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Relative Efficiency of Rhizobacteria for Auxin Biosynthesis

Azeem Khalid, Muhammad Arshad, Zahir Ahmad Zahir and Muhammad Khalid
Department of Soil Science, University of Agriculture, Faisalabad, Pakistan

Abstract: Thirty-one cultures of bacteria (W_1, W_2, ... W_{31}) were isolated from the rhizosphere of different varieties of wheat and twenty-eight (R_1, R_2 .... R_{28}) from the rhizosphere of rice growing at different sites. They were tested in the liquid medium and in soil to evaluate their relative efficacy for auxin biosynthesis by colorimetry. Among different rhizobacterial isolates, W_9 showed significantly higher auxin production in liquid culture both in the presence and absence of its precursor, L-tryptophan (L-TRP). Four isolates showing maximum auxin production from each crop were selected. They were further tested for their ability to produce L-TRP (6.0 g kg^{-1} soil)-derived auxins both in non-sterilized and sterilized soils at 7.0 pH, 35°C temperature and 48 hours incubation period under static and shaking (150 rev. min^{-1}) conditions. L-Tryptophan-derived auxin biosynthesis was also measured by supplementing with glucose (6.0 g kg^{-1} soil) under similar conditions. Results revealed that isolates W_{11} and W_9 showed maximum auxin production at 6.0 g L-TRP kg^{-1} soil which was 66.3 and 27.5 $\mu g\ g^{-1}$ soil in non-sterilized and sterilized soils, respectively, under static conditions. However, auxin synthesis at shaking condition was doubled to that produced under static environment. Rice isolates R_{20} and R_{27} were most active in producing auxins when soils were supplemented with glucose along with L-TRP. Glucose application had also positive effect on L-TRP-derived auxins over L-TRP alone.

Key words: Auxins, rhizobacteria, environmental factors

Introduction

Auxins are a class of hormones that largely function in growth regulation in plants. The principal naturally occurring auxin is indole acetic acid (IAA). Like other phytohormones, auxins are synthesized endogenously by plants and their hormonal effects have been elucidated largely by their exogenous application. Numerous soil microorganisms are actively involved in the synthesis of auxins in pure culture and in soil (Arshad and Frankenberger, 1993; Sarwar and Kremer, 1995). Generally, microorganisms isolated from the rhizosphere and rhizoplane of various crops have more potential of producing auxins than those from the root free soil (Arshad and Frankenberger, 1998; Patten and Glick, 1996). Barea *et al*. (1976) found that among bacterial isolates obtained from the rhizosphere of various plants, 86 % produced auxins-like substances. Sarwar and Kremer (1995) screened 16 different bacterial isolates belonging to the genera *Enterobacter, Xanthomonas, Pseudomonas, Alcaligenes* and *Agrobacterium* originating from the rhizosphere of various plants. They found that all of the rhizosphere isolates were efficiently produce auxins than the non rhizosphere soil isolates. Likewise, Rossi *et al*. (1984) found that auxin-like compounds were greater in the rhizosphere soil of maize compared with non-rhizosphere soil, especially during seedling emergence.

Soils may differ in their auxins synthesizing capacity depending upon the environment (Chandramohan and Mahadevan, 1968). Auxin production in the rhizosphere soil is most likely due to abundance of substrates and microorganisms. Auxin biosynthesis is greatly affected by various environmental factors (e.g., substrate concentration, carbon source, temperature, aeration, pH, etc.) as evidenced by the reports of several researchers (Purushothaman *et al*., 1974; Sarwar *et al*., 1992). Auxin production in root zone is also controlled by the genetic and physiological properties of the microorganism(s) and the plant(s).

Tryptophan, an essential amino acid, also serves as a physiological precursor for biosynthesis of auxins in plants and in microbes. Root exudates are the only natural source of TRP for rhizosphere microflora as Kravchenko *et al*. (1991) and Martens and Frankenberger (1994) found detectable amounts of TRP in root exudates of some but not in all varieties of wheat. This indicates that not all the plants can release TRP in adequate quantities into the rhizosphere for microbial production of auxins. Therefore, a low availability of TRP could be a limiting factor for auxin production in the rhizosphere. Many researchers have reported an increased biosynthesis of auxins in rhizosphere soil amended with TRP reflected in plant growth and development (Purushothaman *et al*., 1974; Frankenberger and Poth, 1987).

