvantages, which are well known in general although applying which one of them leads to production of different bioproducts, which, however, depend on the media and microorganisms used [7,16,17]. Solid state fermentation and submerged liquid fermentation differently affect microbial growth and microbial metabolic activity and in some cases the control and management of the overall microbial development in both processes are environmentally dependent. For example, recently we found that a simple medium buffering increases the growth of *Paenibacillus polymyxa* in conditions of liquid submerged fermentation (unpublished results, see the Acknowledgements).

However, the same strain showed higher spore formation in solid-state than in submerged fermentation, with the number of CFU/ml always depending on the type of the solid substrate. Our experience confirmed the results of other authors that solid-state fermentation is advantageous comparing to submerged process offering easier final formulation combined with product viability after longer periods of storage [18-20]. On the other hand, the liquid submerged fermentation is easier to control and can produce more rapidly the desired biomass and/or plant beneficial microbial metabolites. Liquid-medium-based agitated processes provide many advantages compared to solid substrate-based fermentations [21]. One of the main advantages is the homogenous distribution of both nutrients and oxygen in the bioreactor. Manipulation of the environmental conditions in liquid agitated bioprocess affects the respective microbial behavior and this process is easier in comparison with solid-state fermentation. In the field of microbial production there are still many unexploited biotechnological schemes. For example, fed-batch mode of fermentation, which is used in some biotechnological small- and large-scale processes, is not tested in the production of many biofertilizer and biocontrol microorganisms. Similarly, processes with immobilized cells, biomass recycling, and continuous fermentations are not applied in this field although they offer a number of technological and economic advantages [7]. Here, we should mention the fact that microbial active cells are usually attached to surfaces or immobilized within soil particles [7] and some of the above processes can rely on these cell properties.

Formulation Connection

Microbial products are formulated as solids, liquids, or slurries. However, the process of microbial growth and formation of spores to great extent determines the mode of formulation and the type of the commercial product [5,22]. For example, inoculum from Petri dishes consisting of 1 ml (1×10^6 spores ml^{-1}) suspension is added to the planting hole [20]. In some experiments, the microbial mass grown in Erlenmeyer flasks and separated from the fermentation liquid is mixed with soil (1 g biomass in 100g soil) [15]. However, many studies, following these strategies in controlled and small open field trials with plants, showed controversial results and opinions [24].

- The final products of solid-state fermentation consist of [7]:The solid, partially degraded particles, usually lignocellulosic waste materials, which in many cases serve simultaneously as a support and substrate;

- Microbial biomass (including spores) in the form of more or less well-developed mycelium layer or bacterial cells within the substrate pores and/or on their surface;

- Metabolites, produced during the fermentation process with plant beneficial properties.

After drying and milling, this material could be used directly as a commercial product. Another possibility is to separate the spores of the post-fermentation material and, after mixing with solid cell-viability-enhancing protectors, can be used in soil-plant systems. The third option is to extract the metabolite part of the mixture and form a cell-free product [18,22]. While the first two options are well known, the third one is still in its infancy. However, our opinion is that the development of such kind of cell-free microbial plant beneficial metabolite-based bio formulates is the future of the bio-based agriculture by many reasons. The main one is the lack of necessity of a cell- or spore-based formulations for further adaptation and development in a soil-plant system. Fermentation cell-free liquids (broths) contain many metabolites, some of which, could stimulate growth and activity of other microorganisms in soil or in bioreactors. In a highly complex substratum such as soil, with millions of microorganisms in small volumes, interchanging metabolites and specific growth substances is a natural process within a given microbial community, which also affects phenomena such quorum sensing, biofilm formation and interactions between plants and plant beneficial (or pathogenic) autochthonous/introduced microorganisms [25]. Therefore, introducing metabolite-containing cell-free post-fermentation liquid could be a challenge as well and needs additional studies.

Another used technique for formulation is the macro- and micro-encapsulation of cells and spores of plant beneficial microorganisms and particularly of double [26], triple, and multiple microbial gel-based formulations, which could also include phyto- or microbial stimulants [27]. In general, gel-entrapped biological systems offer many advantages such as better survival during storage and slow release of their content in soil while protecting it from harsh soil conditions. Additional advantage is that they could be used also in fermentation processes to produce the above-mentioned metabolites or mineral fertilizers if we can use microorganisms, which solubilize insoluble mineral-bearing materials [28].

Final Remarks: Effect on Microbial Structure and Relation to the Overall Holobiome

An important feature of plant beneficial microorganisms is represented by their abiotic and biotic stress mitigation capabilities. It is another complex issue, which in soil-plant systems depends also on the mode of microbial production and formulation. Analyzing the results which we obtained in this field of research within EU funded projects (see the Acknowledgements), it appeared that the microorganisms are more efficient in immobilized state in conditions of high salinity and high/low pH as sole or combined stress factors [29]. Testing how to alleviate different abiotic and biotic stress factors by microbial formulates in one experimental scheme should be highly recommended as one unique stress factors rarely occurs in real soil. Similarly, the effect of microbial formulations on the belowground biodiversity (bacteria, fungi, protists, and invertebrates) and particularly on the structure of the microbial communities should be noted. One very attractive and useful technique to manage different microbial communities is to develop methods, which could be able to predict how different species assemblages can affect the composition of the community. At this moment, we pay attention on the diverse physical, biological, and ecological processes governing microbial changes, which is based on a highly limited information. However, applying the advantages of deep learning all these constrains and limitations can be eliminated [30].

Overall, we could finish this short story interrelating the human microbiome, animal microbiome, and plant beneficial microorganisms [31]. Following the One Health approach, we should not only register the change of the soil-plant related microorganisms but change the overall strategy of managing the traditional microbial-plant profile. Many studies just show the increase of biodiversity and community changes as a result of soil microbial inoculation but in fact it is a natural process, which can be observed after whichever physical, chemical, and biological change in the soil environment (particularly soil salinity, temperature, and soil pH) [32-34]. In our opinion, what should be more attractive, and challenging, is to use already selected and well-known microorganisms beneficial for humans, such as probiotics, in soil-plant systems. Enrichment of plant biomass with probiotics and their consumption will return the naturally existing cycle of minerals and microorganisms within the holobiomes [35]. Similarly, the future studies should be oriented towards personalized individual and/or complex application of different prebiotics, probiotics, synbiotics, as basic or alternative technologies for developing mixed plant- and pharma-based products, which could improve the overall status and/or treat different human and agricultural deficiencies. In any case, preliminary large studies of microbial functions by multi-omics approach could be important with a subsequent test under various agricultural conditions. Further optimized fermentation processes aiming at rapid production of high microbial biomass or spore density based on substrates which could be included in the formulated products, is the next essential step. Formulation or preparation of the commercial product following the fermentation stage should be always interrelated to both the fermentation process and the soil-plant characteristics, which will determine the method and the composition of the formulate. Therefore, a strong relation between the traditional and novel biotechnological approaches should be further developed (always based on the achievements of previous studies) to obtain highly efficient and multifunctional bio-based formulates which should satisfy the consumer needs for a healthy and tasty [36] agricultural products.

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