APPLICATION OF METABOLITES SECRETED BY PLANT GROWTH-PROMOTING BACTERIA TO SELECTED CROPS AND EVALUATION OF NUTRITIONAL QUALITY THEREOF

Melini Francesca[1,2], Luziatelli Francesca[1], Melini Valentina[2], Anna Grazia Ficca[1], Ruzzi Maurizio[1]

[1] Dept. for Innovation in Biological, Agro-food and Forest systems (DIBAF), University of Tuscia, Via S. Camillo de Lellis, 01100 VT

[2] CREA Research Centre for Food and Nutrition, Via Ardeatina 546, 00178 Roma

II Year PhD student E-mail address: francesca.melini@gmail.com

Introduction

Application of bio-based products such as plant bio-stimulants emerged as a promising alternative to agrochemicals and a new frontier of investigation.

Plant growth-promoting rhizobacteria (PGPR) act as bio-stimulants. They can increase crop tolerance against abiotic stresses and improve nutrient use efficiency, plant health, productivity and yield [1]. Biosynthesis of plant growth (PG) regulators is among the direct mechanisms correlated to PG stimulation. Indole-3-acetic acid (IAA) is the most common plant hormone of the auxin class, regulating various PG processes.

The identification of efficient bacterial IAA producers and other auxin-related compounds has thus become pivotal. Several genera have been investigated for their PGP traits and ability to produce IAA. As the production of these compounds proved to vary from strain to strain, it is crucial to investigate the IAA-production ability and exometabolome of auxin-producing bacteria, i.e., selected *Enterobacter*/*Pantoea* strains.

Aim

The present PhD research project aims to:

- investigate and define the role/potential of selected *Enterobacter* and *Pantoea* strains in the production of novel plant postbiotics, and
- evaluate the effect thereof on the nutritional quality of produces.

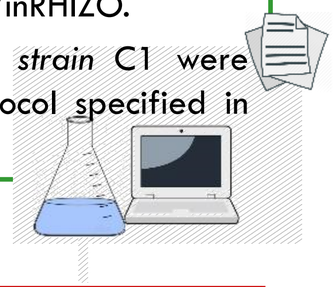
Materials and Methods

PGPR strains used in this work were *Pantoea agglomerans* C1 and *Enterobacter* sp. P36 [2, 4].

Different growth media and conditions were investigated, and optimization thereof for production of metabolites occurred according to the protocol reported in [3, 4, 5]. Strain growth was investigated in Erlenmeyer flasks and in a 2-L stirred tank fermenter.

Application of metabolites to tomato seedlings was done using the quick dip method. After the treatment, seedlings were grown in transparent polypropylene microboxes with wetted quartziferous sand under controlled conditions. Root morphology was investigated by WinRHIZO.

The genomic features of *Pantoea strain* C1 were determined according to the protocol specified in [1, 3].

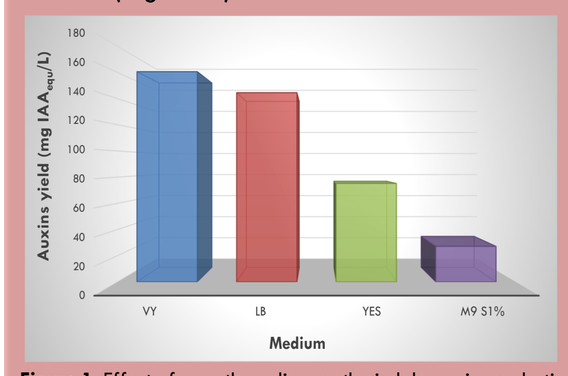


Results and Discussions

Growth media and conditions

Upon growth of *Pantoea agglomerans* strain C1 on different media amended with tryptophan (Trp, 40 mM), it emerged that:

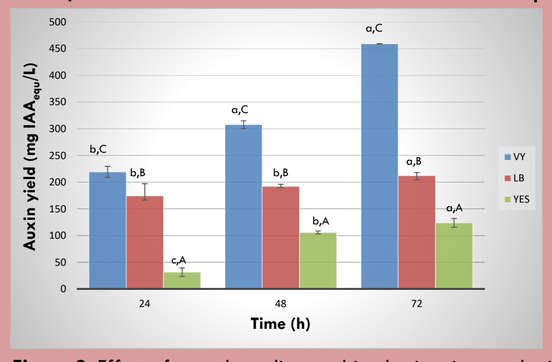
- The vegetal peptone-yeast extract (VY) medium allowed the highest production of indole auxins (Figure 1).



- For all media, it emerged that Trp (40 mM) addition is mandatory for IAA production. Trp is, in fact, a precursor and inducer of the IAA biosynthetic pathway.
- Glycine is not a valuable alternative to Trp.
- A higher carbon source concentration, pre-inoculum volume, OD do not positively affect IAA production (data not shown).

Figure 1: Effect of growth medium on the indole auxin production yield by *P. agglomerans strain* C1. Results are means of three independent experiments.

The ability to produce IAA was also investigated in *Enterobacter* sp. strain P36 upon growth in VY, LB and YES medium amended with Trp and by varying fermentation time [4].