In this experiment, bacteria were isolated from wheat and rice rhizosphere soil to test their potential for auxin biosynthesis in liquid culture and also to assess the biotic and abiotic soil auxin production at optimal environmental factors.

Materials and Methods

Isolation of rhizobacteria: Dilution plate technique was used for isolating rhizosphere bacteria from different varieties of wheat and rice crops at different locations by using Glucose Peptone Agar and Combined Carbon media, respectively. Thirty one bacterial colonies showing prolific growth were selected from wheat and twenty eight from rice and numbered as W_1, W_2, ...W_{31} and R_1, R_2, ...R_{28}, respectively. The cultures were purified by further streaking on fresh plates, maintained on agar, refrigerated at 4°C and shifted to new slants after every 25 days.

Auxin Biosynthesis: Auxin production was determined by colorimetry both in the presence and absence of L-TRP @ 10^3 $\mu g\ mL^{-1}$ liquid medium. For this purpose, 25 mL of glucose peptone medium and combined carbon medium was taken in 100 mL Erlenmeyer flask, autoclaved and cooled. L-Tryptophan was filter sterilized and added at desired concentration in the cooled liquid medium. The flask contents were inoculated with the respective wheat and rice bacterial isolates. The flasks were covered and incubated at 28 ± 2°C for 48 h. Non-inoculated/untreated control was kept for comparison. After incubation, the contents were filtered through Whatman filter paper No. 2. Auxin compounds (IAA-equivalents) were determined by spectrophotometer at 535 nm using Salkowiski reagent as colouring agent as described by Sarwar *et al*. (1992).

After determining the relative efficiency of rhizobacterial isolates of wheat and rice for auxin production in the liquid culture, four active isolates each from wheat (W_9, W_{11}, W_{14},

W_{29}) and rice (R_5, R_6, R_{20}, R_{27}) were selected to see their potential for auxin production in sterilized and unsterilized soil at optimal environmental factors. For this purpose, 3.0 g of rhizosphere soil was placed in 100 mL Erlenmeyer flasks and treated with 6 mL of phosphate buffer solution (0.2*M*, pH 7.0) and L-TRP (6.0 g kg^{-1} soil). The soil was inoculated with selected rhizobacteria. There was also an uninoculated control. The flasks were covered and incubated at 35°C both under static and shaking conditions (150 rev. min^{-1}). After 48 hours incubation, the flask contents were treated with 2.0 mL of trichloroacetic acid (5 g 100 mL^{-1} H_2O) to terminate the reaction and 1.0 mL of $CaCl_2$ (0.5M) to facilitate filtration. The soil solution was then filtered through Whatman filter paper No. 2. Similarly, L-TRP-derived auxin production by these isolates of rhizobacteria was also determined at glucose concentration of 6.0 g kg^{-1} soil at shaking of 150 rev. min^{-1}. Investigations were also under taken to determine auxin biosynthesis derived from L-TRP added to the sterilized soil inoculated with selected rice/wheat rhizobacteria. The soil was sterilized by autoclaving three times at 121°C for 30 min while L-TRP and glucose solutions were filter-sterilized (0.2 μm pore membrane filters, type GS) under aseptic conditions.

The data were analyzed statistically using completely randomized design (Steel and Torrid, 1980) and means were compared by Duncan's Multiple Range Test (Duncan, 1955)

Results

Auxin biosynthesis in liquid culture: Results of both wheat and rice isolates indicate that different cultures of bacteria varied greatly in their efficiency for producing auxins in the liquid growth medium both in the presence and absence of precursor, L-tryptophan. Among 31 isolates of wheat rhizobactaria, 74% (23 isolates) produced auxin (ranging from 0.6 to 12.1 μg mL^{-1} IAA-equivalents) in the absence of L-TRP (Table 1). Bacterial isolate W_9 produced significantly higher amounts of IAA-equivalents (12.1 μg mL^{-1}) compared to all other isolates. Eight bacterial isolates (W_4, W_{15}, W_{16}, W_{17},W_{18}, W_{27} ,W_{28} and W_{30}) did not produce auxins in the absence of L-TRP.

