Predictive Scenario for the Behaviours of Pseudomonas Aeruginosa in the Face of Global Warming

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Abstracts

Microbial biotechnology can provide solutions for sustainable development, including in food supply and in the regulation (e.g. of diseases or greenhouse gas emissions and sequestration) of ecosystem services for humans, animals and plants. The aim of this study was to assess the ability of Pseudomonas aeruginosa to produce plant growth-promoting compounds in response to sudden changes in temperature in order to predict future scenarios of the behaviours of this bacterial species in the face of global warming. The results of this study reveal that Ps. aeruginosa has the ability to biologically fix nitrogen, produce siderophores, solubilize zinc and phosphates at temperature changes from 37 to 45°C. This is the first study of the adaptability of the native strain isolated from arsenic-tolerant tropical pasture rhizosphere and of retaining the production of compounds directly involved in plant growth promotion up to 45°C.

Keywords: Bacteria, global warming, temperature, plant growth promotion.

1. Introduction

According to the work of Cavicchioliet al., (2016), micro-organisms play a key role in carbon and nutrient cycling, in animal (including human) and plant health, in agriculture and in the global food web. Micro-organisms live in all environments on Earth that are occupied by macroscopic organisms and are the only life forms in other environments, such as the deep subsurface and ‘extreme’ environments. Microorganisms date back to the origin of life on Earth at least 3.8 billion years ago and are likely to exist well beyond any future extinction event. Although micro-organisms are central to the regulation of climate change, they are rarely the focus of climate change studies and are not considered in policy making. Their immense diversity and varied responses to environmental changes make determining their role in the ecosystem a challenge.

According to the results of predictive studies conducted (Union of Concerned Scientists, 1992) and reported by the Alliance of the World's Scientists, the scientists' warning movement was established to alert humanity about the impacts of human activities on the climate and the

environment. In 1992, 1,700 scientists signed the first warning, raising awareness that human impact puts the future of the living world at serious risk.

As reported by Ripple, W.J.et al., (2017), by 2017, 25 years later, the second warning was released in a publication signed by more than 15,000 scientists. The movement has continued to grow, with more than 21,000 scientists endorsing the warning. By 2018 (Ripple, W.J.et al., 2018), they predicted that at the heart of the warning is a call for governments and institutions to shift from a policy of economic growth to a conservation economy that halts environmental destruction and allows human activities to achieve a sustainable future.

According to predictive analyses, two of the most important threats to health today - climate change and the spread of antibiotic-resistant bacteria - may be linked. Several scientific studies are analyzing this situation and have found that extreme weather conditions, such as heavy rainfall, flooding or rising temperatures, promote bacterial growth, including that of resistant microorganisms.

Also, in 2018, study by Ripple, W.J.et al., (2018), as a diagnostic result set out as 'Stressor interaction networks suggest that antibiotic resistance is derived from stress and temperature responses', notes that bacteria that have evolved in conditions of extreme cold or heat may be more resistant to certain antibiotics. The researchers tracked how bacteria responded to the stressful effects of heat shocks and observed that gene expression patterns changed similarly for both temperature and type of antibiotic.

As indicated by (Hoffmann A.A. and Sgrò, 2011; Hutchins and Fu, 2017), climate change disrupts interactions between species and forces them to adapt, migrate and be replaced by others or become extinct. Warming, acidification, eutrophication and overuse of the oceans (e.g. fishing, tourism) together cause coral reef decline and may induce ecosystem shifts towards macroalgae (Hughes, 1992; Bellwood et al, 2006; Hoegh-Guldberg et al., 2007; Mumby et al., 2007; Enochs et al., 2015) and benthic cyanobacterial mats (DeBakker et al., 2015; Ford et al., 2018). The ability of corals to adapt to climate change is strongly influenced by the responses of their associated microorganisms, including symbiont microalgae and bacteria (Ziegler et al., 2017; Torda et al., 2017; Quigley et al., 2018).

Based on the current reality of the climate change that we will face in a few years and its repercussions on the plant diversity that sustains animal food and, as a consequence, human food, it is still unknown how the microbial communities associated with the soil and rhizosphere will change in order to support the macroorganism-plant-animal interaction and the nutritional balance and detoxification of the environment, The strategy was to evaluate the behaviours of the bacterial species Pseudomononas aeruginosa isolated from rhizosphere of tropical pastures adapted to environments contaminated with heavy metals and to evaluate in vitro the behaviours of this plant species on an increase in temperature and plant growth-promoting activity in the face of a future scenario of global warming.

