

**Detection of Family Specialty for *Microbacterium* sp. AJ-Z Isolated from Fenugreek (*Trigonella foenum-graecum*) Root Nodules**

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**ABSTRACT**

*Microbacterium* sp. AJ-Z was isolated from fenugreek (*Trigonella foenum-graecum*) root nodules on Yeast Extract Mannitol (YEM) medium. Colonies of the bacteria isolates grown were rod shape, whitish, translucent, sticky nature. The results showed responded of the fenugreek seedlings grown on Nitrogen free medium (NF) to inoculated with *Microbacterium* sp. AJ-Z isolate at 30 min. and successful to form nodules its roots in the rate of 70% after 7 days with rate number of nodes/seedling was 2.9 superiority on the other which grown on Murashige and Skoog (MS) medium and 0.7% agar. Nodules produced have a spherical shape, pinkish and indeterminate in growth and after a few days, its shape becomes elongated.

**Keywords:** Fenugreek, *Microbacterium* sp. AJ-Z, Family Specialty.

## INTRODUCTION

The fenugreek (*Trigonella foenum-graecum* L.) belongs to *Leguminoceae* family (Seasotiya *et al.*, 2014), cultivated in the world for leafy vegetables, condiments and medical (Paul and Pal, 2014). Dry fenugreek seeds have been used in health benefits, such as diuretics, anti-bacterial, anti-oxidant, anti-tumor and diabetes (Moradi-kor *et al.*, 2013). The plants of *Leguminoceae* have important role in increase the fertility of the soil and nitrogen fixation through its symbiosis relationship with rhizobium (Pervin *et al.*, 2017). The fixation of N<sub>2</sub> process by Rhizobium achieved in the root nodules by Nitrogenase that reduce atmospheric nitrogen N<sub>2</sub> to ammonia NH<sub>3</sub> (Singh *et al.*, 2008). The simultaneous infection with rhizobia and rhizosphere bacteria increase nodulation and growth in an extensive variety of legumes (Singh *et al.*, 2013). Use of bacterial inoculums leads to increase the number of root nodules that rise the capability of nitrogen fixation (Bader *et al.*, 2015). The inoculation of Rhizobium increasing highest benefit of the number of leaves per plant, plant dry weight and number of nodules per plant compared with the un-inoculated control (Gendy, 2013).

The genus *Microbacterium* belong to the phylum: Actinobacteria, the class: Actinobacteria, the order: Actinomycetales and the family: *Microbacteriaceae* (Hadjadj *et al.*, 2016), which is positive for the Gram stain. *Microbacterium* is an endophytic bacterium that colonizes the root nodules of leguminous plants, non-symbiotic (Peng *et al.*, 2015). Which have more than 90 species isolated from different sources such as *Microbacterium phyllosphaerae* were isolated from the phyllosphere of grasses (Behrendt *et al.*, 2001). Also from different plants, such as sweet corn, *Zea mays*, cotton (Zakhia *et al.*, 2006), *Ornithopus compressus*. and Fenugreek (Qaddawi, 2021).

They do not symbiotically induce nodules but their coexistence for assisting nodulation ability has been proven by many studies (Martínez-Hidalgo and Hirsch, 2017; Zhang *et al.*, 2018). The aim of this study, detection the family specialty of *Microbacterium* AJ-Z sp. On the Fenugreek (*Trigonella foenum-graecum*).

Present study aimed to characterization the bacteria according to the microbial test and detection of family specialty for *Microbacterium* AJ-Z sp. by re-inoculation with the root of same plant.

## MATERIALS AND METHODS

### The source of *Microbacterium* sp. AJ-Z

The *Microbacterium* AJ-Z sp. provided from plant tissue culture laboratory in department of Biology/ College of Science / University of Mosul/ Iraq, which sub cultured on the solid YEM medium every 20 day (Qaddawi and Mohammed, 2021).

