# STUDY OF INDOLE-3-ACETIC ACID BIOSYNTHESIS PATHWAYS IN *Bradyrhizobium japonicum* BJBV-05

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*SUMMARY*

*The bacterium* Bradyrhizobium japonicum *induces nodulation in* Glycine max *(soy) plants and other legumes. It is considered of great importance, since it is in the nodules that the bacteria are established, contributing to the biological fixation of $N_2$. The process is controlled by nitrogenase, an enzyme produced by the* nif *genes present in the genome of the bacterium. By metabolizing the nitrogenase, the indole acetonitrile transforms it into indole acetic acid (IAA) and releases a nitrogenated molecule. There have been other IAA synthesis routes reported in plants, other genera and species of bacteria, fungi, and algae, named tryptophan-dependent (TRP-D) or tryptophan independent (TRP-I), where this amino acid is the precursor. For TRP-D, there are four pathways to the synthesis of IAA, and only two for TRP-I. The microorganisms may or not have all the routes in their genomes, and the expression of the genes varies with the isolation and the genotype of the host plant. This work reports the results obtained from an* B. japonicum *soy isolate, cultivated in an enriched LB medium, or alternatively, with tryptophan. With the data obtained, we estimate that* B. japonicum *uses both TRP-D and TRP-I routes, since in the former type indole acetamide was detected, and in the latter, indole and anthranilic acid were found. Likewise, the presence of TRP in the medium may alter IAA synthesis routes.*

## Introduction

The *Bradyrhizobium japonicum* bacterium is used as an inoculant for the cultivation of soy (*Glycine max*) since it induces the formation of nodules and increases nitrogen absorption. The nodulation of the root of the soybean plant derives from the contact between *B. japonicum* and the radicular hairs, a phenomenon regulated by the soybean plant's *nod* genes (Zahran, 1999).

In the nodules, indole acetic acid (IAA) is synthesized and released; this induces the growth of the roots and the formation of nodules in the

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soybean plant (Ghosh and Basu, 2002; Sprent, 2007; Remans *et al*., 2008). Five pathways have been proposed for IAA synthesis in bacteria: indole-3-acetamide (IAM), tryptamine (TAM), Trp side-chain oxidase (TSCO), indole-3-pyruvic acid (IPyA) and indole-3-acetonitrile (IAN) pathway; also, one tryptophan-independent pathway from indole-3-glycerol phosphate or indole (Grunennvaldt *et al*., 2018). When this takes place, *B. japonicum*, just like other bacterial species in the rhizosphere, uses a metabolic route in which the indole acetonitrile (IAN) is transformed into IAA, a pathway that is mediated by different enzymes, including nitrile hydratase and nitrilase (Rojas *et al*., 2009; Cassan *et al*., 2014; Rivera *et al*., 2018; Duca and Glic, 2020).

In plants, bacteria, fungi, and other organisms, some IAA synthesis routes have been reported to use tryptophan (TRP) as a precursor, which is why they are considered TRP-dependent (TRP-D). In the end, the TRP-D route synthesizes IAA through four different ways: the indole acetamide (IAM or ACM) route, the pyruvic acid (IPyA) route, the indole acetonitrile (IAN) route and, the tryptamine (TRM) route (Carreño-López *et al*., 2000; Spaepen *et al*., 2007; Mano and Nemoto 2012; Cassán *et al*., 2014). Out of the four routes mentioned, the IPyA route takes place preferably in plants, although it is present in some genera and species of bacteria, such as *B. japonicum* (Jones *et al*., 2007), but it is absent in other bacteria studied in our laboratory, such as *Trichoderma asperellum* and *T. koningiopsis*, as the route was not detected using HPLC (Hernández-Mendoza *et al*., 2008). The TRM and IAM routes have been reported in a wide group of genera and species of bacteria, fungi and plants (Glickman *et al*., 1998; Spaepen *et al*., 2007; Naturatat *et al*., 2016).

In these TRP-D routes, the use of TRP has been described as a precursor of the IAA, which stimulates some routes or inhibits others (Kamilova *et al*., 2006; Idris *et al*., 2007; Spaepen and Vanderlryden, 2011). In *Azospirillum brasilense* the presence of TRP in the culture medium in concentrations of 100 and 200ppm, leads to the intensification of a route such as the TRM one and, on the other hand, the route of ACM or IAM become negatively affected in the culture medium (Carreño-López *et al*., 2000).