2. Material and Methods

Isolation of rhizospheric bacteria

A sample of 10 g of soil was added to 90 mL of a phosphate solution (g/L): 0.2 KH_2PO_4, and 0.8 K_2HPO_4, at pH 7.0, which was kept in agitation at 100 revolutions per minute (rpm)/30 min (Mauricio et al., 2010). Subsequently, serial dilutions up to 10-1 to 10-3 were carried out and the plates were seeded with M9 mineral medium modified by the microbiological research laboratory with the following composition:(g/L): 6.0 Na_2HPO_4, 3. 0 KH_2PO_4, 1.0 NH_4Cl, 0.5 NaCl, 0.5 yeast extract and 10 glucose; further included (mL/L): 2.0 $MgSO_4$-7H2O 1 M, 0.1 $CaCl_4$ 0.1 M, and 0.1 thiamine at a concentration of 1 mg/mL (sterilised by 0.2 μm filtration), pH 7 (Sambrook and Green 2012).

Once the isolation of each bacterial strain had been carried out, tolerance tests at different arsenic concentrations were evaluated.

Arsenic tolerance test

M9 mineral medium without the addition of Na_2HPO_4 and with the addition of 0.3 mM $AsCl_2$ (Massadeh et al., 2005), as modified, was used to estimate the bacterial population tolerant to $AsCl_2$. Plates were incubated at 28 °C for 48 h. Total culturable aerobic bacteria were determined as colony forming units (CFU)/g of soil as described by Brim et al.,1999. Colonies that grew on M9 mineral medium with $AsCl_2$ and showed different macroscopic morphology were isolated and purified by streaking until pure strains were obtained. These were stored at -70 °C in Luria-Bertani (LB) medium (g/L): 10 bactotryptone, 5 yeast extract, 10 NaCl (Atlas 2010) with 50 % glycerol and 0.3 mM AsCl2.

For growth-promoting activity we used the genomic bank of rhizospheric bacteria identified by sequencing strain C85ROsLIM with 100% homology to sequences of the bacterium Pseudomonas aeruginosa, which was incubated separately at temperatures of 35, 37, 39, 41, 43 and 45°C and evaluated for the production capacity of the following assays:

- Siderophore production.

Qualitative assessment of siderophore production will be carried out by direct seeding of each morphotype on the surface of the chromium azurol-S (CAS) medium proposed by Schwyn and Neilands (1987). They were incubated for 7 days at 30°C. The ability of the bacteria to produce siderophores was evidenced by halo formation.

- Nitrogen fixation

A direct surface seeding of the cadmium-tolerant rhizospheric bacteria was performed on selective BURK agar medium: 5 g MgSO4, 20 g KH2PO4, 5 g K2HPO4, 3.25 g CaSO4, 1.45 g FeCl3, 0.253 g NaMoO4, 1000 ml sterile distilled water (Park et al., 2005; Tejera et al., 2005) with no nitrogen source as an evaluator of nitrogen fixing activity, which uses a combined carbon source that allowed the recovery of a greater amount of bacteria with possible fixing activity, selecting only those that present the enzymatic system that allows them to reduce atmospheric

nitrogen and use it in their metabolism. The results were observed according to the growth of the bacteria in the medium.

- Phosphate solubilisation

For isolation, selection or preliminary evaluation of phosphate solubilizing activity, the solid culture medium according to NBRID was used: 10 g Glucose, 5 g Ca3(PO4)2, 5 g MgCl2 * 6H2O, 0.25 g MgSO4 * 7H2O, 0.2 g KCl, (NH4) SO4 in 1000 ml distilled water (Dawwam et al., 2013). This culture medium contains calcium phosphate salts and bromocresol purple as pH indicator. Approximately 6 days after sowing, bacterial colonies were selected and formed a transparent halo, indicative of the acidification process of the medium (turning from red to yellow due to the pH change), which indicated phosphate solubilizing activity.