Bacterial efficiency for auxin synthesis was enhanced several folds when the medium was supplemented with L-TRP (Table 1). The isolate W_9 was most efficient in deriving-auxin from L-TRP. This bacterial isolate produced significantly higher IAA-equivalents (24.8 μg mL^{-1} of liquid medium) compared to other isolates. It was followed in descending order as W_{14} (21.3 μg IAA-equivalents mL^{-1}), W_{29} (19.7 μg mL^{-1}) and W_{11} (16.8 μg mL^{-1}). All other isolates were also able to derive auxin from L-TRP, however, they were relatively less efficient in auxin biosynthesis and their IAA production ranged from 1.8 to 16.2 μg mL^{-1} growth medium.

Among all tested cultures of bacteria isolated from the rhizosphere of rice (Table 2), 93% (26 out of 28 isolates) were able to produce IAA in defined liquid medium in the absence of L-TRP as analyzed by colorimetry. Maximum auxin biosynthesis (7.9 μg mL^{-1} IAA-equivalents) was found with the bacterial isolate R_5 which differed significantly with all other isolates tested. The isolates R_{10} and R_{14} were unable to produce IAA without L-TRP. Auxin biosynthesis by all other isolates varied greatly and ranged from 0.8 to 7.2 μg mL^{-1} growth medium.

Interestingly, all tested isolates showed auxin-producing ability in the liquid medium supplemented with L-Tryptophan (Table 2). Production of IAA-equivalents by these isolates (R_1, R_2...R_{28}) was as high as 8.3 to 22.9 μg mL^{-1}. The most prolific auxin producer isolate was R_{20}, which differed significantly with all other isolates but was at par with the isolate R_{27}. The isolate R_2 was less efficient among all the isolates. Rice isolates also showed several folds increases in auxin production when the liquid medium was supplemented with L-TRP.

Auxin biosynthesis in non-sterilized soil: The tryptophan-dependent auxin production in soil by various isolates of bacteria selected on the basis of auxin biosynthesis in liquid culture. The data on auxin production is given below.

L-Tryptophan: Inoculation of soil with rhizobacterial isolates of wheat and rice significantly increased auxin biosynthesis as compared to uninoculated soil containing only indigenous microflora at tryptophan concentrations of 6.0 g kg^{-1} soil in static environment (Fig. 1). The isolate W_{11} produced maximum increase in auxin biosynthesis (66.3 μg g^{-1} soil) which was 85.7% higher than control and differed significantly with rest of the treatments. Inoculation of other rhizobacterial isolates also produced IAA-equivalents in higher magnitude ranging from 38.8 to 63.3 μg g^{1} soil and had significant difference with control having value of 35.7 μg IAA-equivalents g^{-1} soil.

Inoculation of soil under shaking at 150 rev. min^{-1} resulted in 2-fold greater L-TRP-derived auxin synthesis as compared to static incubation. Most active auxin producer was W_{11} (produced 79.3 μg IAA-equivalents g^{1} soil) which caused an increase of 37.2% over uninoculated control soil (Fig. 2). Rest of the treatments also had significant effect on auxin synthesis (ranging from 3.5 to 26% greater IAA production than control).

Glucose: Inoculation of soil with selected isolates of rhizobacteria caused significant increases in auxin biosynthesis at glucose concentration of 6.0 g kg^{-1} soil (Fig. 5). Maximum production (85.1 μg IAA-equivalents g^{-1} soil) was resulted in soil inoculated with bacterial isolate R_{20} which was 19.2% higher than control under optimal assay conditions. It had significant difference from control and all other treatments except isolate R_{27}. Bacterial isolate R_6 had non significant effect on auxin production over control.