Other pathways can also be used by these microorganisms in the synthesis of IAA and they do not use TRP as a precursor (Ona *et al*., 2005; Idris *et al*., 2007). They are known as TRP-independent (TRP-I), which begin with the incorporation of anthranilic acid (ANA), which comes from the chorismic acid, to then transform it into phosphoribosyl anthranilate, and from there, to indole (IND) (Cohen 1999; Spaepen *et al*., 2007; Remans *et al*., 2008). From this point onwards, there are genes that coordinate the change of this compound into TRP, and thereafter they restart the synthesis of the hormone via the TRP-D routes (Phi *et al*., 2008). Bacterial genera and species such as *A. brasilense* reportedly release ANA and IAA in the culture medium (Hernández-Mendoza *et al*., 2008). The other route beginning at IND is the change into IAA, where no genes have been described to control this reaction (Idris *et al*., 2007). An alternate TRP-I route, of which there are scarce references, is the one involving the change from indole-3-glycerol phosphate directly into indole with the action of indole-3-glycerol phosphate ligase/indole synthase.

According to the above information, *B. japonicum* stimulates the growth of soybean plants and, therefore, the aim of the present work is to analyze the TRP-D or TRP-I routes for the synthesis of IAA used by *B. japonicum* BjBV-05 isolated from soybean and grown in culture media, enriched or not, with tryptophan.

## Materials and Methods

The *B. japonicum* BjBV-05 strain was isolated from nodules of the vernal variety of soybean and provided by the Plant Biotechnology Laboratory, Genomic Biotechnology Center, Instituto Politécnico Nacional, Mexico. The culture medium used to activate the strain and prepare the inoculation was yeast-extract mannitol (YEM) incubated at 30°C for 72h at 200rpm. The tests to synthesize metabolites were carried out in 50ml Falcon tubes, with 20ml of LB medium, enriched and non-enriched with 100ppm of tryptophan. Each treatment was carried out in triplicate, incubated in the same way as the inoculant, and samples were taken every 12h from the beginning until the end, 96h later (Hernández-Mendoza *et al*., 2012). Estimations were made of the *B. japonicum* population during incubation using the cell count method in a Neubauer chamber (Olimpus BH-2™ microscope), as well as the estimation by optical density, where 2ml were taken and read in a spectrophotometer at 510nm.

For the analyses using HPLC (Agilent 1100™), the samples were centrifuged at 3500rpm for 10min and the supernatant filtered through 0.45µm (Milipore™) membranes, using an Ultraspher C-18 column 150*4.6mm and 300C (Beckman Ultrasphere™). The volume of injection was 20µl. We used a mobile phase of 80:20 (phosphate buffer: acetonitrile) at a pH of 3.1, a wavelength of 220nm and a flow of 1ml·min$^{-1}$. The compounds analyzed were tryptophan (TRP, Sigma-Aldrich™), indole acetic acid (IAA, Fluka™), tryptamine (TRM, Aldrich™), indole-3-actonitrile (IAN, Aldrich™), indole acetamide (ACM, Aldrich™), indole pyruvate (PIR, Sigma™), anthranilic acid (AA) and indole (IND). A calibration curve was prepared from each compound so as to obtain a factor to determine the parts per million (ppm) of each analyzed sample (Hernández-Mendoza *et al*., 2012).

## Results

The population of *B. japonicum* in the culture medium without TRP (Figure 1) increases rapidly and reaches the peak of growth after 48h, to slowly decrease thereafter. In the culture medium enriched with TRP, a peak was observed after 48h, to decrease slightly after 72h and, from there, begin an exponential growth phase for *B. japonicum*. According to the estimations of population growth by optic density, after 48h of incubation, the *B. japonicum* population was slightly higher, but with no significant statistical difference.

The strain of *B. japonicum* only synthesized IAA in the culture medium enriched with TRP (Figure 2), which confirms that TRP is an enhancement factor for the formation of this plant hormone, since in the culture medium that was not enriched with tryptophan, the hormone was not detected.

When *B. japonicum* was grown in a culture medium without TRP enrichment (Figure 3), the indole acetamide (ACM) route appeared to be the most important for TRP degradation, since that compound, was found in high concentrations. TRM was only detected in minimal amounts after 72h. On the other hand, in the tubes with a culture medium enriched with TRP, the amount of ACM was ten times higher than in the culture medium not enriched with TRP, so it is considered the main route of production of IAA.

Other compounds found in the culture medium with TRP (Figure 2) are ACM and indole acetonitrile (IAN), which maintained a constant production during incubation and the highest point of synthesis was after 72h. In turn, in the same medium enriched with TRP, compounds of route TRP-I, which are key steps in the synthesis for the