### Preparation of *Microbacterium* sp. AJ-Z cell suspension

Suspension of the *Microbacterium* sp. AJ-Z was prepared by taking a lob full of bacteria and placed in a vial contain 20 ml of liquid YEM medium and incubated at 28 °C for 48 hours in the shaker incubator) New Brunswick Scientific Co., Inc. Edison. N.J. USA) with 100 r / min. rotational speed (Godschalx *et al.*, 2017).

### Characterization of *Microbacterium* sp. AJ-Z

Visual characterization of colony morphology was done for isolate of *Microbacterium* sp. AJ-Z isolate.

### Cultural characters

Observed for *Microbacterium* sp. AJ-Z colony color, shape and size (Aneja, 2003).

### Gram's staining

*Microbacterium* sp. AJ-Z suspension with 48 hrs. old was tested to the Gram's staining using aqueous Crystal violet solution (0.5%) and iodine solution. Later observed under research microscope (make: Olympus) at 40X.

### Surface sterilization of fenugreek (*Trigonella foenum-graecum*) seeds

Seeds of fenugreek (*Trigonella foenum-graecum* L.) plant were surface sterilized by soaked in ethanol 96% for 2 min. flowed in 3% sodium hypochlorite (NaOCl) with stirred for 5 min., then finally rinsed with sterilized distilled water three times/ one minute (Al-Mahdawe, 2013). Sterilized seeds placed on the surface of solidified Murashige and Skoog medium (MS), (Murashige and Skoog, 1962), Nitrogen free medium (NF), (Fahraeus, 1957) and 0.7% agar individually. Samples maintained in culture room at  $24 \pm 2$  °C in the dark. After produced the complete seedlings were transported to the same condition but in 1500 lux with 16 hours light / 8 hours dark.

### Detection the family specialty of the *Microbacterium* sp. AJ-Z isolates

Forty of fenugreek seedlings growing with 3 days on each of MS, NF and 0.7% agar medium were inoculated individually by immersion their root in flask contain *Microbacterium* sp. AJ-Z suspension for 15, 30 and 60 min. alone, then the seedlings were dried using sterile filter papers and transferred to the surface of 20 ml of solid NF medium in a 9.0 cm plastic petri dish at a rate of 2-3 seedlings / plate. The plates were sealed with their covers and coated with parafilm and kept vertically in the growth incubator at  $24 \pm 2$  °C under 16 hours light / 8 hours of darkness conditions and intensity of light 2000 lux, with covering the root system with black tape.

## RESULTS AND DISCUSSION

### Cultural characters of *Microbacterium* sp. AJ-Z

The colonies of the isolate *Microbacterium* sp. AJ-Z which grown on solid YEM medium after two days of incubation showed a sticky form, indicate the production of mucous substances., also observed as whitish, colored till 3-4 days of growth and turning yellowish in color after 4 days. This result similar to (Singh *et al.*, 2008; Tsegaye *et al.*, 2015)

### Production of nodules on the fenugreek seedling roots

The results indicated for ability of *Microbacterium* sp. AJ-Z bacteria isolate to re-infected the roots of fenugreek seedlings when treated with them and the success of the root nodule formation process, with different ratio according to the seedling sources, which growing on MS, NF, 0.7% agar and treatment times (Table 1).

