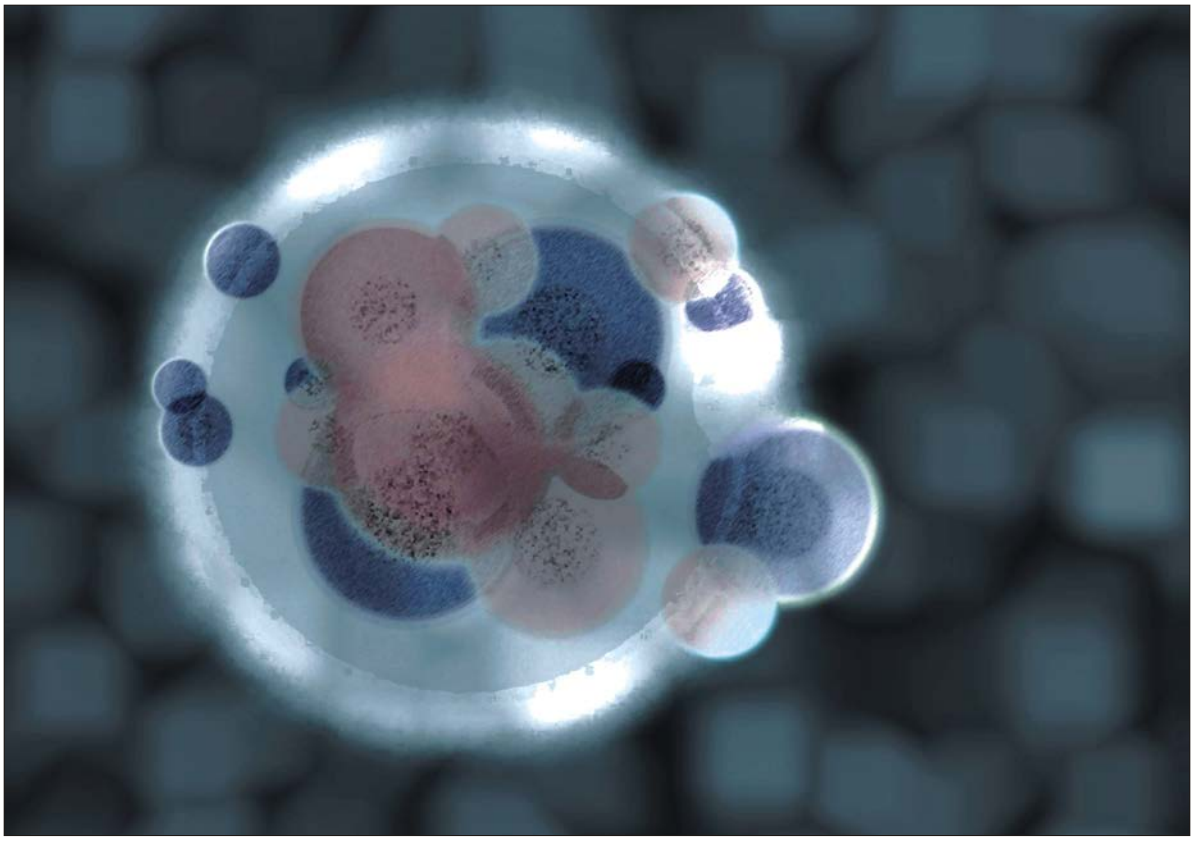

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Nitrogenase Activity of *Pseudomonas corrugata* Isolated from Egyptian Lettuce

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Abstract: In the present study an isolate capable of root colonization of Egyptian Lettuce was isolated from the roots after selective enrichment. The isolated strain was identified as *Pseudomonas corrugata* by using Biochemical and Biolog identification system. The isolate has been found to be positive for nitrogenase activity. This root-colonized bacterium has not been previously isolated from Egyptian Lettuce and could be used as a bio-fertilizer.

Key words: Egyptian lettuce, Kafer Hakiem, nitrogen fixation, *Pseudomonas corrugata,* root-colonization

INTRODUCTION

Microorganisms are important components in all ecosystems; their ubiquity is mainly due to small size and easy dispersal, the ability to grow and multiply[1]. Germinating seeds and growing plants influence the activities of soil microorganisms in the adjoining volumes of soil known as the spermosphere and the rhizosphere, respectively. Conversely, microorganisms in these settings condition the seeds and plants in a number of ways. Some of microorganisms (e.g., the so-called plant-growth promoting rhizobacteria) may enhance plant health and productivity by synthesizing phytohormones; increasing the local availability of nutrients, facilitating the uptake of nutrients by the plants. In addition, they can decrease heavy metal toxicity in the plants, antagonizing pathogens, and including systemic resistance in the plants to pathogens[2,3]. Species of *Pseudomonas* are known to be aggressive root colonizer, possess plant growth-promoting activity and have potential for biocontrol[4-10]. In the present investigation one strain of *Pseudomonas corrugata*, has been isolated and identified from the root of the Egyptian Lettuce.

