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INVITED REVIEW

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Indole-3-acetic acid in plant–microbe interactions

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Abstract Indole-3-acetic acid (IAA) is an important phytohormone with the capacity to control plant development in both beneficial and deleterious ways. The ability to synthesize IAA is an attribute that many bacteria including both plant growth-promoters and phytopathogens possess. There are three main pathways through which IAA is synthesized; the indole-3-pyruvic acid, indole-3-acetamide and indole-3-acetonitrile pathways. This chapter reviews the factors that effect the production of this phytohormone, the role of IAA in bacterial physiology and in plant–microbe interactions including phytostimulation and phytopathogenesis.

Keywords Indole-3-acetic acid (IAA) · Phytohormone · Plant growth promoting bacteria · IAA biosynthesis · IAA regulation

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Introduction

The phytohormone auxin controls nearly every aspect of plant growth and development (Grossmann 2010). It is a fundamental compound that modulates plant growth and development (Halliday et al. 2009). The most crucial natural auxin (and the most common) is indole-3-acetic acid (IAA), produced by bacteria, plants and fungi. In plants, this phytohormone plays a central role in cell division, elongation, fruit development and senescence. IAA initiates roots, leaves and flowers (Phillips et al. 2011). Particularly in dicots, IAA specifically induces lateral-root formation, whereas in monocots it induces adventitious root formation (McSteen 2010). Moreover, IAA coordinates cambial growth and vascular development. In both conifer and angiosperm trees, IAA promotes secondary wall thickness and increases the size of xylem cells (Uggla et al. 1996). IAA also serves as a signal in the plant shade avoidance syndrome. Researchers have observed that plants deficient in IAA production have lost the ability to avoid shade and consequently displayed stunted growth compared to normal plants (Tao et al. 2008).

IAA transport in plants

Most IAA is transported throughout the plant via the phloem, forming concentration gradients and accumulating in different tissues (Eklund et al. 2010; Tromas and Perrot-Rechenmann 2010). This long

distance transport is efficient but cannot be finely regulated. Polar auxin transport between cells involves the diffusion of this molecule across cell walls and membranes. The hydrophobic protonated form (IAAH) can diffuse across cell membranes, however the hydrophilic anionic form (IAA^{-}) cannot (Tromas and Perrot-Rechenmann 2010). Therefore, the anionic form is actively transported via specific auxin–influx carriers and efflux transporters (Tromas and Perrot-Rechenmann 2010). IAA has a pKa of 4.75 and the proportion of the hydrophilic anionic and the hydrophobic protonated forms depends on the local pH (Tromas and Perrot-Rechenmann 2010). Endogenous concentrations of IAA vary greatly among different tissues, depending on the plant species and organ. Typically the concentration is low (5–100 ng/g fresh weight) in leaves and perhaps 10–100 times lower in roots (Reid et al. 2011). Even at low concentrations, IAA is a master regulator of plant development. There are many excellent reviews on IAA transport, some of which have been recently summarized in the book "Polar Auxin Transport" (Chen and Baluška 2013).

The nature of IAA biosynthesis

IAA was first discovered in plants at end of the nineteenth century and this discovery initiated the field of "auxinology" (Spartz and Gray 2008). The German botanist Julian von Sachs first proposed that endogenous organ-forming substances move throughout a plant in response to light and gravity. This idea was supported by Charles and Francis Darwin, who observed the bending of plants toward light due to a signal, which was later identified as IAA (Spartz and Gray 2008; Masuda and Kamisaka 2000). Over the last few decades, several pathways of IAA biosynthesis have been proposed. These pathways are determined by identifying the enzymes that catalyze each reaction and the intermediates involved at each step (Lehmann et al. 2010). The presence of multiple parallel and redundant pathways within a single organism creates a robust IAA biosynthetic network. Different pathways may intersect with one another, which poses difficulties when trying to characterize them. Such a multi-route system compensates for the loss of any particular pathway, as IAA production can be fluxed through an alternate route. Due to the intricate nature of IAA biosynthesis and the importance of having a functioning IAA biosynthetic mechanism within a plant, there is no known plant IAA auxotrophic mutant completely devoid of the ability to produce IAA (Lehmann et al. 2010).

