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ORIGINAL ARTICLE

Characterization of the plant growth promoting bacterium, *Enterobacter cloacae* MSR1, isolated from roots of non-nodulating *Medicago sativa*

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Abstract The aim of the present study was to characterize the endophytic bacterial strain designated MSR1 that was isolated from inside the non-nodulating roots of *Medicago sativa* after surface-sterilization. MSR1 was identified as *Enterobacter cloacae* using both 16S rDNA gene sequence analysis and API20E biochemical identification system (Biomerieux, France). Furthermore, this bacterium was characterized using API50CH kit (Biomerieux, France) and tested for antibacterial activities against some food borne pathogens. The results showed that *E. cloacae* consumed certain carbohydrates such as glycerol, D-xylose, D-maltose and esculin melibiose as a sole carbon source and certain amino acids such as arginine, tryptophan ornithine as nitrogen source. Furthermore, MSR1 possessed multiple plant-growth promoting characteristics; phosphate solubility, production of phytohormones acetoin and bioactive compounds. Inoculation of *Pisum sativum* with MSR1 significantly improved the growth parameters (the length and dry weight) of this economically important grain legume compared to the non-treated plants. To our knowledge,

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this is the first report addressing *E. cloacae* which exist in roots of alfalfa growing in Al-Ahsaa region. The results confirmed that *E. cloacae* exhibited traits for plant growth promoting and could be developed as an eco-friendly biofertilizer for *P. sativum* and probably for other important plant species in future.

1. Introduction

Bacterial endophytes are a group of soil bacterial that inhabit plant tissues and play a pivotal role in plant growth enhancement via direct and indirect mechanisms (Hallmann et al., 1997; Narula et al., 2013). Production of phytohormones, solubilization, of inorganic phosphate, nitrogen fixation and sequestration of iron are different direct ways of endophytic bacteria for plant growth-stimulation. Among the indirect ways are protection against pathogenic microorganisms and amelioration of ecological constraints such as drought, salinity and heavy metals (Rodríguez-Díaz et al., 2008; Dudeja et al., 2012; Rashid et al., 2012; Ji et al., 2013).

Certain *Enterobacter* spp. have been reported as plant-growth enhancers since they possess multiple growth-promoting activities (Kampfer et al., 2005; Deepa et al., 2010; Ramesh et al., 2014). Several endophytic bacteria with traits of plant growth-promoting activities have been isolated from different plant species. Examples are *Enterobacter* spp. from maize (McInroy and Kloepper, 1995); *Enterobacter sakazakii* and *Enterobacter agglomerans* from soybean (Kuklinsky-Sobral et al., 2004); *Enterobacter cloacae* from citrus plants, maize (Araújo et al., 2002; Hinton and Bacon, 1995); *Enterobacter asburiae* from sweet potato (Asis and Adachi, 2003). *E. cloacae* have been recently recovered from the soybean rhizosphere and enhanced significantly the growth of soybean-wheat (Ramesh et al., 2014).

Researchers have given a considerable attention to bacterial endophytes of root-nodules of Alfalfa (*Medicago sativa* L.) due to its significant role in increasing nitrogen input to soils (Gallego-Giraldo et al., 2014). These efforts revealed that root-nodules of alfalfa harbour diverse bacterial endophytes including the nodulating microsymbiont, *Sinorhizobium meliloti* (Young, 2003), in addition to non-nodulating strains of *Endobacter medicaginis* (Ramírez-Bahena et al., 2013) *Micromonospora* (Trujillo et al., 2010), and *Brevibacillus choshinensis* and *Microbacterium trichothecenolyticum* (Stajković et al., 2009). These bacterial species seem to share this ecological niche. A comprehensive study was conducted using cultural-independent methods that revealed a high taxonomic variability among bacterial communities associated with nodules, stem and leaves of *M. sativa* (Pini et al., 2012). They have found that members of *Alphaproteobacteria* are dominant in alfalfa tissues. However, little is known about the bacterial endophytes in roots of non-nodulating alfalfa plants growing in Al-Ahsaa region, Saudi Arabia. Therefore, the current work aimed at isolation and characterization using phenotypic and genotypic characteristics of bacterial endophyte isolated from surface-sterilized roots of alfalfa. In addition, inoculation of *Pisum sativum* with the isolated bacterium species was also assessed.

2. Materials and methods

2.1. Isolation of E. cloacae strain MSR1 from roots

2.1.1. Collection of Alfalfa plants

Alfalfa plants were uprooted along with the rhizosphere from farms of Al-Ahsaa city and brought immediately to the laboratory in sterile plastic bags. To remove soil particles, roots were washed under running tap water. Surface sterilization was carried out according to the method described previously (Vincent, 1970). *E. cloacae* were isolated from alfalfa roots by squeezing surface-sterilized roots between two sterilized glass slides and loopfuls of the exudates were streaked onto yeast-extract mannitol agar (YMA) (Vincent, 1970). The plates were then incubated at 30 °C for 48 h. Well isolated colonies were re-streaked on fresh agar plates and maintained in agar slants and stored at 4 °C for further use.

2.1.2. Morphological characteristics

The colonial characteristics (shape and margin) and pigmentation of MSR1 were determined. The shape of cells and Gram reaction were as described by Arora (2003).

2.1.3. Phenotypic characterization MSR1 using API20 and API50CH kits (Biomerieux, France)

Phenotypic characteristics of the strain MSR1 were investigated using the API20 and API50CH strip kits (Biomerieux, France) according to the manufacturer's instructions. After inoculation, the strips were incubated at 30 °C and results were scored after 24 h hours for API20 and 48 for API50CH. Results of API20E were interpreted using the API 20E software Version 4.1 identification database.

2.1.4. Catalase test

MSR1 was tested for its ability produce catalase enzyme by adding drops of drops of hydrogen peroxide (5%) to an aliquot of an overnight MSR1 culture was smeared on a clean glass slide. Results were recorded as positive when gas bubbles were evolved within few seconds (Table 1).

2.1.5. Production of indole acetic acid (IAA)

The ability of the MSR1 strain to produce IAA was investigated using the method outlined previously (Gordon and Weber, 1951). MSR1 was grown in Bertani broth supplemented with 0.0 and 0.2 mg ml^{-1} of tryptophan and incubated in shaking incubator at 30 °C for 3 days. Then, the cells were harvested after centrifugation at 8000 rpm for 15 min then 1 ml of the supernatant was mixed vigorously with 2 ml of Salkowski's reagent (150 ml of concentrated H_2SO_4, 250 ml of distilled H_2O, 7.5 ml of 0.5 M $FeCl_3{\cdot}6H_2O$) (Ehmann, 1977). The tubes