- Zinc production

The zinc solubilizing capacity of the isolated strains will be evaluated on mineral salt medium (MSM: NaCl 1 g, $CaCl_2$ 0.1 g, $MgSO_4$ 0.5 g, KH_2PO_4 1 g, K_2HPO_4 1 g, yeast extract 4 g, agar 16-18 g in 1 L and pH maintained at 7.2). Different sources of insoluble zinc salts such as zinc oxide (1,244 g/L = 15,23 mM), zinc phosphate (1,3882 g/L = 4,0 mM), zinc carbonate (1,4728 g/L = 11,74 mM) and zinc sulphide (1,124 g/L = 11,54 mM) at a final concentration of 0,1 % shall be added to the medium individually and autoclaved at 121 °C for 30 minutes. A loop filled with bacterial growth shall be spread on MS medium overnight in nutrient broth. Plates shall be incubated at 30 °C for 7 days. Strains showing a clear zone around the colony shall be considered as zinc solubilizing strains. The halo zone shall be measured to determine the zinc solubilisation efficiency (ZnE) of the strains, using the method proposed by (Khanghahi, 2018).

3. Results and Discussion

Table 1 shows the results of in vitro evaluation of Pseudomonas aeruginosa strain response to different temperatures above the optimum growth temperature and the ability to retain biological nitrogen fixation, siderophore production, zinc and phosphate solubilisation at temperature changes from 37 to 45°C.

Table 1. Growth promoting activity of Pseudomonas aeruginosa at different temperatures.

Test	Temperature					
	35°C	37°C	39°C	41°C	43°C	45°C
Sidephore	+	+	+	+	+	+
Fixacion of nitrógen	+	+	+	+	+	-
Solubilization of zinc	+	+	-	-	-	-
Solubilization of phosphate	+	+	+	+	+	+

(+): expression of Activity; (-): No expression of growth-promoting activity

Table 1 shows the ability of Ps. aeruginosa to produce siderophore from 35 to 45°C; nitrogen fixation from 35 to 41°C; zinc solubilisation up to 37°C and phosphate solubilisation up to 45°C.

Figure 1 and 2 show the in vitro ability of Ps. aeruginosa to produce siderophore from 35 to 45°C; nitrogen fixation from 35 to 41°C; zinc solubilisation up to 37°C and phosphate solubilisation up to 45°C.

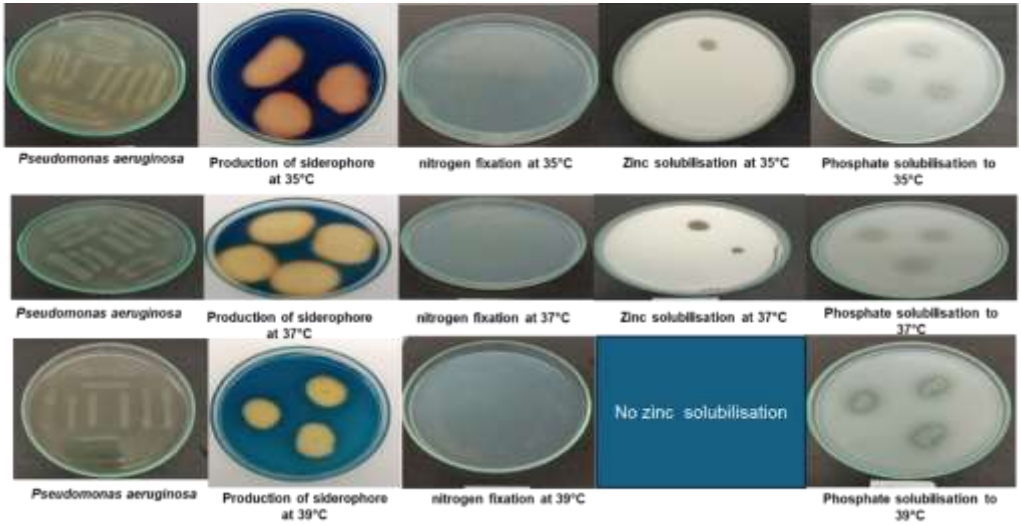

Figure 1. In vitro evaluation of the ability of Pseudomonas aeruginosa to produce siderophore, fix nitrogen, solubilize zinc and phosphate from 35 to 39 °C

