Indole-3-Acetic Acid Production by Pseudomonas Fluorescens LC830621.1 under High Temperature Conditions

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Abstract

The aim of the present study was to evaluate in vitro indole-3-acetic acid production by Pseudomonas fluorescens LC830621.1 under high temperature conditions. The rhizospheric bacterial strain identified as Pseudomonas fluorescens LC830621.1, which was isolated from rhizosphere of rice soils contaminated with heavy metals, was taken as reference. Growth kinetics of the bacterium was performed at three temperatures (30, 32 and 45°C). Subsequently, in vitro assay of indole-3-acetic acid production was performed at 32 and 45°C for 26 hours. The results show that the production of indole-3-acetic acid by Pseudomonas fluorescens LC830621.1 at 32°C 1.29 from zero hour to 17.15 mg/L up to 26 hours of incubation. While at 45°C the production of the compound was 0.29 at 0 hours up to 7.5 mg/L up to 26 hours. The results obtained infer the ability of Ps. fluorescens LC830621.1 to produce indole-3-acetic acid up to 45°C and the behaviours and adaptation of growth and auxin expression as a compound involved in plant growth promotion.

Keywords: Bacteria, rhizosphere, auxin, temperature.

1. Introduction

Pseudomonas fluorescens is a Gram-negative bacterium with strictly aerobic energy metabolism and chemoorganotrophic nutrition that does not require growth factors. According to Santoyo et al. (2016), P. fluorescens adapt easily to the soil to survive and colonize the root system of plants. Although the rhizosphere is a hostile microenvironment for the organisms that inhabit it, Pseudomonas species of the fluorescens group contain an arsenal of compounds to combat and fight to occupy the best niches, those where nutrients are found.

According to Hernández-León et al. (2015), Pseudomonas fluorescens has direct plant growth-promoting effects through the synthesis and excretion of phytohormones. In vivo studies show that the use of Ps. fluorescens strains UM16, UM240, UM256 and UM270 increase shoot and root weight, as well as chlorophyll concentration in the plant species M. truncatula. UM16 and UM270 produce high concentrations of indole-3-acetic acid (IAA), with 22 and 10.6 µg mL-1,

respectively, aiding root growth and plant development. The plant growth-promoting effects of the Pseudomonas fluorescens strains indicate the great potential of the bacteria for use in agriculture, increasing crop yields and reducing the use of exogenous applications of plant growth promoters.

Pseudomonas fluorescens has long been reported as a plant growth promoting bacterium (Adesemoye et al., 2009). The incorporation of bacteria favors soil exploration, improves water accessibility, reduces nutrient loss processes, improves water stress, inhibits the attack of phytopathogens and maintains active growth rates of the crop by improving its photosynthetic capacity (Kah and Brown, 2006; Díaz-Zorita and Fernández, 2008).

Taking into account the different scientific reports on the importance of the Pseudomonas fluorescens strain as a plant growth promoter, it was proposed as a strategy to evaluate in vitro the production of indole-3-acetic acid at different times by the rhizospheric bacterium Pseudomonas fluorescens LC830621.1 under high temperature conditions.

2. Materials and Methods

Growth of Pseudomonas fluorescens. For the evaluation of the present study, C17ROsLIM, stored in the genome bank of the Bioprospecting research group of the Faculty of Agricultural Sciences, University of Sucre, was used, which presents 99% homology with sequences of the Genbank with the species LC830621.1 Pseudomonas fluorescens.

Pseudomonas fluorescens strain LC830621.1 was grown on medium supplemented with: 1 g/L $(NH_4)SO_4$, 1.5 g/L KH_2PO_4, 1 mL/L sln 20% (w/v) $MgSO_4.7H_2O$, 1 mL/L sln 1% (w/v) $CaCl_2.2H_2O$ and 1 mL/L of a micronutrient solution, salts and micronutrients are Merck reagent grade (Jiang et al., 2008; Pijuan et al., 2009; Thakor et al., 2005). Analytical grade glucose from Merck and molasses produced by Ingenio Castilla for Arco Iris Natural Products with a glucose content of 12% and fructose content of 13% were used as carbon source.

