EFFECT OF ANTIBIOTICS ON INDOLE-3-ACETIC ACID PRODUCTION

BY KLEBSIELLA PNEUMONIAE, BACILLUS SUBTILIS AND

BACILLUS AMYLOLIQUEFACIENS

VIJENDRA KUMAR MISHRA¹&ASHOK KUMAR²

¹Department of Biotechnology, Faculty of Science, Lalit Narayan Mithila University, Kameshwarnagar, Darbhanga, India

²Microbial Biotechnological Unit, School of Biotechnology, Faculty of Science, Banaras Hindu University, Varanasi, India

ABSTRACT

The present study is aimed to investigate the effect of six antibiotics, namely spectinomycin(10µg/ml), chloramphenicol (20µg/ml), tetracycline(20µg/ml), kanamycin(20µg/ml),ampicillin (100µg/ml) and gentamycin (20µg/ml) on Indole-3-acetic acid (IAA) production by *Klebsiella pneumonia*,*Bacillus amyloliquefaciens* and *Bacillus subtilis* under in vitro condition.Quantitative estimation was performed after 96h at 37°C in L.B. medium exogenously supplemented with L-tryptophan (200µg/ml). Diverse levels of IAA were observed in the culture supernatant. The IAA level was significantly lower in the presence of antibiotics and L-tryptophan added medium as compared to only L-tryptophan added medium, by all the six strains. Among the sixstrains *Klebsiella pneumonia* strain name MR-M1, followed by *Bacillus amyloliquefaciens*, MR-AI and four *Bacillus* sp. name MR-Z1, RR-R2, MR-SP and WR-W2, were the most efficient strain in terms of its capability to produce IAA in presence of antibiotics. Taken together, the result shows that all these strains significantly reduce the levels of IAA production in presence of antibiotics.

KEYWORDS: IAA, *Bacillus*, Antibiotics & PGPR

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INTRODUCTION

Colonization and physiologically activeness of the microbial population in rhizospheric soil is considerably higher than that of nonrhizospheric soil. The rhizosphere is a site with complex interactions between the root and associated microorganisms and high microbial diversity (Khan, 2005). Since the nutritional substance released by the root and its surfaces are useful for microbial growth. Moreover, plant roots influence soil -borne microbial communities via several mechanisms, including excretion of organic compounds, competition for nutrients, and providing a solid surface for attachment. Plant growth promoting bacteria (PGPR) are rhizospheric bacteria that can exert Phytostimulatory potential via different mechanisms (Chabot *et al.*, 1996; Rodriguez and Fraga, 1999). One of the direct mechanism of plant growth promotion is through the release of phytohormones, like indole acetic acid (IAA). Which regulate various metabolic processes during plant growth and development. Indirect mechanisms of plant growth promotion includes antibiotic production (Glick *et al.*, 1999). *Bacillus amyloliquefaciens* has been recognized as a good plant growth promoter and root colonizer (Fan *et al.*, 2011). Antimicrobial activity of *Bacillus amyloliquefaciens* has been well documented (Meng *et al.*, 2012). *Bacillus amyloliquefaciens* is a closely related species to *B. subtilis* (Arguelles-Arias *et al.*, 2009). In addition, the genetic capacity for the synthesis of secondary metabolites in *B. amyloliquefaciens* (340 kb) is larger than that in *B. subtilis* (180 kb) (Chen *et al.*, 2009). The number of *Bacillus* sp. are reduced immediately following stress conditions, but they quickly recover and through their aggressive growth and/or

production of antibiotics are believed to be major contributors to disease suppression also (Stapleton and Devay, 1984). In addition, the formation of endospores makes the *Bacillus* genus highly resistant to adverse environmental conditions (Abrioulet *et al.*, 2011). *B. subtilis* is able to synthesize more than 60 different types of antibiotics, mainly polypeptides and belonging to an iturin family, possessing antimicrobial effects (Phae and Shoda, 1991). There are several antimicrobial compounds released by *Bacillus* sp. Such as iturins, fengycins and surfactants. On the other hand phytostimulatory potential of *Klebsiella pneumonia* has also been well documented (Mishra and Kumar, 2015). The present study is aimed to evaluate the effect of six antibiotics on IAA production under *in vitro* conditions by the six strains.

MATERIALS AND METHODS

Isolates, Culture, Media and Incubation Condition

To isolate most efficient IAA producing PGPR, soil samples were collected from rice, wheat and maize agricultural crop field and the procedure was followed as described in Mishra and Kumar (2015a).