To date, the majority of our understanding of IAA biosynthetic pathways is based on studies in bacteria. Genetic approaches are effective for evaluating whether a proposed route is important for IAA biosynthesis and to determine the contribution of each route to the overall pool of IAA. If a particular gene plays a crucial role in IAA biosynthesis, the inactivation of that gene is expected to disrupt the pathway, leading to a decrease in IAA production. The resultant phenotype would result in dramatic developmental defects in the plant (Soeno et al. 2010). Biochemical approaches aim to isolate and purify the enzymes that catalyze IAA biosynthesis. Substrate feeding assays may reveal the role and specificity of each enzyme. Determining the structure and function of proteins involved in IAA biosynthetic pathways is a cornerstone in understanding how these pathways work.

IAA produced by bacteria

Many different bacteria are capable of synthesizing IAA, including soil, epiphytic, endophytic, marine, methylotrophs and cyanobacteria (Sergeeva et al. 2002). Some of these bacteria are detrimental to a plant's health while others are advantageous. Plants harbor millions of IAA-producing bacteria from genera such as *Pseudomonas*, *Rhizobium*, *Azospirillum, Enterobacter, Azotobacter, Klebsiella, Alcaligenes, Pantoea* and *Streptomyces* (Apine and Jadhav 2011). Bacterial IAA stimulates root hair formation while increasing the number and length of lateral and primary roots when it is within an ideal concentration range. However, at higher concentrations, bacterial IAA can also inhibit primary root growth (Davies 1995). A well-developed root system is imperative for water and nutrient uptake and for anchoring plants in the soil. The plant reciprocates by providing the bacterial partner with exudates (containing nutrients) and an ideal shelter (Ahmed and Hasnain 2010). IAA is a reciprocal signaling molecule in plant–microbe interactions, sustaining the symbiotic relationship that has evolved between host plants and their microbial allies (Malhotra and Srivastava 2009).

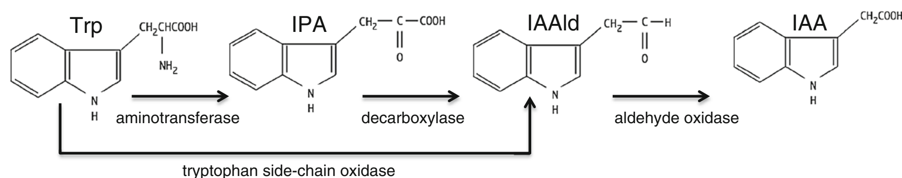

Fig. 1 The indole-3-pyruvic acid pathway. Trp tryptophan, IPA indole-3-pyruvic acid, IAAld = indole-3-acetaldehyde, IAA indole-3-acetic acid. (Patten and Glick 1996)

The IAA biosynthesis pathways

Bacterial IAA biosynthesis may be tryptophan-dependent, or independent of tryptophan. In tryptophan-dependent IAA biosynthesis, the following pathways have been postulated utilizing tryptophan as a precursor:

The indole-3-pyruvic acid pathway

Both plants and bacteria can synthesize IAA via the indole-3-pyruvic acid (IPA) pathway. Initially, L-tryptophan is deaminated to IPA by an aminotransferase. Subsequently, a decarboxylase enzyme converts IPA into indole-3-acetylaldehyde (IAAld), which is then oxidized to IAA by aldehyde dehydrogenase, mutase or oxidase enzymes (Fig. 1) (Rajagopal 1971; Pollmann et al. 2006). Aromatic amino acid aminotransferases have been reported in several plant-associated bacteria (Table 1) (Kittell et al. 1989; Koga et al. 1994). A bacterium may possess several different isoforms of the enzyme, each often able to utilize multiple substrate amino acids, including all three aromatic amino acids as well as aspartate (Kittell et al. 1989). This suggests that these enzymes may function within metabolic pathways other than IAA synthesis (Kittell et al. 1989; Pedraza et al. 2004).