Kinetic assays. For inoculum preparation 0.25 L nutrient broth (Merck), previously sterilized in an autoclave, was inoculated with Pseudomonas fluorescens and incubated at 30, 32 and 45°C °C for 26 h. Every 2 h, the growth performance of the strain was evaluated by performing absorbance measurements at 600 nm.

Production and quantification of indole-3-acetic acid. The indole acetic acid production capacity of Pseudomonas fluorescens LC830621. 1, was evaluated, using the liquid medium Burk Korea: 0.41 g KH2PO4, 0.52 g K2HPO4, 0.05 g Na2SO4, 0.2 g CaCl2, 0.1 MgSO4 * 7H2O, 0.01 g Fe SO4 * 7H2O, 0.0025 g NaMoO4, in 1000 ml distilled water, and supplemented with 0.1 g tryptophan (indole acetic acid precursor). The D.O. of the cultures was measured, the isolates were brought to the same concentration of 106 CFU/tissue, then seeded in the described medium and incubated at 150 rpm for 72 hours, after which time 1 ml of the bacterial suspension was taken and centrifuged at 12000 rpm for 5 min. Then an aliquot of the supernatant was taken and Salkowski's reagent was added (Dawwam et al., 2013; Gordon and Weber, 1951), this was incubated in the dark for 30 min after which the absorbance of the sample was measured at

450nm. For quantitative determination, the standardized curve was used using standard solutions of pure 3-indole acetic acid proposed by (Lara et al., 2011; Sarwar et al.,1992).

3. Results and Discussion

For the evaluation of the present study, C17ROsLIM, stored in the genome bank of the Bioprospecting research group of the Faculty of Agricultural Sciences, University of Sucre, was used, which presents 99% homology with sequences of the GenBanck with the species LC830621.1 Pseudomonas fluorescens (figure 1).