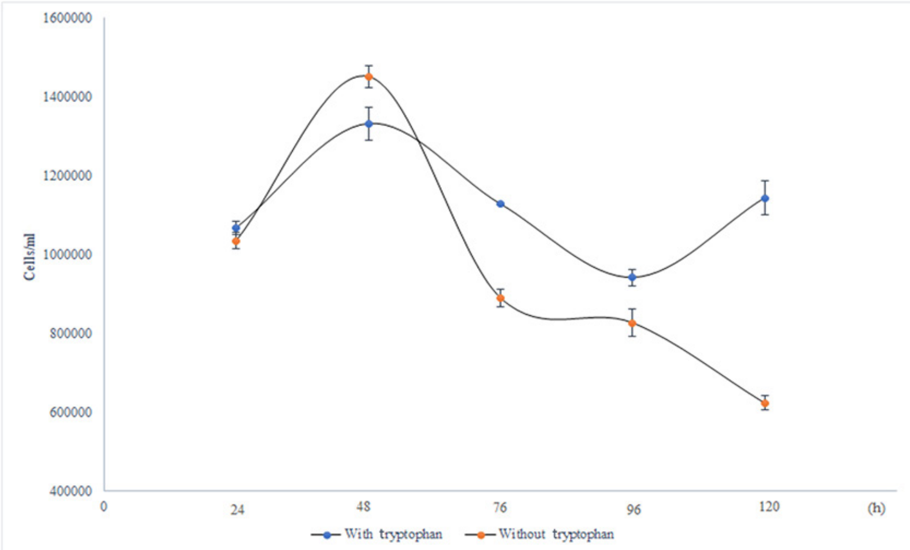


Figure 1. Growth kinetics of *B. japonicum* BjBV-05 in LB culture medium, with and without tryptophan enrichment, by cell count during incubation.

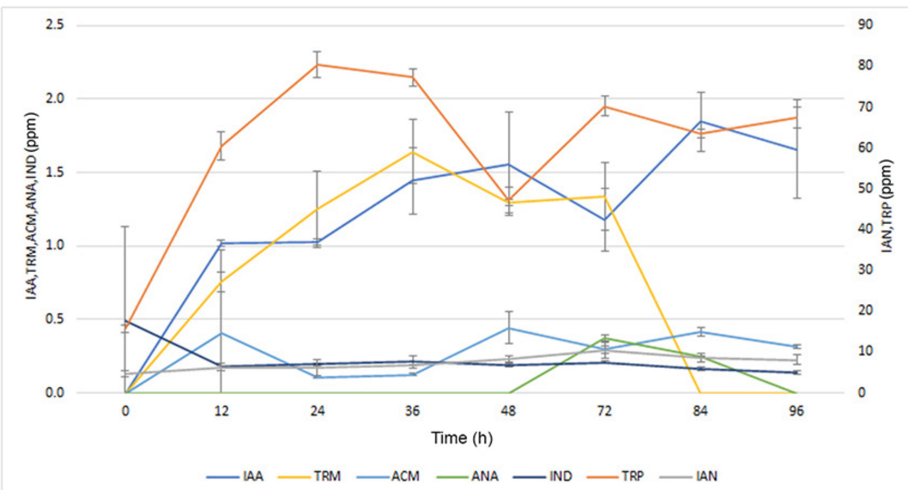


Figure 2. Kinetics of the production of auxinic compounds by *B japonicum* BjBV-05 in LB culture medium enriched with tryptophan.

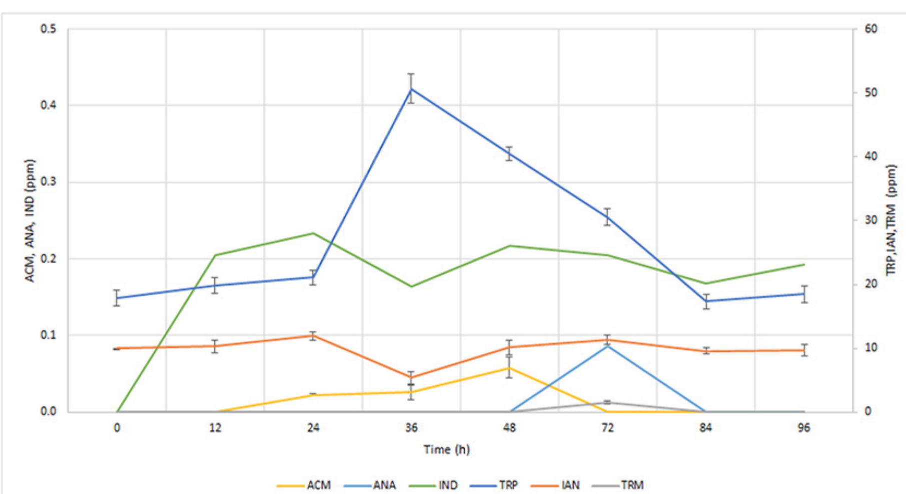


Figure 3. Kinetics of the production of metabolites by *B. japonicum* BjBV-05 in LB culture media not enriched with tryptophan.

generation of IAA, were identified. Anthranilic acid (ANA) reached the peak of production after 72h and indole (IND) was generated during all of the *B. japonicum* incubation period.

In the case of the auxinic compounds found in the culture medium without TRP (Figure 3) the compounds detected in the amounts generated were observed to be different to those found in the culture medium with TRP. Here, although TRM, ACM and IAN were found, IAA was not. In this case, as in the media with TRP, TRM was found in small amounts and IAN, showed no variations during the entire time of incubation. The results of the chromatograms of the analyzed samples did not indicate the presence of indole pyruvic acid, and it is therefore estimated that, at least with this methodology, this pathway may not exist in *B. japonicum*.

