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Effect of *Ulva lactuca* on Indole-3-acetic acid, Siderophores and Phosphatase Production by *Azospirillum brasilense* NH Growing in Seawater as Natural Medium

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Abstract: In this paper we aim to synthesize a new culture medium similar to the seawater, and test the osmotolerant strain *Azospirillum brasilense* NH for IAA and siderophores-production and phosphate solubilization in presence of a natural osmoprotectant *Ulva lactuca* (marine alga). This is in order to reveal the capacity of this strain to restore a plant growth irrigated with seawater or brackish water in the future. *Azospirillum brasilense* NH a new isolate from Algerian soil known as an osmotolerant bacterium was tested with natural medium containing seawater (32g/l NaCl) in presence of ethanol algal extracts and absence of other carbon source such as malic acid, for growth and indole acetic acid production. The results obtained showed that bacterium NH increased it growth at 25% of algal extracts and produced high concentration of auxin phytohormone indole acetic acid from tryptophan (64.61µg/ml) in absence of NH4Cl. Siderophores production and inorganic phosphates solubilization were tested then positive results were obtained.These results demonstrated that *A.brasilense* NH get it natural environment in seawater in presence of algal extracts. So, the capability of the strain to keep the Plant Growth Promoting Rhizobacteria characters under seawater using *U.lactuca* to save it growth, let us think about an eventual application as a biofertilizer in agricultural soils irrigated with saline water or menaced with salinity. As we demonstrated already, that this strain stimulated a wheat growth under saline conditions, but, here, we have to look for an application of the bacterium in large scale. Because, now we need to save the crop invaded by seawater and other saline waters.

Keywords: :*Azospirillum brasilense* NH, Seawater -*Ulva lactuca*, IAA-production, Siderophores, phosphatase.

1 Introduction

In our time, aridity and water missing become factors threatening soil. Many countries suffer from the consequences of theses biotic stress. Algeria is one of the countries where the aridity touches more than three quarters of its surface. So, the use of osmotolerant bio-fertilizer bacteria (e.g., *Azospirilla*) and marine algae (e.g., *Ulva*), could provide a solution for problem.

The selection of plant growth promoting rhizobacteria (PGPR) will be a good idea in the application of the biological fertilizer. The effect of *Azospirillum* on plant growth has been intensively studied, as reported by many authors [3,6,11,12].*Azospirillum* Sp. is known by it improvement of crop yield with inoculation of plants roots, this plant responses to *Azospirillum* inoculation is attributed to the indole-3-acetic acid (IAA) production by these bacteria [2,16].

Various authors suggested direct promoting mechanisms in addition to IAA production [17], and osmotic stress response in plants [1,4,12], phosphate solubilization [20] and siderophore production [18].

Aridity and lack water in the soil inhibit the plant growth. Therefore, our study focused on synthesis of medium containing Seawater which could be a sustainable solution to fight against water missing, in one hand, and the combination of the marine alga *Ulva lactuca* with the halophilic and osmotolerant PGPR to challenge the osmotic stress due to the salinity pressure of Seawater, in other hand. So, the osmoprotective effect of the alga

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allows the restoration of IAA (auxin-phytohormone) synthesis; siderophores production and inorganic phosphate solubilization.

The algal extracts contain high amounts of different betaines, amino-acids, proteins, dimethylsulphoniopropionate and nitrogen source [7]. IAA is the most important naturally occurring of plant growth and development [17]. In this paper we tried to show the effect of marine *U. lactuca* on restoration of production of IAA in Seawater.

2 Materials and Methods

Microorganisme: *Azospirillum brasilense* NH isolated from Algeria [13].Vegetal material: the marine alga *Ulva lactuca* isolated from the Gulf of Bejaia in Algeria. Ethanol algal extracts was obtained according to[7]. Semisolid medium contains: 1l of Seawater preliminary autoclaved (110°C for 30 min), and 5ml 0.5 % alcoholic solution of bromothymol blue (BBT), algal extracts (25 %) and agar (1.75 g). Another medium was prepared without algal extracts as control. pH was adjusted with KOH at 6.8. Media were inoculated with 1ml of bacterial solution of *A. brasilense* NH then, incubation conditions were monitored at 32°C for 72 h. Solid medium was prepared with same composition cited above, but without BBT. Agar was added (15g/l). One colony was removed from the semisolid medium containing algal extracts to inoculate the solid medium. A control was used without algal extracts.

2.1 Indole Acetic Acid synthesis

The protocol was inspired from [5], but the medium is modified here by using Seawater instead of distilled water. Bacteria of *A. brasilense* NH were grown in the liquid medium prepared with water Sea, algal extracts and tryptophan (0.5 mg/l), but without NH4Cl under shaking conditions (100 rpm) at 32°C to the late stationary phase. The same medium was prepared but without algal extracts as control and inoculated with *A. brasilense* NH. Culture supernatant was obtained by centrifugation at 10.000 rpm for 15 min. Determination of concentration of IAA produced was performed according to [5] and [13].

2.2 Phosphate solubilization

The NBRIP medium was utilized [14] containing the following composition in g/l of Seawater: Glucose (10 g), $Ca_3\ (PO4)_2$ (5g), $MgCl_2$ x $6H_2o$ (5g), Mg SO_4 x $7H_20$ (0.25g), KCl (0.2g), $(NH_4)2SO_4$ (0.1g), algal extract (25%) and Agar (15g).The pH was adjusted to 6.8 then the medium was autoclaved 120°C/20min.

