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Indole Acetic Acid Production by *Rhizobium* sp. Isolated from Pea (*Pisum sativum* L. ssp. *arvense*)

Bezelye (*Pisum sativum* L. ssp. *arvense*)'den İzole Edilen *Rhizobium* sp. Tarafından İndol Asetik Asit Üretimi

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Abstract

In this study, isolates of the genus *Rhizobium* were isolated from root nodules of peas (*Pisum sativum* L. ssp. *arvense*) which were taken from pasture of Şanlıurfa, Turkey. Indole acetic acid (IAA) production by *Rhizobium* sp. was determined and IAA production of tested isolates ranged from 20.4 to 165.6 mg/ml. The highest IAA production and specific activity was found in isolate P2. Maximum IAA production for P2 isolate was obtained 96 h of inculbation time. IAA production by P2 isolate was determined in the media containing different carbon and nitrogen sources. Sucrose and glycine as carbon and nitrogen source, respectively gave the best result for P2 isolate.

Keywords: Indole acetic acid, *Rhizobium* isolate, carbon and nitrogen sources

Öz

Bu çalışmada, *Rhizobium* cinsine ait izolatlar Türkiye, Şanlıurfa meralarından toplanan bezelyelerin (*Pisum sativum* L. ssp. *arvense*) kök nodüllerinden izole edilmiştir. *Rhizobium* sp. tarafından indol asetik asit (IAA) üretimi belirlenmiş ve test edilen izolatların IAA üretimi; 20.4-165.6 mg/ml arasında değişmiştir. En yüksek IAA üretimi ve spesifik aktivite P2 izolatında bulunmuştur. P2 izolatı için maksimum IAA üretimi inkübasyonun 96. saatinde incelenmiştir. P2 izolatı tarafından IAA üretimi farklı karbon ve azot kaynaklarının bulunduğu ortamlarda belirlenmiştir. Karbon ve azot kaynakları olarak sırasıyla; sukroz ve glisin, P2 izolatı için en iyi sonucu vermiştir.

Anahtar Kelimeler: İndol asetik asit, *Rhizobium* izolatı, karbon ve azot kaynakları

1. Introduction

In symbiotic nitrogen fixation plants benefit directly from the presence *Rhizobium* bacteria, since approximately 90 % of the fixed nitrogen is translocated from the bacteria to plant. The agricultural systems, in which legumes are used, input of nitrogen increases into the soil (Avcıoğlu et al. 2009). This crops a source of protein for human and animal consumption (Hansen 1994). The use of pea as a forage legume in Turkey required the introduction of the corresponding symbionts. This species has wide adaptation and produces high quality forage under grazing regimes (Hansen 1994). The energy value of feed grains as feed peas is quite high. Açıkgöz (1991) reported that wherein seeds have 26.5% crude protein, 1.7% crude fat, 7.3% crude fiber and 59.8% N. Protein and lysine contents of fodder peas grain are rich. After the harvest, it provides plenty of straw. This straw is valuable when compared to grain as animal feed (Açıkgöz 1991). Protection of soil organic matter and that followed for increasing yield of the main product feed peas can also be used as green manure crops such as legumes other single year. Forage peas are planted alone or in a mixture with cereals, especially in coastal areas are used as pasture in early spring (Zahran 1997; Avcıoğlu et al. 2009).

Since the most Turkey soils are nitrogen deficient N_2 fixing *Rhizobium* bacteria could increase yield at a low coast and preserve water resource from pollution by nitrates (Zahran 1997). The formation of root nodules results from interactions between the bacteria and the plant and requires the growth and differentiation of both partners. Indole acetic acid (IAA) has been reported to play an important role in formation of nodule development (Dullaart 1970; Bhattacharya and Basu 1992; Fukuhara et al. 1994).