Regarding the synthesis of IAA with the route of TRP-I present in *B. japonicum*, this was confirmed to be possible, since it was detected by HPLC, anthranilic acid (ANA) and indole. Indole was detected in the first 12h and it remained present in the same levels up to 96h, while ANA showed a peak of high production at 72h of incubation, to later disappear.

Some kinetics are described in particular ways, as they are considered more important in the studies performed. Figure 4, for example, shows that *B. japonicum* did not synthesize IAA in the absence of TRP, confirming that TRP acts a promoter in this genus and species of bacteria.

On the other hand, IAN production is related to the biological fixation of nitrogen, and the data obtained show that *B. japonicum* did not synthesize more IAN when the culture medium did not contain TRP; yet, after 48h of incubation, concentrations were practically similar (Figure 5).

Regarding indole-3-hydroxyethyl (tryptophol), it was observed that its concentration was low in the absence of precursor TRP in the culture medium (Figure 6) and its synthesis began in the first hours of incubation, whereas in the presence of the precursor, the formation increased until it reached 35ppm after 82h.

Based on the data presented, Figure 7 outlines the routes used by *B. japonicum* towards the synthesis of IAA, both the tryptophan-dependent and the tryptophan-independent ones, since the auxinic compounds involved in both routes were detected in this study.

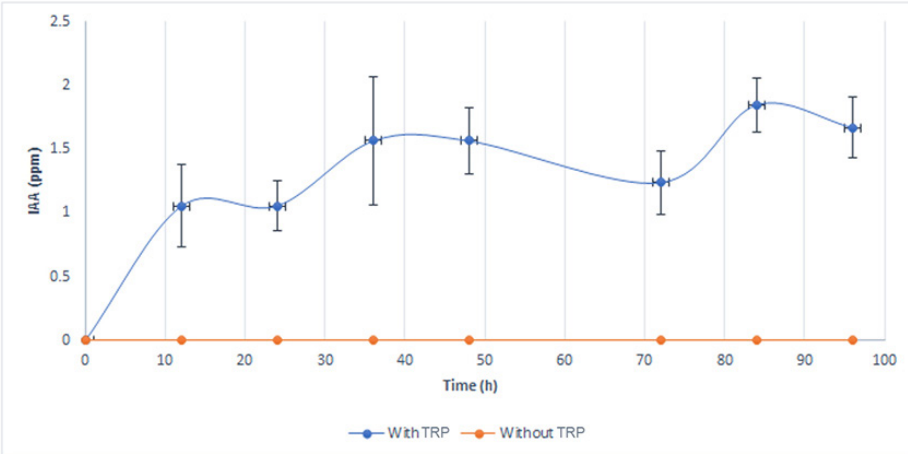


Figure 4. Production of the IAA by *B japonicum* BjBV-05 in an LB culture medium, with or whitout TRP.

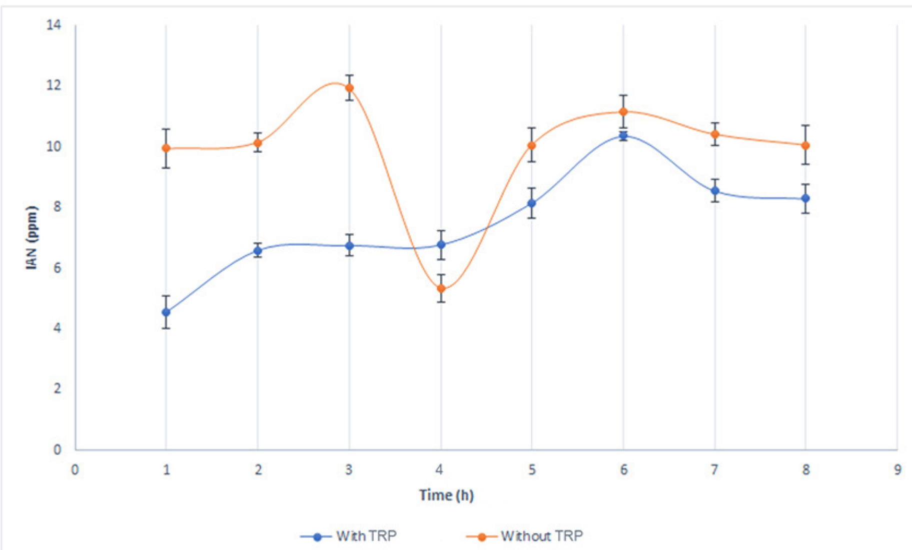


Figure 5. Kinetics of production of indole acetonitrile by *B japonicum* BjBV-05 cultivated in an LB culture medium, with or without tryptophan as a precursor of the synthesis of indole acetic acid.