The gene responsible for the aldehyde oxidation step of the IPA pathway remains elusive in bacteria. Putative NADP-dependent indole-3-acetaldehyde dehydrogenase genes have been identified in the genomes of *Bacillus amyloliquefaciens* FXB42 and *Bacillus subtilis* 168 (Kunst et al. 1997; Idris et al. 2007). However, disruption of this gene in *B. amyloliquefaciens* FXB42 did not significantly affect its IAA production, suggesting that the gene product does not play a role in IAA synthesis (Idris et al. 2007). Xie et al. (2005) identified an *aldA* gene in *Azospirillum brasilense* Yu62 that had significant homology (80 and 78 %) to the aldehyde dehydrogenases from *Bradyrhizobium japonicum* USDA 110 and *Mesorhizobium loti* MAFF303099, respectively. The *aldA* gene was disrupted by Tn*5* insertion and the resulting mutant produced less IAA. This implies that the *aldA* gene product catalyzes the aldehyde oxidation step in the IPA pathway (Table 1) (Xie et al. 2005). Aldehyde dehydrogenases have been identified in bacteria such as *Gluconacetobacter diazotrophicus*, however this enzyme showed narrow substrate specificity toward aliphatic aldehydes such as acetaldehyde and was not tested on the aromatic indole-3-acetaldehyde substrate (Gómez-Manzo et al. 2010). While some aldehyde dehydrogenases are highly specific for a handful of substrates, others show broad substrate specificity. All aldehyde dehydrogenases require either NAD or NADP as a cofactor (Lindahl 1992; Yoshida et al. 1998; Perozich et al. 1999).

An alternative pathway exists in which tryptophan is directly converted into IAAld by a tryptophan side chain monooxygenase enzyme (Fig. 1). This is known as the tryptophan side chain oxidase (TSO) pathway (Oberhänsli et al. 1991). The TSO pathway has been reported in bacteria such as *Pseudomonas fluorescens* HP72, *P. fluorescens* strains such as *P. fluorescens* CHA0 and *P. fluorescens* Pf-5 (Suzuki et al. 2003; Oberhänsli et al. 1991; Whistler et al. 1998).

The *ipdC* gene codes for the indolepyruvate decarboxylase enzyme, which catalyzes the key step in the IPA pathway (Patten and Glick 2002). This gene has been cloned from several bacteria (Table 1) and disrupted to create IAA-deficient mutants (Ryu and Patten 2008; Patten and Glick 2002; Fedorov et al. 2010; Malhotra and Srivastava 2008; Costacurta et al. 1994; Carreño-Lopez et al. 2000; Yagi et al. 2001; Koga et al. 1991; Brandl and Lindow 1996; Zimmer et al. 1994). The *ipdC*-encoded enzyme is an α-keto acid decarboxylase and these enzymes are known to have broad substrate specificity. While the indolepyruvate decarboxylases from some bacteria have high

Table 1 Summary of IAA biosynthesis pathways that have been identified in different bacteria