Rhizobia are capable of producing physiologically significant levels of IAA during free living growth in the absence of exogenous tryptophan (Ghosh and Basu 2006) and could conceivably contribute to the increased IAA accumulation observed in nodules. Understanding the

biochemical nature of IAA production in *Rhizobium* isolates is a first step in determining whether bacterially produced IAA is involved in nodule development. The purpose of this study was to determine the influence of various carbon and nitrogen sources on the growth and indole acetic acid production in culture by *Rhizobium* sp. isolated from root nodules of pea (*Pisum sativum* L. ssp. *arvense*) grown on pasture from semiarid region of Turkey.

2. Materials and Methods

Rhizobium sp. was isolated from root nodules of field pea (*Pisum sativum* ssp. *arvense)* plants. For the isolation of *Rhizobium* sp.; healthy root nodules from pea were selected, washed 5 times in distilled water and sterilized with 0.1% $HgCl_2$, crushed and growth in sterile yeast extract mannitol agar (Vincent 1970). Inoculated medium was incubated 28 ^oC for 5-6 days. Pure cultures were obtained and cultures were grown at 28 ^oC for 24 h. The culture was maintained on YEMA at 4 ^oC.

The IAA production by the isolates was performed by the method of Gordon and Weber (1951). For this activity, the isolates were grown in Yeast Extract Broth containing different carbon (1%) or nitrogen (0.1%) sources. Bacterial isolates were grown (10^8 cfu/ml) in media. Different carbon sources such as arabinose, mannitol, glucose, fructose and sucrose were added separately to the tryptophan supplemented broth medium omitting mannitol at the beginning and glycine, ammonium sulphate, glutamic acid as nitrogen sources were separately to the tryptophan medium omitting yeast extract at the beginning. Also, the growth of the isolate was measured at 540 nm. The medium was centrifuged; the supernatant was used for IAA, which was determined by UV absorption at 280 nm (Gordon and Weber 1951).

3. Results and Discussion

In this study, we determined the IAA production of *Rhizobium* sp. isolates. Isolates were identified as *Rhizobium* sp. according to Bergey's Manual of Systematic Bacteriology (data not shown). The IAA production and specific activity by *Rhizobium* sp. isolates were shown in Table 1. Productions of IAA were obtained during the growth of isolates in YEM broth. IAA was produced under all conditions. In this study, the production of IAA production by 15 isolates ranged between 15.6-165.6 µg/ml with a specific activity of 21.1-96.2 (Table 1). The highest level of IAA production (165.6 µg/ml) and specific activity (96.2) was observed in P2 isolate.

IAA production of the P2 isolate was detected between 24 h-120 h in YEM broth medium (Fig. 1). It was determined that the IAA production by P2 isolate increased at 96 h. After 96 h of incubation, IAA production started to decrease (Fig. 1). Kumar and Ram (2012) reported that the highest IAA production by *Rhizobium* isolates from *Vigna trilobata* was determined at 72h, according to other isolates Similarly, it was reported by Ghosh and Basu (1996), Datta and Basu (1998), Mandal et al. (2007) and Verma et al. (2015) that incubation period had effect on IAA production and found positive interaction between the growth of *Rhizobium* strains and IAA production.

Table 1. Effects of tryptophan on IAA production and growth of isolates

Strains	Growth (OD at 540 nm)	IAA (µg/ml) Production	Specific activity (IAA Prod./growth)
P2	1.72	165.6	96.2
P3	1.50	66.7	44.5
P4	1.12	30.5	27.2
P6	1.25	32.4	25.9
P11	1.25	47.8	38.2
P12	1.32	87.6	66.3
P13	1.30	30.0	23.0
P14	1.10	80.4	73.1
P18	1.82	90.3	49.6
P21	0.75	15.8	21.1
P22	0.92	75.6	82.1
P25	0.64	20.4	31.9
P34	1.27	75.4	59.3
P35	1.45	92.4	63.7
P42	0.47	15.6	33.1