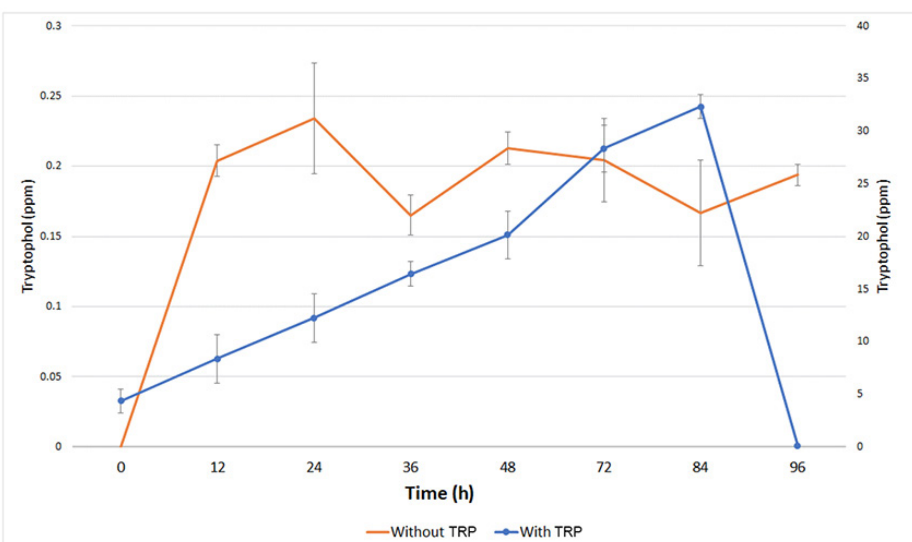


Figure 6. Kinetics of the production of Indole-3-hydroxyetil by *B japonicum* BjBV-05 in an LB medium, with or without tryptophan.

**Discussion**

Rhizobacteria synthesize indol acetic acid (IAA) among the compounds that they release into the growth culture medium, and in the case of *B. japonicum* high amounts of IAA are produced, since these bacteria are important synthesis centers (Bashan *et al*., 2014; Ona *et al*., 2005; Tsavkelova *et al*., 2007). *B. japonicum* modulates the soybean plant roots to carry out the biological fixation of nitrogen, where it synthesizes IAA, the main plant growth hormone.

In this study, tryptophan (TRP) was used for enrichment of the LB culture medium, as it can be naturally found among the compounds that make up the radicular exudates (Kravchenko *et al*., 2004) and it has been reported to be a precursor in the synthesis of IAA (Ghosh and Basu, 2002; Idris *et al*., 2007; Guruprasad *et al*., 2011; Mohite, 2013). The results obtained show that the media in which TRP was added is where the highest amount of IAA wass produced.

IAA synthesis pathways are divided depending on the use or not of TRP as a precursor. One of them, TRP-D, depends on this amino acid, and four routes are known for it, according to the key auxinic compounds generated: the route of indole pyruvic acid (IPyA), the indole acetamide (IAM o ACM) route, the route of tryptamine (TRM) and the route of indole acetonitrile (IAN) (Normanly, 2010; Mano and Nemoto, 2012; Nonhebel, 2015; Naturatat *et al*., 2016; Li *et al*., 2018). Out of these, routes of IAM, ACM and IAN were detected in this work and they correspond to the TRP-D pathway.

The TRM route has previously been described in genera and species of *Bacillus cereus* and *Azospirillum brasilense* (Hartman *et al*., 1983; Remans *et al*., 2007), and is absent in other genera such as Arthrobacter pascens strain ZZ21 (Li *et al*., 2018). In yeast such as *Rhodosporidiobolus fluviales* DMKU-CP293 and *R. paludigenum*, this route is also reported in a wide range of organisms that used TRM for the synthesis of IAA. Also, fungi such as *Fusarium graminearum* use this same route (Luo *et al*., 2016; Bunsangiam *et al*., 2019). The route of indole acetamide (ACM or IAM) has been described in *B. japonicum* (Spaepen and Vanderlryden, 2011; Glickmann *et al*., 1998) and in other microorganisms; this compound was detected in the present study.

Pyruvic acid (IPyA) was not present among the compounds detected in this work, and we therefore assume that the strain used does not use this path of the synthesis of IAA. This TRP-D

