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Indole-3-acetic acid: a reciprocal signalling molecule in bacteria–plant interactions

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Auxins were discovered early in the twentieth century as plant-regulating substances. Indole-3-acetic acid (IAA) is a naturally occurring auxin with broad physiological effects. Although many plant genes that are transcriptionally regulated by IAA have been characterized in recent years, our understanding of the auxin signal transduction pathway(s) in plants is still incomplete. IAA biosynthesis in plants can occur via different pathways¹, which are classified according to their intermediates: indole-3-acetamide (IAM), indole-3-pyruvate (IPyA), tryptamine, and indole-3-acetonitrile². To further our understanding of plant growth and development in nature, microbially released auxins, which can have a pronounced effect on plants, should be considered. The two most common routes for IAA biosynthesis in bacteria are the IAM and the IPyA pathways.

Phytopathogenic bacteria

Hyperplasia-inducing bacteria were the first plant-associated bacteria

in which IAA biosynthesis pathways were studied because of the suspected role of microbially released auxins in disturbing plant morphology and development³. *Agrobacterium tumefaciens*, *Agrobacterium rhizogenes*, *Pseudomonas savastanoi* and *Erwinia herbicola* all possess the IAM pathway, and DNA sequence analysis of the *iaaM* gene suggests a common evolutionary origin³. In all of these bacterial species, IAA is involved in pathogenesis. The production of IAA via the IAM pathway can vary considerably and is controlled by different mechanisms, including tryptophan supply and feedback inhibition of *iaaM* activity by IAA and IAM

(Ref. 4). *E. herbicola* pv. *gypsophilae* uses two pathways for IAA biosynthesis: the IAM and IPyA pathways⁵. Expression of *iaaM* is high when the bacteria are in the apoplast, whereas expression of the *ipdC* gene, the key gene of the IPyA pathway, is high when bacteria are on the leaf surface⁵. Apparently, the IAM and IPyA pathways in this bacterium seem to be adapted for expression in different environments. The IAM pathway is involved in gall size, whereas the IPyA pathway determines epiphytic fitness⁵.

It is now recognized that many bacteria are able to produce IAA, including bacteria that are phytopathogenic (not only hyperplasia inducing), as well as those that are plant-growth promoting. Except in a few cases, the link between IAA synthesis and plant phenotype has not been demonstrated or at the least it remains ambiguous. Some plant-growth-promoting rhizobacteria (PGPR) stimulate root proliferation by IAA biosynthesis. Thus, these bacteria enhance uptake of soil minerals

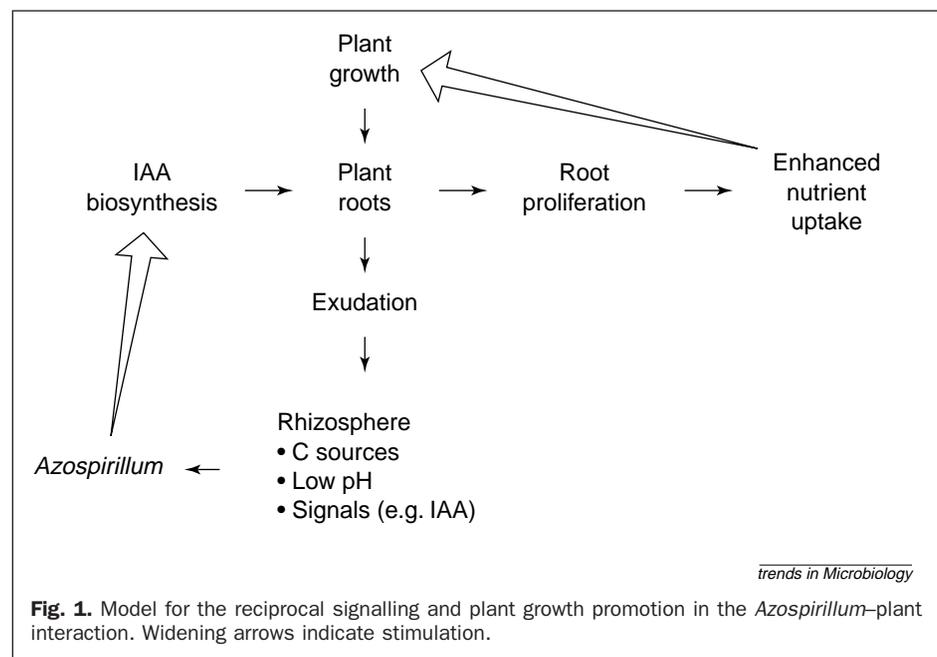
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and nutrients by the host plant. Most beneficial bacteria synthesize IAA via the IPyA pathway. In general, IAA biosynthesis pathways in plant-beneficial bacteria are inducible² and the intermediates of these pathways have also been identified in plants¹.

***Azospirillum*–plant root association**

Azospirillum brasilense is a Gram-negative, nitrogen-fixing bacterium found in the rhizosphere of many plant species. The plant growth promotion observed after inoculation with *A. brasilense* is mainly caused by biosynthesis and secretion of bacterial IAA (Ref. 6). At least three pathways for IAA biosynthesis exist in *A. brasilense*, two tryptophan-dependent pathways (IAM and IPyA) and one tryptophan-independent pathway⁷. Our recent data indicate that indole-3-pyruvate decarboxylase (encoded by the *ipdC* gene) is a common enzyme for both the IPyA pathway and the tryptophan-independent pathway (the precursor of which is so far unknown), suggesting that both pathways merge at the IPyA intermediate (branched IPyA pathway). Expression of the *A. brasilense ipdC* gene is upregulated by IAA (Ref. 8); this is the first description of a bacterial gene that is specifically induced by auxins. The *A. brasilense ipdC* promoter contains an auxin-responsive element (AuxRE), which is similar to the AuxRE found in the promoters of plant auxin-inducible genes⁹.