MATERIALS AND METHODS

Study site: The bacteria were isolate from an Egyptian governate called Kafer Hakiem (about 10 km from Cairo), one of the most famous places cultivating Lettuce in Cairo.

Isolation of bacteria from Lettuce roots: Isolation of root-colonized bacteria was carried out by surface sterilization of roots by using 70% ethanol. A sterile needle was introduced into the swollen area of the roots and a streak was made on agar plates. Also, a *Pseudomonas* isolation agar were used (all from Al-Gomhorea, Cairo, Egypt).

Identification and characterization of the selected Bacteria: One pure type of isolate was obtained and selected for detailed study. The selection was based on various physiological and root-colonizing properties. This bacterium was first identified to the genus level based on growth characteristics, microscopy and biochemical tests identification to the species level was based on Biolog GN Microplate method[11]. The microplates were incubated at 28°C for 24 h before recording results on a computer-based reader. The isolate was finally identified as *Pseudomonas corrugata.*

The isolate was tested for nitrogenase activity by the acetylene reduction test at 28°C according to Somasasegaran and Hoben[12].

RESULTS AND DISCUSSION

Identification of the bacterial isolate: A presence of swelling areas in roots of Egyptian lettuce was observed. The plants containing such swelling areas were healthy in relation to the size of the leaves, the weight of the whole plant and the absence of any symptoms indicating any presence of Pathogen; than the other plants without swelling areas (data not shown). It was interesting to study this phenomenon especially, the root-colonized Egyptian Lettuce has not been recorded before. For that reasons, a samples from the swelling area of the roots were taken under aseptic conditions as previously mentioned at the material and methods. The isolate was streaked and grown on nutrient agar medium plates at 28°C. The plates showed pure bacterial isolate similar in terms of their colony morphology, growth characteristics and for various biochemical properties.

The bacteria developed entire, circular, mucoid, raised colonies of 2-3 mm diameter on *Pseudomonas* isolation agar plates at 28°C after 24 h incubation. The isolate was Gram-negative oval rods. The bacterium was aerobic, made a thin layer on the surface of the medium. The bacterium was positive for gelatin liquefaction, catalase and oxidase and were unable to hydrolyse starch, Tween-80, *O*-nitrophenol β -galactosidase or to ferment lactose. Based on these key characters the isolate was placed in the genus *Pseudomonas*. The species level identification was achieved as a result of 95 tests, based on the ability of bacteria for carbon source utilization and obtained by the Biolog GN Microplate system. The isolate showed similarity with *Pseudomonas corrugata*.

Other properties of Pseudomonas corrugata: The nitrogenase activity was estimated to be 9 nmol of C_2H_2 produced/mL/h by *Pseudomonas corrugate*.

Influence of *Pseudomonas corrugata* on root-colonization and growth promotion due to nitrogenase activity: Associative root colonization has been studied with various nonsymbiotic bacteria, mainly such as *Pseudomonas*, *Azospirillum*, or *Bacillus* (Known as associative root colonizers)[13]. Nitrogen-fixing symbioses involving microorganisms other than rhizobia occur in a variety of nonleguminous plants. The first stable product of N_2 fixation is ammonia and the ammonia assimilated into organic nitrogen compounds. Our finding that the *Pseudomonas corrugata* able to colonize the root of Egyptian lettuce and its nitrogenase activity of great important and agree with our observation. Since, the root-colonized plants were more healthy than the non-root colonized plants. Because nitrogen deficiencies often occur in unfertilized bare soils, the use of *Pseudomonas corrugata* as biofertilizer in the fields of Egyptian lettuce is of great importance. This shall show an increase in yield after inoculation of the fields. Since, lettuce is one of the most crops exported from Egypt to other countries.

Further studies should be done to study the mechanisms of *Pseudomonas corrugata* to colonize the roots of lettuce the its effect on the metabolic activity of lettuce plant.

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