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# Importance of Microbial Exo-Metabolites as Postbiotics for Sustainable Agriculture

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**Mini Review**

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## Abstract

Plant growth-promoting (PGP) microorganisms are bacteria and fungi associated with the plant holobiont that can positively affect the health and growth of plants. Their beneficial activity is associated with a mixture of organic compounds produced and released outside the cell, including phytohormones such as auxins and auxin-related compounds. This mini-review describes the importance of using microbial exo-metabolites as postbiotics for developing innovative second-generation plant biostimulants for sustainable agriculture.

**Keywords:** Plant Growth-promoting; Plant Holobiont; Plant Postbiotics

**Abbreviations:** PGP: Plant growth-promoting; IAA: Indole-3-acetic Acid; IBA: Indole-3-butyric Acid.

## Mini Review

Overusing chemical pesticides and fertilizers in agriculture gave rise to environmental pollution and biodiversity loss. According to the European Commission, approximately 60-70% of soils in the EU are unhealthy due to improper use of chemicals, soil erosion, and unbalanced crop rotations. Furthermore, in the last years, the situation has been aggravated by global climate change that has determined irreversible modifications in the agroecosystems. Concerned with these challenges, the EU's strategies on the Green Deal, the Farm to Fork, and the Bioeconomy aim to preserve and improve European natural resources. One of the central pillars of the European Green Deal is reducing the use of fertilizers and chemical pesticides by 20% and 50%, respectively, before 2030. Within this framework, approaches based on new technologies and bio-based products, such as plant biostimulants, are promising alternatives to agrochemicals to pursue global food security and agricultural sustainability.

At the European level (European Regulation no. 1009 of June 5, 2019); biostimulants have been defined as *"any product that enhances plant nutritional efficiency and tolerance to abiotic stress independently of its nutrient content."*

Based on this definition, biostimulants are classified into two different categories: 1) bioactive natural substances (animal and vegetal protein hydrolysates, humic and fulvic acids, seaweed extracts, and silicon); 2) beneficial microorganisms (mycorrhizal fungi and N-fixing bacteria belonging to the genera *Rhizobium*, *Azotobacter*, and *Azospirillum*). The same EU regulation on fertilizing products prevents using Plant Growth- Promoting (PGP) microorganisms from species different from those mentioned before (https://eur- lex.europa.eu/legal-content/EN/

TXT/?uri=CELEX:32019R1009).

The recent literature demonstrates that the ability to promote plant growth is associated with several bacteria and fungi. PGP bacteria include a heterogeneous group of endophytic and epiphytic microorganisms that actively colonize plant tissues or surfaces, positively affecting plant development through direct and indirect mechanisms [1]. Indirect mechanism affects plant's health by protecting against pathogens through the production of siderophores, antibiotics, cell wall degrading enzymes, and soluble and volatile metabolites [2-5]. Direct plant growth-promoting mechanisms exert a positive effect by increasing the availability of essential nutrients (e.g., nitrogen, phosphorus, iron) and producing and regulating compounds involved in plant growth (e.g., phytohormones) [6-8]. Among plant regulators, indole-3-acetic acid (IAA) is the most abundant member of the auxin family [9].

In plants and bacteria, IAA biosynthesis proceeds through different pathways that can be classified into two categories: tryptophan (Trp)–dependent or Trp-independent. In the bacteria systems, the indolic compounds can be excreted outside the cells with other metabolites enhancing auxin activity [10]. The production level of auxin and the array of related molecules are strictly strain dependent. The production of these molecules is influenced by culture conditions, growth stage, and substrate availability. The presence of tryptophan, vitamins, salt, oxygen, pH, temperature, carbon source, nitrogen source, and growth phase contribute to regulating auxin/IAA biosynthesis [10]. In *Azospirillum brasilense*, the expression of the indole-3-pyruvate decarboxylase (the key enzyme involved in the IAA biosynthesis) is upregulated by: IAA and synthetic auxins, such as 1-naphthaleneacetic acid, 2, 4-dichlorophenoxypropionic acid, and p-chlorophenoxyacetic acid [11]; daylight [12]; nitric oxide [13]; abiotic stress effectors, including abscisic acid and 1 - aminocyclopropane 1-carboxylic acid [12]. Aerobic growth has differential effects on the IAA production in different PGP bacteria: it inhibits IAA synthesis in *A. brasilense* [14]; stimulates its production in *Pantoea agglomerans* [15].