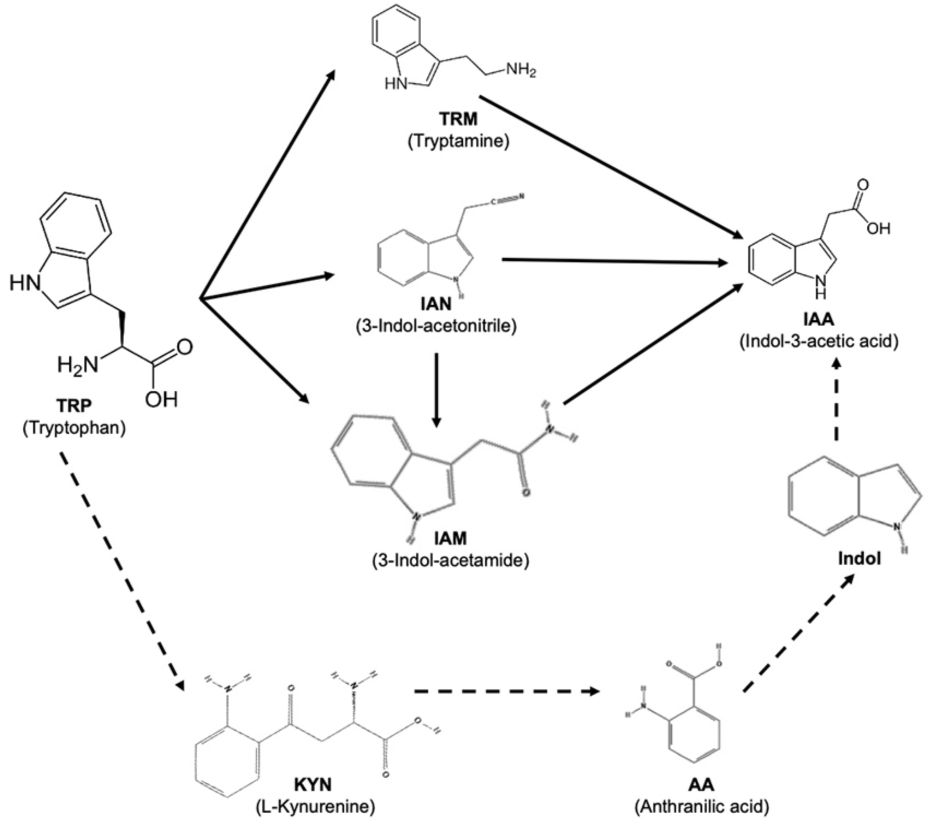


Figure 7. Auxinic compounds that intervene in the synthesis of indole acetic acid, and which were detected in *B japonicum*. The names in color are those compounds that were detected in this work. Solid lines indicate the TRP-D route, and dotted lines, TRP-I.

route has been reported in plants and fungi such as *Neurospora crassa*, where it seems to be one of the main routes for IAA synthesis (Phi *et al*., 2008; Sandar and Kempken 2018).

Indole (IND) and AAN, reported as precursors of IAA, were detected in the present study. Therefore, we estimate that they are produced by the studied strain which, along with fungi and plants, has the ability to produce IAA using TRP-I pathways (Normanly, 2010; Nonhebel, 2015; Naturatat *et al*., 2016; Li *et al*., 2018).

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**REFERENCES**

Bunsangiam S, Sakpuntoon V, Srisuk N, Ohashi T, Fujiyama K, Limtong S (2019) Biosynthetic pathway of indole-3-acetic acid in basidiomycetous yeast *Rhodosporidiobolus fluvialis*. *Mycobiology 47*: 292-300.

Bashan Y, Bashan LE, Prabbu SR, Hernandez JP (2014) Advances in plant growth-promoting bacterial inoculant technology formulation and practical perspectives. *Plant Soil 378*: 1-33

Carreño-López R, Campos-Reales N, Elmerich C, Baca BE (2000) Physiological evidence for differently regulated tryptophan-dependent pathway for índole -3-acetic acid synthesis in *Azospirillum brasilense. Mol. Gen. Genet. 264*: 521-530.

Cassan F, Vanderleyden J. Spaepen S (2014) Physiological and agronomical aspects of phytohormone production by model plant growth promoting rhizobacteria (PGPR) belonging to the genus *Azospirillum. J. Plant Growth Regul. 33*: 440-459.

Cohen JD (1999) An in vitro system form maize seedling for tryptophan - independent indole -3-acetic acid biosynthesis. *Plant Physiol. 119*: 173-178.

Duca DR, Glick BR (2020) Indole-3-acetic acid biosynthesis and its regulation in plant-associated bacteria. *Appl. Microbiol. Biotechnol. 140*: 8607-8619.

Ghosh AC, Basu P. (2002) Growth behavior and bioproduction of indole acetic acid by a *Rhizobium* sp isolated from root nodules of a leguminous tree *Dalbergia lanceolaria*. *Indian J Exp Biol. 40*: 796-801.

Glickmann E, Gardan L. Jacquet S, Hussain S, Elasri M, Petit A, Dessaux Y (1998) Auxin Production Is a Common Feature of Most Pathovars of *Pseudomonas syringae*. *Mol. Plant Microbe Interact. 11*: 156-162.

Grunennvaldt RL, Degenhardt-Goldbach J, de Cássia Tomasi J, Dos Santos GD., Vicente VA, Deschamps C (2018) *Bacillus megaterium*: bacteria endofítica de callos de *Ilex paraguariensis* con actividad de promoción del crecimiento. *Biotecnol. Veget. 18*: 3-13.