Auxin biosynthesis in sterilized soil

L-Tryptophan: Investigation in sterilized soil also showed significant positive effect on auxin biosynthesis in response to inoculation with rhizobacteria (Figs. 3 and 4). The isolate W_9 was most efficient in auxin production (27.5 μg g^{-1} soil) compared with all other isolates and control, at tryptophan concentration of 6.0 g kg^{-1} soil. The magnitude of auxin biosynthesis in other isolates ranged from 12.4 to 17.6 μg g^{1} soil and differed significantly with control. Isolates W_1, W_{29}, R_5 and R_{20} were statistically similar with each other but they were significantly different from control.

Under abiotic conditions, shaking of 150 rev. min^{-1} resulted in an increase in auxin production compared with static conditions. Maximum auxin (43.4 μg g^{-1} soil) was produced by wheat isolate W_9 and it was statistically different from control and rest of the treatments. It was followed in descending order by R_{20}, R_{27}, W_{14}, R_5, W_{29}, W_{11} and R_6 producing (R5), 27.1, 24.5, 24.4, 20.2, 18.8, 15.9 and 14.9 μg g^{1} soil amounts of auxin, respectively.

Glucose: Glucose concentrations @ 6.0 g kg^{-1} soil had additive effect on the efficiency of most of the rhizobacteria for auxin biosynthesis under sterilized conditions (Fig. 6). Most active auxin producer was the culture R_{27} isolated from rice and produced 58.0μg IAA-equivalents g^{-1} soil. This isolate was

Table 1: *In-vitro* auxin production by various isolates of wheat rhizobacteria (Average of three repeats)

Rhizobacteria	IAA-equivalents (μg ml^{-1})	
	Without L-TRP	With L-TRP
W_1	6.2cd	9.8m
W_2	1.8ij	2.7t
W_3	1.1jk	13.4i
W_4	0.0l	5.9pq
W_5	4.1g	15.1g
W_6	1.8ij	7.8o
W_7	2.0hij	12.2j
W_8	1.2jk	4.9r
W_9	12.1a	24.8a
W_{10}	3.0h	13.0i
W_{11}	5.0efg	16.8de
W_{12}	5.0efg	14.3h
W_{13}	2.8hi	10.6l
W_{14}	10.7b	21.3b
W_{15}	0.0l	1.8u
W_{16}	0.0l	3.7s
W_{17}	0.0l	5.5qr
W_{18}	0.0l	4.9r
W_{19}	6.2cd	16.4def
W_{20}	6.1cde	15.8fg
W_{21}	6.2cd	16.3de
W_{22}	5.1defg	13.8hi
W_{23}	6.3c	16.0ef
W_{24}	4.3fg	11.2kl
W_{25}	5.2cdefg	15.6fg
W_{26}	5.0efg	11.6jk
W_{27}	0.0l	6.5p
W_{28}	0.0l	8.4no
W_{29}	5.4cdef	19.7c
W_{30}	0.0l	2.9t
W_{31}	0.6kl	9.1mn

Table 2: *In-vitro* auxin production by various isolates of rice rhizobacteria (Average of three repeats)

Rhizobacteria	IAA-equivalents (μg ml^{-1})	
	Without L-TRP	With L-TRP
R_1	4.6e	19.4cdef
R_2	1.2ijk	11.4k
R_3	2.5g	19.6cdef
R_4	4.3ef	17.3gh
R_5	7.9a	20.3bcd
R_6	6.1c	21.4b
R_7	1.8hi	18.2fg
R_8	2.3gh	19.7cde
R_9	5.7cd	18.3efg
R_{10}	0.0l	12.4jk
R_{11}	1.1jk	20.6bc
R_{12}	5.6cd	19.2cdef
R_{13}	3.7f	16.6h
R_{14}	0.0l	8.3l
R_{15}	0.9k	13.5ij
R_{16}	6.2c	19.3cdef
R_{17}	5.4d	19.8cde
R_{18}	2.9g	20.4bcd
R_{19}	4.6e	16.1h
R_{20}	5.9cd	22.9a
R_{21}	1.6ij	19.1def
R_{22}	0.8k	16.1h
R_{23}	6.2c	20.0cd
R_{24}	1.7hi	17.4gh
R_{25}	3.8f	18.3efg
R_{26}	4.2ef	17.4gh
R_{27}	7.2b	22.7a
R_{28}	1.3ijk	13.8i

statistically similar with the wheat isolate W_9 which synthesized auxin up to 57.5 μg g^{-1} soil but had significant difference with rest of the treatments. Auxin production in other treatments ranged from 8.7 to 40.7 μg g^{-1} soil and differed significantly with each other. Slight amounts of auxin (1.4 μg g^{-1} soil) were also observed in uninoculated sterilized soil.