For this reason, there is an increased interest in characterizing the exo-metabolome of PGP bacteria using both target and untargeted metabolomics approaches [16,17]. This goal can be achieved by analyzing the profile of the secreted auxins and identifying other molecules that can modulate the uptake of the exogenous phytohormones. PGP bacteria can also promote plant growth by inducing the expression of auxin-responsive genes in host- plant roots using elicitors different from auxin. These molecules, alone or in combination with bacterial auxins, determine transcriptional changes in hormone and cell wall plant-related genes induce root elongation, increase root biomass development, and decrease stomata size and density [18].

Interestingly, it was demonstrated that using PGP-selected strains such as *P. agglomerans* C1 allows for obtaining a combination of metabolites that work in synergy with IAA to stimulate the adventitious root formation. Luziatelli, et al. [19] demonstrated that the treatment of *Pyrus communis* shots with exo- metabolites from strain C1 determined an earlier development and the change in the root architecture compared to a control treatment with the synthetic hormone indole-3-butyric acid (IBA) regularly used in commercial plant propagation systems. The successful application of the plant postbiotics biostimulants is based on the possibility of developing eco- friendly and more sustainable products concerning ingredient origin and process cost. In particular, the formulation of the culture medium must satisfy the following requirements: (i) free from animal-derived components; (ii) have similar nutritional benefits compared to soy and milk proteins, and (iii) provide a highly nutritious general-purpose range of media for the growth of bacteria and fungi.

Comparative metabolomics studies in *Enterobacteria* revealed a significant effect of the culture medium on balance between IAA and other metabolites secreted by the microorganism. In the defined medium, the carbon source affects the release in the culture medium of peptides and cyclopeptides. These compounds are known to crosstalk with auxins and affect auxin transport and turnover [10]. The carbon source also significantly affects the production level of IAA and IAA-related compounds (indole-3-carbinol and IAA-leucine), which modulate the IAA availability in the plant system [20,21]. Several authors demonstrated that the shift from rich to defined medium stimulates the excretion of metabolites with antimicrobial activity and affects the hypoxanthine/xanthine ratio, which has positive effects on the plant's response to auxin and environmental stresses and the plant purine salvage pathway [15,22].

In a contest of green chemistry, replacing the traditional culture media containing ingredients of animal origin with plant-based culture media will be of great interest. The plant-based media have been successfully used to optimize the *in vitro* cultivability of rhizobacteria and their recovery from plant-soil environments [23]. More recently, Luziatelli, et al. [19] demonstrated that, in a peptone-based medium, replacing animal-tryptone with vegetable peptone determined a significant increase in the auxins yield and specific productivity by the PGP strain *Enterobacter* sp. P-36. These results indicate that the culture medium has a central role in exploiting the metabolic potential of PGP microorganisms and is critical in producing plant postbiotics.

## Conclusion

Bioactive compounds produced by PGP microorganisms under controlled fermentation conditions can represent a valuable approach to developing new microbial biostimulants with higher safety and a lower environmental impact. The use of plant postbiotics as an alternative to conventional microbial inocula can facilitate the safety assessment of these new products and have a lower impact on the plant holobiont. In addition, the plant postbiotics offer the opportunity to overcome the limitation of species allowed to be marketed as biofertilizers. Finally, with the aim of creating multifunctional bioproducts, the plant postbiotics offer the opportunity to combine the metabolic potential of different PGP microorganisms that exhibit complementary and synergistic effects.

## Conflicts of Interest

The authors declare that there is no conflict of interest regarding this study.

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