Bacterium	Pathway	Genes identified	Enzymatic activity	Intermediates identified	References
Azospirillum brasilense Yu62	IPA	aldA	Aldehyde dehydrogenase		Xie et al. (2005)
Pantoea agglomerans	IPA		Aminotransferase		Sergeeva et al. (2007)
Rhizobium meliloti			Aminotransferase		Kittell et al. (1989)
Rhizobium sp. NGR234	IPA	y4wE y4wF		ILA, IAld, IEt, IPA	
Azospirillum brasilense UAP14			Aminotransferase		Baca et al. (1994)
Azospirillum lipoferum ATCC 29708			Aminotransferase		Ruckdäschel et al. (1988)
Rubrivivax benzoatilyticus JA2	IPA		Aminotransferase	IPA, IAld	Kumavath et al. (2010)
Azospirillum brasilense Sp7	IPA	hisC1	Aromatic amino acid Aminotransferase		Castro-Guerrero et al. (2012)
Azospirillum brasilense Sp245, *Azospirillum brasilense* R07, *Azospirillum lipoferum* USA5a, *Pseudomonas stutzer*i A15, *Gluconacetobacter diazotrophicus* PAL5, *Gluconacetobacter azotocaptans* CFN-Ca 54T, *Gluconacetobacter johannae* CFN-Cf55T	IPA		Aminotransferase		Pedraza et al. (2004)
Azospirillum brasilense Sp7	IPA	ipdC			Zimmer et al. (1998)
Azospirillum brasilense Sp245	IPA	ipdC	Indolepyruvate decarboxylase		Costacurta et al. (1994)
Enterobacter cloacae	IPA		L-Tryptophan 2-oxoglutarate aminotransferase		Koga et al. (1994)
Paenibacillus polymyxa E681			Indolepyruvate decarboxylase		Phi et al. (2008)
Azospirillum lipoferum FS		ipdC	Indolepyruvate decarboxylase		Yagi et al. (2001)
Enterobacter cloacae FERM BP-1529	IPA	ipdC	Indolepyruvate decarboxylase		Koga et al. (1991)
Enterobacter cloacae NCIMB 11461, *Enterobacter cloacae* NCIMB 11463, *Enterobacter agglomerans* 333, *Enterobacter agglomerans* 339, *Klebsiella aerogenes* DSM 681	IPA	ipdC			Zimmer et al. (1994)
Pseudomonas putida GR12-2	IPA	ipdC			Patten and Glick (2002)

Table 1 continued

Bacterium	Pathway	Genes identified	Enzymatic activity	Intermediates identified	References
Enterobacter cloacae UW5	IPA	ipdC			Ryu and Patten (2008)
Azospirillum brasilense SM	IPA	ipdC			Malhotra and Srivastava (2008)
Erwinia herbicola R299		ipdC			Brandl et al. (1996) and Brandl and Lindow (1996)
Pseudomonas fluorescens HP72	IPA			IAld, IPA	Suzuki et al. (2003)
Methylobacterium extorquens AM1	IPA				Fedorov et al. (2010)
Rubrivivax benzoatilyticus JA2	IPA		Aminotransferase	IAld, IPA	Kumavath et al. (2010)
Rhodococcus fascians				IAld, IPA, ILA	
Pseudomonas fluorescens Psd	IAM	iaaM iaaH	Tryptophan monoxygenase	IAM	Kochar et al. (2011)
Agrobacterium tumefaciens	IAM	Tms-1 Tms-2			Thomashow et al. (1984), Rausch et al. (1985), Morris (1986) and Van Onckelen et al. (1986)
Pseudomonas savastanoi	IAM	iaaM iaaH			Comai and Kosuge (1980) and Yamada et al. (1985)
Pseudomonas syringae	IAM				Comai and Kosuge (1980)
Rhizobium fredii	IAM		Indole-3-acetamide hydrolase		Sekine et al. (1988)
Bradyrhizobium japonicum J1063	IAM	bam		IAM	Sekine et al. (1988) and Sekine et al. (1989)
Rhizobium sp. NGR234	IAM			IAM	Theunis et al. (2004)
Erwinia herbicola pv.gypsophilae	IAM	iaaM iaaH			Clark et al. (1993) and Manulis et al. (1998)
*Erwinia chrysanthem*i 3937	IAM	iaaM iaaH			Yang et al. (2007)
Ralstonia solanacearum	IAM	iaaM iaaH	Tryptophan monooxygenase		Salanoubat et al. (2002) and Kurosawa et al. (2009)
Rubrivivax benzoatilyticus JA2	IAM		Tryptophan monoxygenase	IAM	Mujahid et al. (2010)
Vibrio sp.	IAM			IAM	Gutierrez et al. (2009)
SStreptomyces exfoliatustreptomyces violaceus	IAM			IAM	Manulis et al. (1994)
Streptomyces En-1	IAM	iaaM iaaH		IAM	Lin and Xu (2013)