Guruprasad BK, Shirshailnath SS, Karegoudar TB (2011) Pathogenicity of indole-3- acetic acid producing fungus *Fusarium delphinoides* strain in GPK towards chickpea and pigeon pea. *Eur. J. Plant Pathol. 131*: 355-369.

Hartman A, Singh M, Klingmuller W (1983) Isolation and characterization of *Azospirillum brasilense* mutants excreting high amounts of indoleacetic acid. *Can. J. Microbiol. 29*: 916-923.

Hernández-Mendoza JL, Quiroz Velásquez JD, Moreno-Medina VR, Mayek Pérez N (2008) Biosíntesis de ácido antranílico y ácido indolacético a partir de triptofano en una cepa de *Azospirillum brasilense* nativa de Tamaulipas, México. *Rev AIA. 12*: 57-67.

Hernández-Mendoza JL, Quiroz-Velázquez JD, Díaz-Franco A, García-Olivares JG, Bustamante-Dávila AJ, Gill-Langarica HR (2012) Detection of metabolites in Flor de Mayo common beans (*Phaseolus vulgaris* L.) and their response to inoculation with *Trichoderma harzianum*. *Afr. J. Biotechnol. 11*: 11767-11771.

Idris EE, Iglesias DJ, Talón M, Borris R (2007) Tryptophan dependent production of índole-3-acetic acid (IAA) affects level of plant growth promotion by *Bacillus amyloliquefaciens* FZB42. *Mol. Plant Microbe Interact. 20*: 619-623.

Jones KM, Kobayashi A, Davis BW, Toga E, Welker GC (2007) How *Rhizobium* symbionts invade plants: the *Sinorhiobium-Medicago* model. *Nature Rev. 5*: 619-633.

Kamilova F, Kravchenko LV, Shaposhnikov AL, Azarova T, Makarova N, Lugtenberg B (2006) Organic acid, sugars, and L-tryptophan in exudates of vegetables growing on stonewool and their effects on activities of rhizosphere bacteria. *Mol. Plant Microbe Interact. 19*: 250-256.

Kravchenko LV, Azarova TS, Makarova NM, Tikhonovich IA (2004) The effect of tryptophan present in plant root exudates on the phytostimulating activity of rhizobacteria. *Microbiology 73*: 156-158.

Li M, Guo R, Yu F, Chen X, Zhao H, Li H, Wu J. (2018) Indole-3-acetic acid biosynthesis pathways in the plant-beneficial bacterium *Arthrobacter pascens* ZZ21. *Int. J. Mol. Sci. 19*: 443. doi:10.3390/ijms19020443.

Luo K, Rocheleau H, Qi PF, Zheng YL, Zhao HY, Ouellet T (2016) Indole-3-acetic acid in *Fusarium graminearum*: Identification of biosynthetic pathways and characterization of physiological effects. *Fungal Biol. 120*: 1135-1145.

Mano Y, Nemoto K (2012) The pathway of auxin biosynthesis in plants. *J. Exp. Bot. 25*: 2853-2872.

Mohite B (2013) Isolation and characterization of indole acetic acid (IAA) producing bacteria from rhizospheric soil and its effect on plant growth. *J. Soil Sci. Plant Nutr. 13*: 638-649.

Naturatat P, Srisuk N, Arunrattiyakorn P, Limtong S (2016) Indole-3-acetic acid biosynthesis pathways in the basidiomycetous yeast *Rhodosporidium paludigenum*. *Arch. Microbiol. 198*: 429-437.

Nonhebel HM (2015) Tryptophan-independent indole-3-acetic acid synthesis: Critical evaluation of the evidence. *Plant. Physiol. 169*: 1001-1005.

Normanly J (2010) Approaching cellular and molecular resolution of auxin biosynthesis and metabolism. *Cold Spring Harb. Perspect. Biol. 2*: 1-17.

Ona O, Van Impe J, Prinsen E, Vanderleyden J (2005) Growth and Indol-e-acetic acid biosynthesis of *Azospirillum brasilense* Sp245 is environmentally controlled *FEMS Microbiol. Lett. 246*: 125-132.

Phi QT, Park YM, Ryu CM, Park SH, Ghim SY (2008) Functional identification and expression of indole-3-pyruvate decarboxylase from *Paenibacillus polymyxa* E681. *J. Microbiol. Biotechnol. 18*: 1235-1244.

Remans R, Beebe S, Blair M, Manrique G, Tovar E, Rao I, Croonenborghs A, Torres- Gutierrez R, El-Howeity M, Michiels J, Vanderleyden J (2008) Physiological and genetic analysis of root responsiveness to auxin-producing plant growth-promoting bacteria in common bean (*Phaseolus vulgaris* L). *Plant Soil 302*: 149-161.

Rivera D, Mora V, López G, Rosas S, Spaepen S, Vanderleyden J, Cassan F (2018) New insights into indole-3-acetic acid metabolism in *Azospirillum brasilense. J. Appl. Microbiol. 125*: 1774-1785.