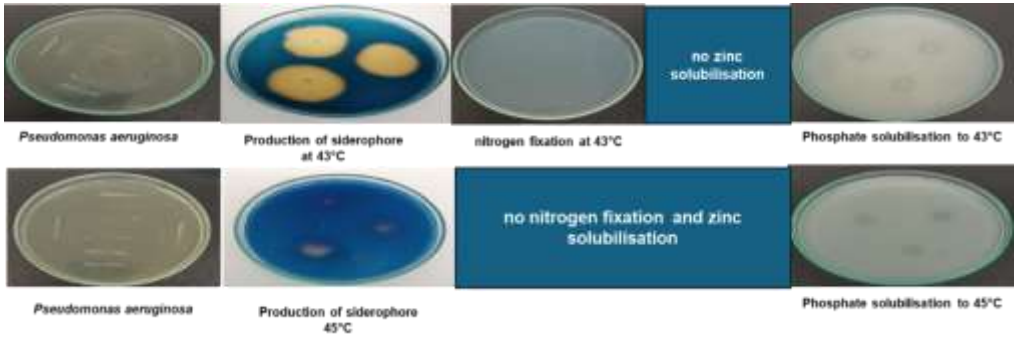

Figure 2. In vitro evaluation of the ability of Pseudomonas aeruginosa to produce siderophore, fix nitrogen, solubilize zinc and phosphate from 43 to 45 °C.

In the recommendations of the team of scientists in their manuscript prepared by Cavicchioliet al., (2016), the framework for quantitative models exists, but these models largely lack mechanistic details of microorganisms present in marine and terrestrial environments. The reason for this deficiency has more to do with the paucity of physiological and evolutionary data that allow robust predictions of microbial responses to environmental change than with the actual

process of model building at the mathematical level. A focused investment in expanding this mechanistic knowledge represents a critical pathway towards generating the global models essential for comparing, scaling and parameterizing Earth system model predictions of current and future climate.

Likewise, the research team Cavicchioliet al., (2016), justify that with an increase in temperature in ecosystems, the biodiversity of micro-organisms is rapidly decreasing due to human activity, suggesting that the biodiversity of certain host micro-organisms of animal and plant species will also decrease. An immediate, sustained and concerted effort is required to explicitly include micro-organisms in research, technological development, policy and management decisions. Micro-organisms not only contribute to the rate at which climate change is occurring, but can also contribute greatly to its effective mitigation and to our adaptation tools.

Itakura et al., (2013; Bakken and Frostegård, (2017), state that to understand the predicted global warming scenario, it is necessary to review studies carried out to date, for example, in the agricultural sector, progress in understanding the ecophysiology of microorganisms that reduce N2O to harmless N2 provides options to mitigate emissions. On the other hand, (Itakura et al., 2013). The use of bacterial strains with higher N_2O reductase activity has decreased N_2O emissions from soybean, and both natural and genetically modified strains with higher N_2O reductase activity provide avenues to mitigate N_2O emissions. Another serious scenario as stated by (Henderson et al., 2015), manipulation of rumen microbiota and breeding programmes that target host genetic factors that generate variations in microbial community responses (Roehe et al., 2016) are possibilities for reducing methane emissions from livestock.

In agriculture, as stated by (Godfray et al., 2010), increasing temperatures and droughts strongly affect the ability to produce crops. Fungal-based soil food webs are common in extensive agriculture (e.g. grasslands) and are abler to adapt to drought than bacterial-based food webs, which are common in intensive farming systems (de Vries et al., 2012; de Vries et al., 2018).

This is discussed by (Jing et al., 2015; Delgado-Baquerizo et al., 2016) who conclude that variations in climate can influence the structure and diversity of microbial communities directly (e.g. by seasonality and temperature) or indirectly (e.g. by plant composition, litter and root exudates). Soil microbial diversity influences plant diversity and is important for ecosystem functions, including carbon cycling.

4. Conclusion

The present study tested the ability of Pseudomonas aeruginosa strains adapted from tropical pasture rhizosphere environments growing in high arsenic concentrations and the ability to maintain biological nitrogen fixation activity, siderophore production, zinc and phosphate solubilisation at temperature changes from 37 to 45°C. This first study envisions the behaviours of this bacterium isolated from arsenic-contaminated environments and a possible in vivo behaviours under a global warming scenario.

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

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