Discussion

Results indicated that different rhizobacterial isolates varied greatly in their auxin producing ability both in liquid culture and in soil and a higher percentage of rhizosphere bacteria were capable of producing auxins. Rhizobacterial cultures W_9 and R_{20} isolated from wheat and rice rhizosphere soil, respectively, showed maximum concentrations of IAA-equivalents in the liquid medium. Results are also similar to the findings of Barea *et al.* (1976) who reported the production of auxin like compounds by a good number of bacteria (86 %) among the total isolated from the rhizosphere soil of various plants. Similarly, Sarwar and Kremer (1995) found that bacteria belonging to different genera isolated from rhizosphere of various plants were more active in auxin production than the bacteria of bulk soil due to the abundance of substrate as released by the plant roots providing favourable environment for biotransformations including IAA synthesis.

The IAA production increased several folds by the addition of L-TRP in the inoculated medium. L-Tryptophan is an efficient precursor of auxin in higher plants as well as for microbial biosynthesis of auxins. Therefore, its low availability could be limiting factor as all the plants can not release TRP in sufficient quantities in the rhizosphere (Martens and Frankenberger, 1994). Sarwar *et al.* (1992) while evaluating the potential of California soils to produce IAA observed 61 fold increase in biosynthesis of IAA by L-TRP application (5.3 g kg^{-1} soil) as compared to control. Asghar *et al.* (2000) isolated 100 cultures of rhizosphere bacteria from different cultivars of rapeseed and, they found a higher production of IAA-equivalents in the presence of L-TRP as compared to that without L-TRP. Results are also in confirmation with the findings of many other researchers (Javed and Arshad, 1999). Auxin production in soil was also significantly affected by various isolates of wheat and rice rhizosphere bacteria and it was found that inoculation had additional benefit over indigenous microflora present in the soil. This indicated that inoculated microorganisms responded even in the presence of indigenous populations. Results were in accordance with the findings of Fallik *et al.* (1989) who reported that inoculated roots of maize had higher amounts of both free and bound IAA as compared to control. GC-MS analysis revealed the presence of both IAA and IBA (indole butyric acid) in the two week old inoculated seedling roots.

Significantly higher amounts of auxins were detected in sterilized soil when inoculated with rhizobacteria as compared to the uninoculated control. Indole compounds and other biologically active substances have been reported in sterilized soil inoculated with *A. chroococcum* (Elwan and El-Naggar, 1972). Several studies demonstrated the auxin production in soil to be a biotic process and such compounds could not be detected in sterilized soils amended with sterilized solution of TRP (Sarwar *et al.*, 1992).

Glucose application (6.0 g kg^{-1} soil) increased the TRP-derived production of IAA. This increase was due to the fact that glucose serves as an energy source for microorganisms and auxin biosynthesis in soil could be enhanced by altering the environmental conditions and the most favourable site for such

transformations was rhizosphere, densely rich in organic C (root exudates) and populated with microorganisms (Sarwar *et al.*, 1992). Furthermore, glucose metabolism by micro-organism yields NAD, NADP or FAD those increase the biosynthesis of IAA from Indole-3-acetaldoxime, formed from L-TRP by plasma membrane bound enzyme oxidation (Ludwig–Muller and Hilgenberg, 1988).

Shaking of inoculated soil promoted oxygen availability that si required for some enzymatic transformation of L-TRP to IAA, so auxin biosynthesis increased under shaking conditions. For instance, the intermediates, indole-3-acetamide, indole-3-acetaldehyde derived from L-TRP required oxidation for their conversion to IAA (Reinecke and Bandurski, 1987). Enhanced microbial production of auxins derived from L-TRP by shaking a synthetic medium was also reported by Bailey and Gentile (1962).

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