In our study, the production of IAA in *Rhizobium* sp. P2 strain, which produced the maximum IAA (Table 1), was determined in different carbon and nitrogen sources (Table 2). The production of IAA in P2 isolate was determined in different carbon and nitrogen sources (Table 2). Growth was high, when tryptophan was used. Different carbon sources were effects to IAA production. Glucose, fructose, mannitol and sucrose tested as carbon sources; the isolate P2 produced 178.2, 183.4, 196.2 and 250.7 µg/ml IAA, respectively. The type of nitrogen source has an influence on IAA production. Growth was high, when glutamic acid was used as nitrogen source, followed by glycine and ammonium sulphate, respectively. The production of IAA by P2 isolate was the highest in sucrose (250.7 µg/ml) and glycine (242 µg/ml), respectively. While the specific activity of IAA in strain P2 was lower with glutamic acid in YEM broth, the highest level of specific activity was observed in the media with ammonium sulphate and glucose as nitrogen and carbon sources (respectively, 177.2 and 162).

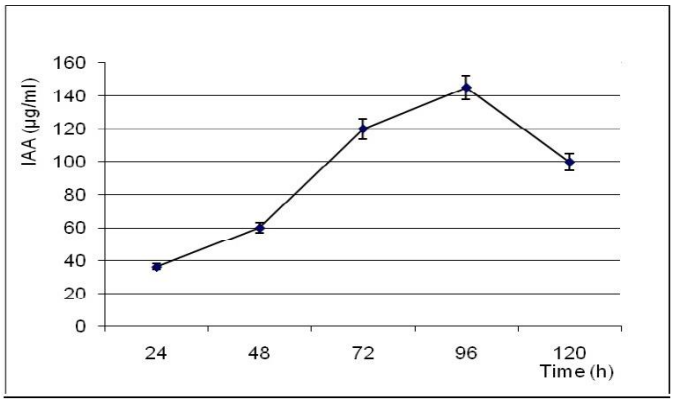

Figure 1. Effect of incubation time on IAA production of *Rhizobium* P2 in culture

Many studies of IAA production by bacterial isolates (Gordon and Weber 1951; Datta and Basu 1998; Leveau and Lindow 2005; Mandal et al. 2012; Mohite 2013) have shown that IAA production is stimulated by the presence of tryptophan. Similarly result was also in this study (Table 2). Also, mannitol used as carbon source in the production IAA has been found to affect (Table 2). This result is similar to that by reported Gosh and Basu (2006), Kumar and Ram (2012) in which *Rhizobium* produced high levels of IAA when grown on mannitol.

Table 2. The production of IAA of *Rhizobium* P2 on media with different carbon (1%) and nitrogen (0.1%) sources

	Sources	Growth (540 nm)	IAA (µg/ml)	% Increase	Specific activity (IAA/growth)
Carbon sources	Control	0.70	96.0	-	137.1
	Glucose	1.10	178.2	85.6	162
	Fructose	1.20	183.4	91.0	152.8
	Mannitol	1.80	196.2	104.2	109
	Sucrose	2.00	250.7	161.1	125.4
	Arabinose	1.75	198.7	106.9	113.5
Nitrogen sources	Glycine	1.75	242.8	138.0	138.7
	Ammonium sulphate	1.30	230.4	125.8	177.2
	Glutamic acid	1.90	200.4	96.4	105.4
	Control	0.95	102.0	-	110

In rhizosphere area, carbohydrates are abundantly available (Dulaart, 1970; Ghosh et al., 2015), suggesting that in rhizosphere environmentals, bacterial isolates would be abundant able to utilize available IAA (Patten and Glick, 1996; Spepen and Vanderleyden, 2011). The growth and IAA production by isolate, type of carbon and nitrogen sources was affected. This result is similar to that by reported Data and Basu (1981), Mandal et al. (2007) in which *Rhizobium* produced high levels of IAA when grown on mannitol, sucrase, maltose, lactose, glutamic acid, KNO_3, asparagine as carbon and nitrogen sources.

IAA produced by this isolate from pea appears benefit the pea and its symbiont, and be of potential commercial value. As a pioneer, IAA productions from bacterial isolates were obtained to different researchers (Bhattachayra and Basu, 1992; Ghosh and Basu, 2006). Further experiments will be carried out to identify produced IAA in *Rhizobium*–pea symbiosis.

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