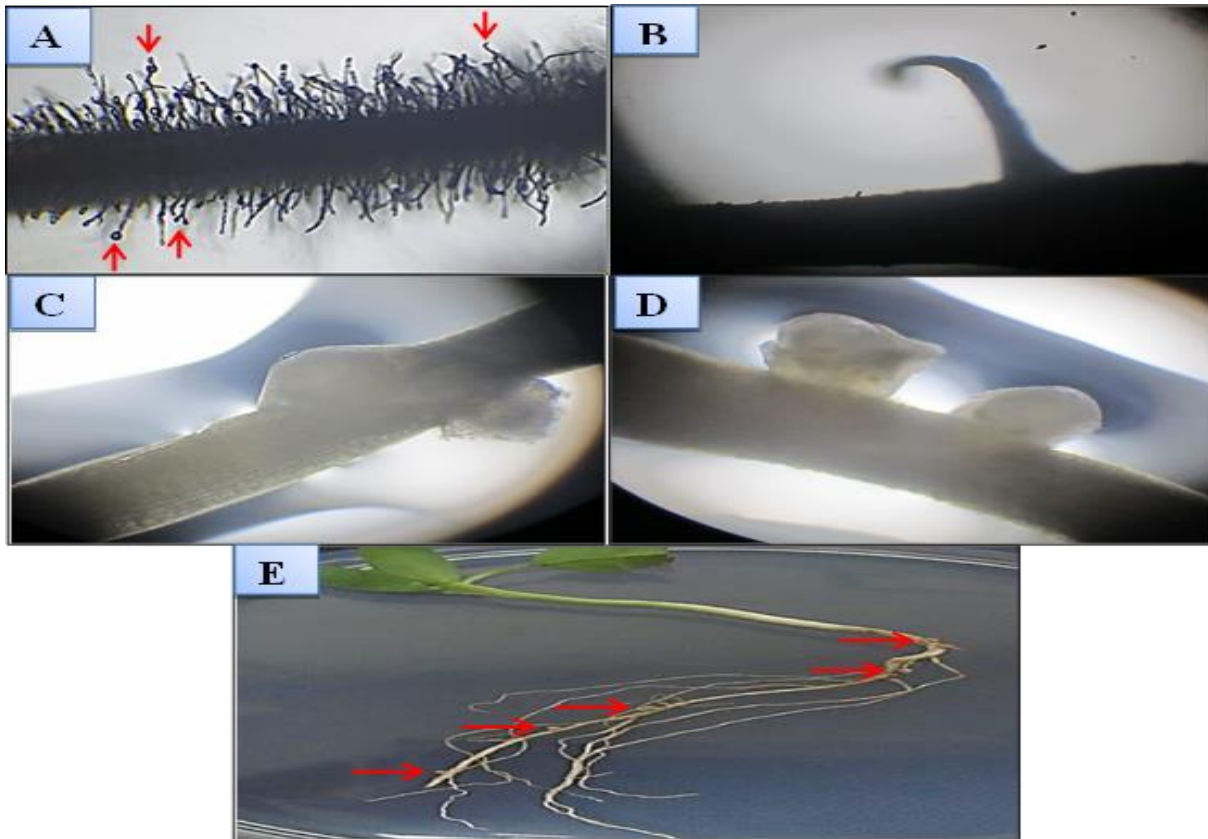
**Table 1: Ability of *Microbacterium* sp. AJ-Z to produce fenugreek root nodules**

Medium	Inoculation time (min.)	Number of seedlings infected	Percentage of infected seedling (%)	Number of nodules	Rate of Number nodes/seedling
MS	15	10	25%	14	1.4
	30	14	35%	26	1.8
	60	8	20%	10	1.25
NF	15	20	50%	54	2.7
	30	28	70%	82	2.9
	60	16	40%	36	2.2
0.7% Agar	15	14	35%	23	1.6
	30	18	45%	36	2
	60	12	30%	26	2.1

### Number of seedlings inoculated: 40

The period of treatment 30 min. was the most prominent for the root nodule formation, which reached to 35%, 70%, and 45% more than other treatment times. The seedlings growing on NF medium showed a superiority in their response to the inoculation process by formed nodules with 70% with 2.9 of nodules/seedling more than other types on seedlings. The hairs of their root's appearance various changes after 3 days of inoculated with bacteria Fig. (A) represented by their transformed from a straight shape to a curved shape (Fig. B). which continued to grow and form a spherical shape represented by a nodule Fig. (C). After two weeks have elongated oval shape, it

developed into a complete root node Fig. (D). Root nodules formed on fenugreek seedlings was thus successful Fig. (E).