Biochemical Characterization of Rhizobacteria

Selected isolates of *Bacillus* spp. and *Klebsiella pneumoniae* were biochemically characterized by Gram's reaction, urease test, catalase test, MR-VP test as per the standard methods (Cappuccino and Sherman, 1992).

Quantitative Estimation of IAA Production

Calorimetric estimation of IAA production was carried out following the method developed by Gordon and Weber (1952) with slight modifications. All the test isolates were grown in L.B. medium (Sambrook and Russell, 2001). 1×10^6 concentrations of inoculums were transferred into 10ml of the freshly prepared medium, incubated in 50 ml Borosil glass tubes with three replicates at 37°C on a rotary shaker for 92-96h. The antibiotics and its concentrations used were Spectinomycin (10µg/ml), Chloramphenicol (20µg/ml), Tetracycline (20µg/ml), Kanamycin (20µg/ml), ampicillin (100µg/ml) and Gentamycin (20µg/ml). Isolates were grown in L.B medium containing tryptophan (200µg/ml).

Identification of the Isolates

Identification of the isolates was performed through the 16S rRNA gene sequencing and using NCBI tool as described in our previous publication Mishra and Kumar (2015a).

RESULTS AND DISCUSSIONS

Over the last decade, a significant research interest has been generated around *Klebsiella pneumonia* and *Bacillus* sp. PGPR. However, hardly any study has been reported to date regarding the effect of antibiotics on the IAA production. In the present study diverse levels of IAA was observed by the six strains as represented in figure: 1. shows that the antibiotic spectinomycin followed by gentamycin proved to be least effective for the IAA production activity. The sensitivity test result of antibiotics showed resistance to all antibiotics in all the six strains. Interestingly, the sensitivity patterns of antibiotics among *Bacillus* sp. and *Klebsiella pneumonia* were very similar. Soils can be regarded as one of the richest natural sources of antibiotics. It is evident from the result that antibiotics doesn't completely blocks IAA biosynthesis rather than inhibited as compared to control containing tryptophan only. The inhibition of IAA level may be due to the interference of antibiotics in the IAA biosynthetic pathway or direct inhibition of protein synthesis. Antibiotics evidently inhibit tryptophan induced auxin production by all the strains. As the concentration of antibiotics was increased the greater inhibiting effectiveness on growth as well as IAA was observed. Chloramphenicol and other antibiotics would

be a less effective growth inhibitor. The highest amount of IAA production was observed in the supernatant of MR-M1 strain (25.36 µg/ml) followed by MR-Z1 (17 µg/ml) in the presence of tryptophan and without antibiotic test culture tube. However, diverse levels of IAA production were observed in the presence of various antibiotics. The effect of antibiotics in IAA production by *Klebsiella pneumoniae* and *Bacillus sp.* has not yet been well documented. As the concentration of antibiotics increased the IAA level was observed decreasing accordingly by the six strains. The level of IAA was too low to be represented here. That might be due to bacterial growth inhibition. Altogether, a significant amount of IAA production was observed in the presence of six antibiotics by the six strains. Several reports revealed the detrimental effect on the PGP activity by use of herbicides. The importance of our study of PGPR is not only an area to find the beneficial microbes, but also to search better beneficial vigor cum multiple resistances PGPR. Complete inhibition of IAA production in the presence of ampicillin (100 µg/ml) may be due to inhibition of indole-3-pyruvate decarboxylase encoding protein synthesis by β-lactamase activity. However, bacterial growth might be due to availability of the ampicillin^R gene in the plasmid. On the other hand tryptophan-¹⁴C radioactive labeled experiment suggests chloramphenicol doesn't affect IAA production. Rather the amount of IAA increase, which might be due to chloramphenicol acts as a carbon compound (Kefeli 1978). The concentration of other antibiotic was below LD50.

Figure:1. Quantitative estimation of IAA production by the six bacteria.

CONCLUSIONS

In conclusion, our work reveals that antibiotics could significantly inhibit the IAA production in rhizobacterial strains. To our knowledge this is the first report showing IAA production in the presence of antibiotics by the six strains *Klebsiella pneumoniae* MR-M1, *Bacillus amyloliquefaciens* strain WR-W2 and four different *Bacillus spp* strains MR-AI, MR-SP, RR-2 and MR-Z1 under *in vitro* conditions.

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