The majority of substrates for microbial activity in the rhizosphere are derived from the plant: depending on the plant species and environmental conditions, the exudated substrates can account for up to 40% of the dry matter produced by plants. The substrates include amino acids, organic acids, sugars, vitamins, nucleotides, enzymes and other plant metabolites, including auxins¹⁰. The presence of plant-derived IAA in the rhizosphere could be sufficient for *A. brasilense* to enhance the expression of the *ipdC* gene. Once expression of the *ipdC* gene is switched on, bacterial synthesis of IAA would increase, provided



there are sufficient IAA precursor molecules (such as tryptophan) available. Bacterial IAA would then stimulate plant root proliferation. The key elements of this model, that is positive feedback regulation of *ipdC* expression and stimulation of lateral root and root hair development by external IAA, have been validated^{8,11}. However, it has also been demonstrated that above a certain concentration of exogenously supplied IAA, root development is inhibited. Therefore, the IAA amplification loop, in which root-colonizing, plant-growth-promoting *A. brasilense* cells play a key role, as suggested in the model (Fig. 1), must be restricted, additionally controlled or compensated by other factors¹¹. Limitation could be at the level of IAA-precursor concentration; additional control could be at one or various genes or enzymes in the branched IPyA pathway; and compensation for too-high levels of IAA could occur by means of regulation of IAA metabolism. To further test the postulated model, *A. brasilense* mutants that are completely deficient in IAA biosynthesis are needed (an *ipdC* mutant still produces 10% of wild-type IAA levels). Such mutants, containing an *ipdC-gusA* reporter-gene fusion, could be used to quantify the spatial-temporal patterns of *ipdC* expression in the

plant rhizosphere. Such a strain could also be used as a reporter system or biosensor for the presence of IAA in the soil.

Rhizobia–plant symbioses

Most *Rhizobium* species have been shown to produce IAA (Ref. 12), and many studies indicate that changes in auxin balance are a prerequisite for nodule organogenesis¹³. Nevertheless, the overall role of IAA in the different stages of *Rhizobium*–plant symbiosis is still unclear. Inhibitors of polar auxin transport induce pseudonodules on alfalfa roots¹⁴. Moreover, recent evidence suggests that Nod factors (lipo-chitin oligosaccharides or LCOs), which are produced by rhizobia upon triggering of *nod* gene expression by plant-derived flavonoids, act as auxin-transport inhibitors¹³. The expression of the auxin-responsive reporter construct *GH3::gusA* in a transgenic white clover plant was rapidly and transiently downregulated after inoculation by *Rhizobium leguminosarum* bv. *trifolii* followed by an upregulation at the site of nodule initiation¹³. This suggests that local lowering of the auxin balance is required for the initiation of nodule primordia.

Other observations nevertheless indirectly suggest that microbially released IAA could play a role in rhizobia–plant symbiosis. It was

demonstrated that the *nod* inducers, the flavonoids, also stimulate the production of IAA by *Rhizobium*¹⁵. Combined application of *Rhizobium* and *Azospirillum* can enhance nodulation, as demonstrated in several studies and recently further explored¹⁶. Better root development could explain the increased nodulation but it cannot be excluded that additional factors could be involved, such as changes in flavonoid metabolism¹⁷. *A. brasilense* caused a significant increase in the *nod*-inducing activity of crude alfalfa root exudates. When analysed, these exudates contained different profiles of flavonoids, as compared with exudates of non-inoculated plants¹⁷.

Root nodules have been shown to contain more IAA than non-nodulated roots^{12,18}, and auxins could be important for maintaining a functional root nodule¹². However, the origin of IAA in the nodules is still not clear. It has been suggested that elevated levels of IAA in nodules are derived from the prokaryotic micro-symbiont because a mutant of *Bradyrhizobium japonicum* that produces 30-fold more IAA than the wild-type strain has a higher nodulation efficiency¹⁹. Bacteroids of plants inoculated with mutant *B. japonicum* strains overproducing IAA produce high amounts of IAA in comparison with wild-type bacteroids²⁰, suggesting that increased IAA biosynthesis in nodules is of prokaryotic origin.

Conclusion

The response of plants to external (microbially released) IAA can vary from beneficial to deleterious effects, depending on the concentration of IAA in the plant root. The levels of IAA released by rhizosphere bacteria depend on bacterial growth, metabolic activity and the expression of genes encoding enzymes for IAA biosynthesis. For a beneficial bacteria-plant interaction, exemplified by *Azospirillum*-plant root association, expression of the *ipdC* gene is highly regulated, including regulation by IAA itself. Further studies on the IAA signal transduction pathway in *A. brasilense* should help us to

further unravel the signalling mechanism of IAA in bacteria-plant interactions. In a more general way, IAA regulation of gene expression in *A. brasilense* should encourage a more systematic search for IAA-regulated genes in other IAA-producing and/or IAA-exposed phyllosphere or rhizosphere microorganisms.

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