**Fig. 1: Production of nodules on the fenugreek seedling roots inoculated with *Microbacterium* sp. AJ-Z**

**A: Root hairs malformed after 4 days of inoculation (arrows)**

**B: Root hair curling (like shepherd's hook)**

**C: Produced the primary root nodule after 7 days**

**D: Mature root nodule formed after 12 days**

**E: Nodules on the main fenugreek seedling root (arrows)**

The formation of root nodules of the fenugreek seedlings when treated with *Microbacterium* sp. AJ-Z bacteria represents the success of the symbiotic relationship between them (Meena *et al.*, 2018). Also, it begins with the occurrence of abnormalities in the root hairs as a result of their secretion of flavonoids that stimulate the specialized genes and encoding those genes for production the nod factor and linking its chitin part in a specialized way with the fenugreek lignin, The incidence of infection and node formation (Andrews and Andrews, 2017). A number of studies indicated the inability of the genus *Microbacterium* to establish a symbiotic relationship with leguminous plants (Wang *et al.*, 2006; Palaniappan *et al.*, 2010; Dudeja *et al.*, 2012), while it is endophytic and only has the ability to fix atmospheric nitrogen because it possesses the *nif* H gene ( Gtari *et al.*, 2012; Yadav *et al.*, 2018). Its ability to form root nodules may explain to what Zakhia (2006) mentioned that the sequences of nitrogenous bases in the *nif* H gene of the bacterial genera *Microbacterium*, *Agromyces*, *Starkeya* and *Phyllobacterium* isolated from the root nodules of leguminous plants are more similar to the sequences of the *nif* H gene of *S. meliloti* as it reached The percentage of congruence according to the NCBI site between the *nif* H gene of *S. meliloti* and the *nif* H gene of three strains of *Microbacterium*, namely, ORS 1417b, ORS 1418b and ORS 1472b to 94%, 93% and 96%, respectively, and the possibility of the evolution of endophytic bacteria into

symbiotic bacteria by It acquires symbiosis genes within root nodules through horizontal gene transfer, which occurs naturally in the soil when appropriate conditions are provided for the conjugation process (Taghavi *et al.*, 2005). The superiority of the growing seedlings on the NF medium in nodule formation ratios may be explained by the fact that it does not contain nitrogen sources and thus stimulates the seedlings to be infected (Saito *et al.*, 2014).

### CONCLUSIONS

The isolated bacteria from root nodules of fenugreek were sticky, whitish color and success in forming nodules on the root of fenugreek after inoculation and superiority MS medium with 30min for inoculation on the rest media which used in this study.

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**التحري عن التخصص العائلي لبكتريا *Microbacterium sp. AJ-Z*  
المعزولة من العقد الجذرية للحلبة**

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**المخلص**

تم عزل بكتريا *Microbacterium sp. AJ-Z* من العقد الجذرية لنبات الحلبة (Fenugreek) على وسط مستخلص الخميرة مانيتول Yeast Extract Mannitol (YEM). كانت مستعمرات العزلات البكتيرية النامية على قضيبيية الشكل، بيضاء، شفافة، لزجة. اظهرت النتائج استجابة بادرات الحلبة النامية على الوسط الخالي من النيتروجين (NF) Nitrogen Free medium للتلقيح بالبكتريا المعزولة مدة 30 دقيقة، ونجحت في تكوين العقد الجذرية على جذور البادرات بنسبة 70% بعد سبعة ايام من التلقيح وكان معدل عدد العقد 2.9 عقدة / بادرة، متفوقة بذلك على البادرات النامية على وسط موراشيغ وسكوك (MS) Murashige and Skoog ووسط الاكار 0.07%. العقد الجذرية شكل دائري، لون وردي وغير محدودة النمو وبعد عدة ايام أصبح شكل العقد متطاوول.

**الكلمات الدالة:** الحلبة، بكتريا *Microbacterium sp. AJ-Z*، التخصص العائلي.