Rojas MM, Rodriguez AJ, Trujillo DJ, Heydrich N (2009) Relación de la fijación de nitrógeno y la producción de auxinas de *Gluconoacetobacter diazotrophicus* procedentes de diferentes cultivos. *Rev. Colomb. Biotecnol. 15*: 84-93.

Sandar P, Kempken F (2018) Characterization of indole 3 pyruvic acid pathway mediated biosynthesis of auxin in *Neurospora crassa. Plos One. 13*(2): e0192293.

Spaepen S, Vanderleyden J (2011) Auxin and plant-microbe interactions. *Cold Spring Harb. Perspect. Biol. 3*: a001438.

Spaepen S, Vanderleyden J, Remans R (2007) Indole-3-acetic acid in microbial and microorganism-plant signaling. *FEMS Microbiol. Rev. 31*: 425-448.

Sprent J (2007) Evolving ideas of legume evolution and diversity: a taxonomic perspective and diversity on the occurrence of nodulation. *New Phytol. 174*: 11-25.

Tsavkelova EA, Cherdyntseva T, Klima SY, Sheslakov PT, Biotina SG, Netrusov AT (2007) Orchid-associated bacteria produce indole 3 acetic acid promote seed germination and increase their microbial yield in response to exogenous auxin. *Arch. Microbiol. 188*: 655-664.

Zahran HH (1999) *Rhizobium*-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. *Microbiol. Mol. Biol. Rev. 63*: 968-989.

## ESTUDIO DE LAS VÍAS DE BIOSÍNTESIS DEL ÁCIDO INDOL ACÉTICO EN *Bradyrhizobium japonicum* BJBV-05

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*RESUMEN*

*La bacteria* Bradyrhizobium japonicum *induce la nodulación en plantas de* Glycine max *(soya) y otras legumbres. Esto es considerado de gran importancia, ya que em los nódulos es donde las bacteria se establecen, contribuyendo a la fijación biológica de* $N_2$. *El proceso es controlado por la nitrogenasa, una enzima producida por los genes* nif *presentes en el genoma de la bacteria. Al metabolizar la nitrogenasa, el indol acetonitrilo la transforma en ácido indol acético (IAA) y libera una molécula nitrogenada. Se han reportado otras rutas de síntesis de IAA en plantas y en otros géneros y especies de bacterias, hongos y algas, denominadas dependientes de triptófano (TRP-D) o independientes de triptófano (TRP-I), donde este aminoácido es el precursor. Para TRP-D, hay cuatro vías para la síntesis de IAA y solo dos para TRP-I. Los microorganismos pueden tener o no todas las rutas en sus genomas, y la expresión de los genes varía con el aislamiento y el genotipo de la planta huésped. Este trabajo reporta los resultados obtenidos a partir de una cepa de* B. japonicum *aislada de soja, cultivado en un medio LB enriquecido, o alternativamente, con triptófano. Con los datos obtenidos, estimamos que* B. japonicum *utiliza ambas rutas TRP-D y TRP-I, ya que en la primera vía se detectó indol acetamida, y en la segunda, indol y ácido antranílico. Asimismo, la presencia de TRP en el medio puede alterar las rutas de síntesis de IAA.*

## ESTUDO DAS VIAS DE BIOSSÍNTESE DO ÁCIDO INDOLACÉTICO EM *Bradyrhizobium japonicum* BJBV-05

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*RESUMO*

*A bactéria* Bradyrhizobium japonicum *induz a nodulação em plantas de* Glycine max *(soja) e outras leguminosas. Isto é considerado de grande importância, já que é nos nódulos onde as bactérias se estabelecem, contribuindo para a fixação biológica do* $N_2$. *O processo é controlado pela nitrogenase, uma enzima produzida pelos genes* nif *presentes no genoma da bactéria. Ao metabolizar a nitrogenase, a indol acetonitrila a transforma em ácido indolacético (AIA) e libera uma molécula de nitrogênio. Têm sido relatadas outras rotas de síntese de AIA em plantas e em outros gêneros e espécies de bactérias, fungos e algas, denominadas dependentes do triptofano (TRP-D) ou independentes do triptofano (TRP-I), onde este aminoácido é o precursor. Para TRP-D, há quatro vias para a síntese de AIA e apenas duas para TRP-I. Os microrganismos podem ter ou não todas as rotas em seus genomas, e a expressão gênica varia com o isolamento e o genótipo da planta hospedeira. Este trabalho relata os resultados obtidos a partir de uma cepa de* B. japonicum *isolada de soja, cultivada em um meio LB enriquecido, o alternativamente, com triptofano. Com os dados obtidos, estimamos que* B. japonicum *utiliza ambas as rotas TRP-D e TRP-I, já que na primeira via foi detectado indol acetamida, e na segunda, indol e ácido antranílico. Da mesma forma, a presença de TRP no meio pode alterar as rotas de síntese de AIA.*