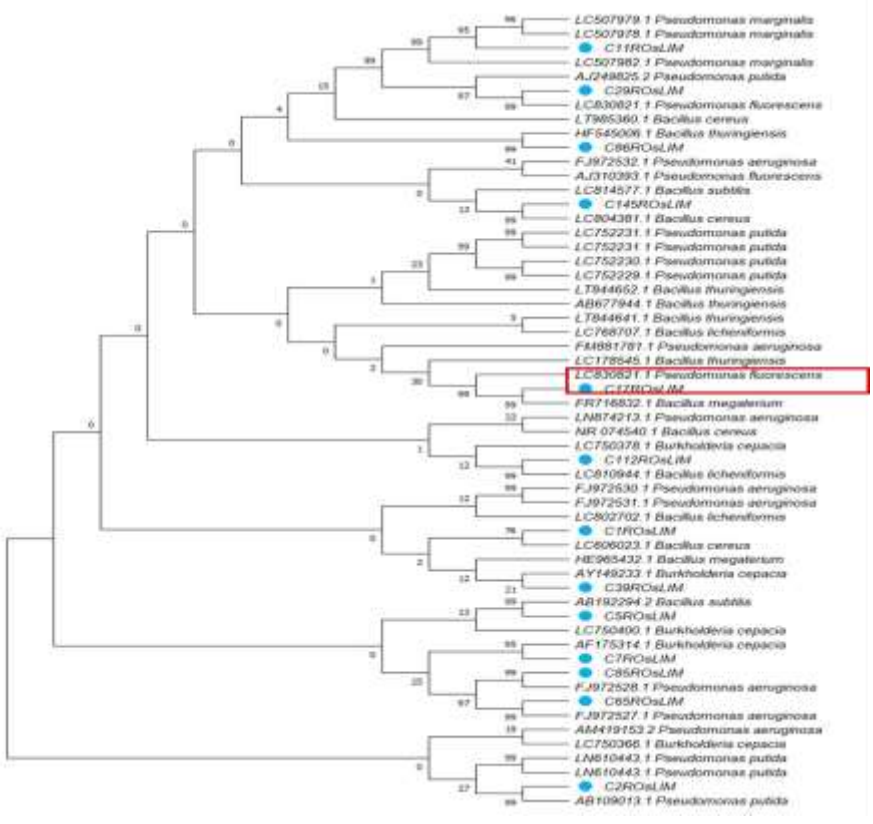

Figure 1. Dendogram derived from the analysis of 16 rDNA gene sequencing of rhizospheric bacteria and homology with bacterial sequences from the NCBI genome bank.

Figure 2 shows the growth behaviours of the Pseudomonas fluorescens LC830621.1 strain at 32 and 45°C at different incubation times.

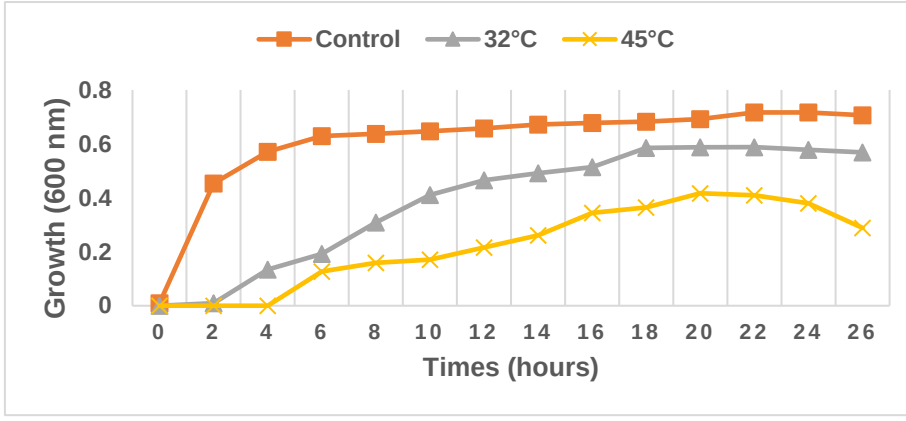

Figure 2. Growth behaviours of Pseudomonas fluorescens LC830621.1 at 32 and 45 °C.

The observed results infer that Ps. fluorescens LC830621.1 has adaptability up to 45°C. Faced with a future scenario of increasing environmental temperature due to global warming, this strain of bacteria could have a response to the problem of climate change. The results were compared with the control strain, which was grown at a temperature of 30°C.

The growth curves show differences in the exponential phase, the maximum biomass concentration and the duration of the exponential phase, when the optimal growth temperature is modified. Temperatures above 30°C generate a drastic change in the stationary phase which is due to protein denaturation and cell death (Price and Sowers, 2004).

Figure 3 shows the production of indole-3-acetic acid by Pseudomonas fluorescens LC830621.1 at 32 and 45 °C. The results show that the production of indole-3-acetic acid by Pseudomonas fluorescens LC830621.1 at 32°C 1.29 from zero hour to 17, 15 mg/L until 26 hours of incubation. While at 45°C the production of the compound was 0.29 at 0 hours up to 7.5 mg/L up to 26 hours.

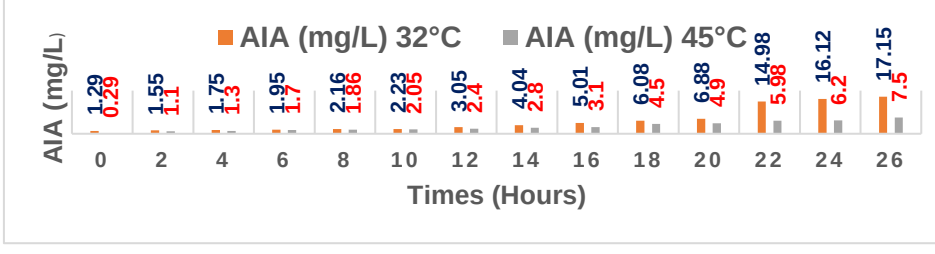

Figure 3. Production of indole-3-acetic acid by Pseudomonas fluorescens LC830621.1 at 32 and 45 °C.