2.3 Siderophores production

Siderophores production was carried out according to [19], but the ingredients are dissolved in Seawater instead of distilled water and added with algal extract of *U. lactuca* (25%).

3 Results

3.1 Growth on the medium with Seawater

Bacteria *Azospirillum brasilense* NH formed a subsurface growth pellicle 2-5mm deep in the semisolid medium containing algal extracts and the color was changed to blue brilliant, but there was non growth on the medium without algal extracts (control). On solid medium containing algal extract, small dense colonies appeared on the surface and non growth was shown on the solid medium without algal extracts (control). The growth in the both media semisolid and solid, confirm that the algal extract replaces all carbon source, such as malic acid.

3.2 Indole Acetic acid Production

A. brasilense NH produced high concentration of IAA (64.61 µg/ml) (Fig.1B) better than culture on NFB medium at 200 mM/l NaCl (55.78 g/ml) [5]. We should note that NH4Cl and carbon source addition to the liquid medium for bacterial growth is not necessary (Figs.1 A).

3.3 Phosphate solubilization and siderophores production

Both of media showed positive results, NBRIP transparent hallos were appeared (figure 2A). The same result is obtained with siderophores, where yellow hallos around the spots are manifested (Figure 2B).

4 Discussion

A. brasilense NH growth in the medium containing only algal extracts and without malic acid or other carbon sources, lets us a conclude that *U. lactuca* provides the bacterial strain by carbon source in one hand, and by osmoprotectant to challenge the osmotic stress in the Seawater (32 g/l) in other hand. *A. brasilense* NH showed a best growth in presence of algal extracts at 600mM NaCl [5], and many bacteria like *E. coli* used the algal extracts as osmoprotectant [7]. It has been reported that marine macroalgae are rich in organic matter and nutrients.[15] showed that 1kg of dry mass of marine algae contained 7890 mg NH^+ 1110-22000 mg total-N, 1370-13400 mg total-P. Bacteria can use the different

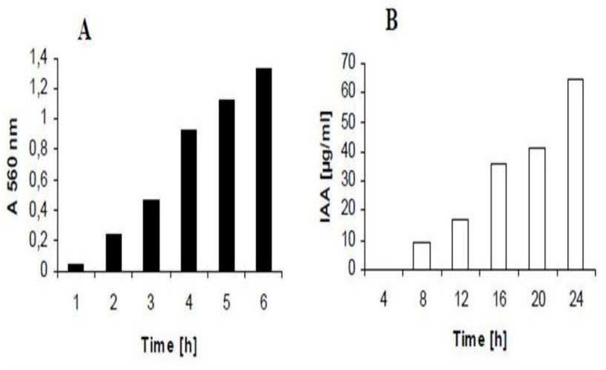

Fig. 1: Growth of *A. brasilense* NH liquid medium containing Seawater and evolution of IAA production.

A: growth (A560nm), B: IAA production (g/ml)

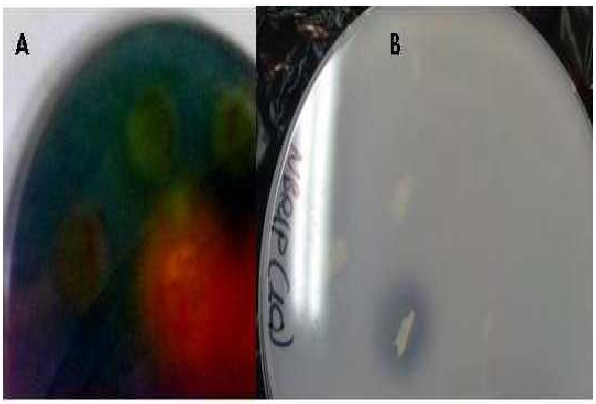

Fig. 2: Phosphatase and Siderophore production by *A. brasilense* NH

A: Siderophores and B: Phosphatase on NBRIP Medium.

nitrogen source and Cl^- presents in the water sea, so, the NH4Cl should be added to the medium is replaced. Seawater is rich with mineral source replacing all mineral necessary for bacteria growing. Inoculation of the medium containing the water sea and algal extracts (10 and 20%) with *A. brasilense* NH does not show any growth (data not shown) it is due to the dominance of several minerals which can inhibit the growth of bacteria. Na^+ and Cl^- are very dominants in this environment, *A. brasilense* NH is known to use *Ulva lactuca* extract as osmoprotectant to challenge osmotic stress [13] and the marine alga has been reported to be rich in various betaines, amino-acids and dimethylsulphoniopropionate [7]. Different betaines are tested like osmoprotectants by *E. coli* [9,7], glycine betaine by *Azospirillum sp*. [8], and glycine betaine by A. brasilense NH [13]. The same case for the dimethylsulphoniopropionate which has an osmoprotective effects on bacteria in Seawater (*Roseabacterdenitrificans*)[10].

Concerning production of high level of IAA in the same medium in presence of algal extracts in the absence of NH4Cl like nitrogen source, and comparatively to NFB medium at 200mM NaCl [12], *A. brasilense* NH used algal extracts like osmoprotectants nitrogen source for the strain like as the growth. It has been reported [17] that IAA production it depends on the environmental conditions (temperature, carbon source and aeration). Algal extracts contain many carbon sources (amino-acids, betaines and DMSP).

5 Conclusion

These results demonstrated that *A.brasilense* NH get it natural environment in seawater in presence of algal extract. In the future it will be probable to use the Seawater to irrigation of wheat, especially in the arid soil messing water, because the strain has been shown that it promoted the wheat growth under saline condition in combination to *Ulva lactuca* in the previous study.

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