Results showed that

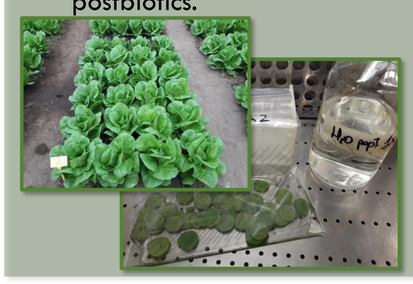
- the highest auxin yield occurred in VY and LB medium (Figure 2);
- the highest auxin/IAA level was obtained when the *Enterobacter* sp. strain was grown for 72 hours. The use of the VY medium determined a two-fold increase compared to the animal-based peptone (LB).

Figure 2: Effect of growth medium and incubation time on the indole auxin production yield by *Enterobacter* sp. strain P36 grown in shake flasks. Results are means±SD of three independent experiments. Differences in letters indicate that the values are significantly different ($p<0.05$). Lower-case (a,b,c) letters are referred to values of the same series (growth medium), while capital (A,B,C) letters indicate statistically significant differences among values of different series (same incubation time)

Application of *P. agglomerans*' metabolites to selected crops (pot and farm trials)

Upon rooting test on tomato cuttings (5 different treatments: i-ii) metabolites by *P. agglomerans* strain C1 and DSM3493; iii) LB; iv) LB+Trp; v) standard fertilizer), it emerged that treatment with:

- strain C1 metabolites were more efficient than metabolites by another strain;
- LB gave non-homogenous results among the different cuttings;
- the commercial fertilizer gave poor results compared to the postbiotics.



Biosafety experiments were also performed by treating lettuce plants, artificially contaminated with *E. coli*, with strain C1 metabolites and other biostimulants to compare efficacy.

Data are currently under analysis.

Upon cultivation of the *Enterobacter* sp. strain P36 in a 2-L fermenter with VY medium amended with Trp, under batch operating mode, it emerged that at an initial inoculum of 0.5 x 10^9 cells/mL (predicted OD_{600} of 0.2), the highest indole auxin production occurred at 48h (Figure 3A), while when the inoculum was set as 1.0 x 10^9 cells/mL (predicted OD_{600} of 0.4), the highest yield was obtained after 24h growth (Figure 3B).

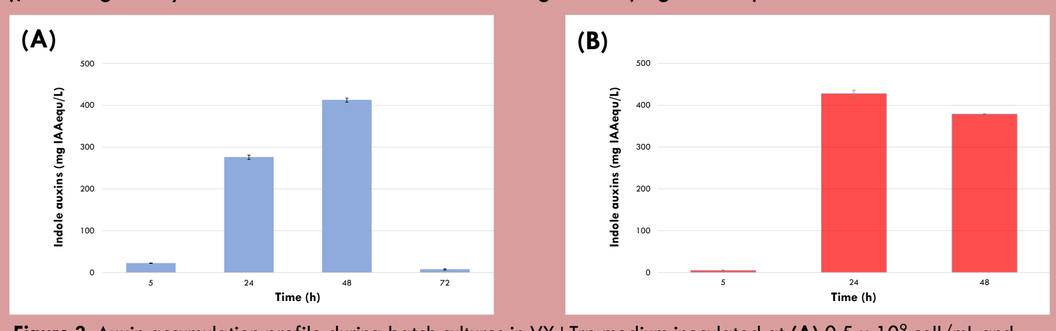


Figure 3: Auxin accumulation profile during batch cultures in VY+Trp medium inoculated at **(A)** 0.5 x 10^9 cell/mL and **(B)** 1.0 x 10^9 cell/mL of *Enterobacter* sp. Strain P-36. The error bars represent standard errors of the means (n=3). Differences in letters indicate that the values are significanly different ($p<0.05$).

Upon analysis of *P. agglomerans* strain C1, a number of genes related to resistance to toxic metals (i.e., arsenic, copper and cadmium) was identified (Figure 4) [1].

These results point out that strain C1 has the potential to survive and grow in environments contaminated by heavy metals and can thus be used as a PGP bacterium in heavy metal polluted soils.

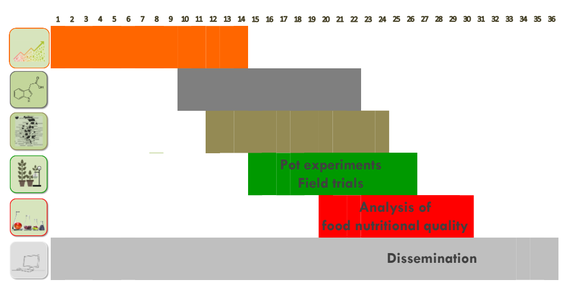
Figure 4: Organization of the heavy metal gene (MRG) cluster of *P. agglomerans* C1 and comparison with other *Pantoea* genomes that have the same 19-gene cluster and with pEM02 plasmid from *Erwinia* sp. EM595 (GenBank reference: LN907829.1). Genes with unknown function are indicated in white.

Conclusions

To conclude, the two strains of PGPR investigated in this research work proved to be good auxin producers. However, further investigations were scheduled for the 3rd year to gain a more comprehensive insight into their potential as biostimulants.

The 3rd year will be also focused on the determination of the nutritional and antioxidant profile of selected vegetal produces treated with metabolites from the selected PGPR strains.

Key results will be continued to disseminate.



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