Indole-3-acetic acid (IAA) is the major native auxin of higher plants. IAA is involved in plant growth and development, mainly in a number of physiological processes including cell

elongation and division, tissue differentiation, phototropism, gravitropism and in defensive responses (Santner et al., 2009; Leveau and Lindow, 2005), highlighting an important role in xylem and root formation (Davies, 1995).

AIA biosynthesis is not limited to higher plants. Microorganisms such as bacteria, fungi and algae are capable of synthesizing AIA, which can affect plant growth and development (Lee et al., 2004). In bacteria, AIA production is a relevant capacity of both plant growth promoting bacteria (PGPBs) and plant pathogenic bacteria (Patten and Glick, 1996; Patten and Glick, 2002). Higher plants exude, among other components, the amino acid tryptophan, which is the main precursor for microbial AIA biosynthesis (Kravchenko et al, 2002; Kravchenko et al, 2004; Kamilova et al., 2006; Idris et al., 2007).

AIA-producing bacteria have the potential to interfere with AIA incorporation processes in plants. The consequence for the plant depends on the amount of AIA produced and the sensitivity of the plant tissue to changes in AIA concentration (Leveau and Lindow, 2005), which is associated with the biosynthetic pathway used by plant-associated bacteria (Spaepen et al., 2007a). Tryptophan is the main precursor in AIA biosynthetic pathways in bacteria (Spaepen et al., 2007a, Tsavkelova et al., 2006). Different AIA synthesis pathways have been identified in bacteria. Five of these anabolic pathways are tryptophan-dependent. The most important and widely distributed AIA synthesis pathways are the indole-3-pyruvate (IPA) and indole-3-acetamide (IAM) pathways (Spaepen et al., 2007b). The IPA pathway has been reported mainly in PGPB, while the IAM pathway has been described in plant pathogenic bacteria (Spaepen et al., 2007a, Spaepen et al., 2007b).

AIA produced by PGPBs has a major impact on their plant growth-promoting activity. Several bacteria of the taxonomic classes α-Proteobacteria, β-Proteobacteria, δ-Proteobacteria and Bacilli are AIA-producing PGPBs. The application of Pseudomonas fluorescens CHA0 stimulates root tissue development by auxin production, which under in vitro culture conditions with the precursor tryptophan synthesizes AIA (Oberhänsli et al., 1991).

4. Conclusion

The results show that the production of indole-3-acetic acid by Pseudomonas fluorescens LC830621.1 at 32°C was 1.29 from zero hour to 17.15 mg/L up to 26 hours of incubation. While at 45°C the production of the compound was 0.29 at 0 hours up to 7.5 mg/L up to 26 hours. Likewise, growth kinetics of Pseudomonas fluorescens LC830621.1 was found at 30, 32 and $5°C, and growth adaptation of this strain was observed up to 45°C without losing the capacity to produce indole-3-acetic acid.

On the other hand, Pseudomonas fluorescens is considered an important biocontrol agent in the field of sustainable agriculture of the future, allowing satisfactory yields to be obtained without harming the environment. Currently, soils are degraded, decreasing organic matter content, increasing soil compaction and erosion, and there is a high degree of salinization, alkalization and contamination of groundwater. The future of food involves generating higher yields and

being able to feed the population, with Pseudomonas fluorescens LC830621.1 being a fundamental bacterium for producing indole-3-acetic acid and increasing organic matter content and crop yields.

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Author contribution. Alexander Perez Cordero: experiment execution, data analysis. Donicer Montes V and Yelitza Aguas M, conceptualization, writing - revision and editing. All authors have read and approved the manuscript.

Conflict of interest. All the authors of the manuscript declare that they have no conflictof interest.

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