Table 1 continued

Bacterium	Pathway	Genes identified	Enzymatic activity	Intermediates identified	References
Streptomyces scabies	IAM	iaaM			Hsu (2010)
		iaaH			
Rubrivivax benzoatilyticus JA2	IAN			IAN	Mujahid et al. (2010)
Alcaligenes faecalis JM3	IAN		Indoleacetonitrilase		Nagasawa et al. (1990) and Kobayashi and Shimizu (1994)
Pseudomonas syringae B728a	IAN		Indoleacetonitrilase		Howden et al. (2009)
Pseudomonas sp. UW4	IAN	nit	Indoleacetonitrilase		Duca et al. (unpublished)
		nthAB	Nitrile hydratase		
Pseudomonas fluorescens EBC191	IAN		Indoleacetonitrilase		Kiziak et al. (2005)
Alcaligenes faecalis ZJUTB10	IAN		Phenylacetonitrilase		Liu et al. (2011)
Alcaligenes sp. ECU0401	IAN		Phenylacetonitrilase		Zhang et al. (2011)
Alcaligenes faecalis MTCC 10757	IAN		Indoleacetonitrilase		Nageshwar et al. (2011)
Bacillus subtilis ZJB-063	IAN		Phenylacetonitrilase		Zheng et al. (2008)
			Nitrile hydratase (PAN)		
			Amidase		
Bradyrhizobium japonicum USDA110	IAN		Phenylacetonitrilase		Zhu et al. (2007)
Burkholderia cenocepacia J2315	IAN		Indoleacetonitrilase		Wang et al. (2013)
			Phenylacetonitrilase		
Pseudomonas fluorescens DSM 7155	IAN		Phenylacetonitrilase		Layh et al. (1998)
Agrobacterium tumefaciens, *Rhizobium loti*, *Rhizobium leguminosarum*, *Rhizobium meliloti*	IAN/IAM		Nitrile hydratase and amidase		Kobayashi et al. (1995)
R. rhodochrous J1	IAN/IAM		Indoleacetamide/phenylacetamide Amidase		Kobayashi et al. (1993a, b)
			Nitrile hydratase		
Rhodococcus ruber CGMCC3090	IAN/IAM		Nitrile hydratase		Zhang et al. (2009)
Rhodococcus equi TG328-2	IAN/IAM		Nitrile hydratase (PAN)		Rzeznicka et al. (2010)
Rhodococcus AJ270	IAN/IAM		Nitrile hydratase (PAN)		Blakey et al. (1995)
Bacillus amyloliquefaciens FZB42	IAN	yhcX	Indoleacetonitrilase		Idris et al. (2007)
Bacillus sp. OxB-1	IAOX/IAN	oxd	Phenylacetaldoxime dehydratase and Phenylacetonitrilase		Asano and Kato (1998) Kato et al. (2000) and Kato et al. (2005)
Rhodococcus globerulus A-4	IAOX/IAN	oxdRG	Phenylacetaldoxime dehydratase and Nitrile hydratase		Xie et al. (2003) and Kato et al. (2005)
		Nha1			

Table 1 continued

Bacterium	Pathway	Genes identified	Enzymatic activity	Intermediates identified	References
Pseudomonas sp. K-9	IAOX/IAN/IAM	oxdK nha1	Phenylacetaldoxime dehydratase/Indoleacetaldoxime dehydratase and Nitrile hydratase		Kato and Asano (2006) and Kato et al. (2005)
Rhodococcus sp. YH3-3	IAOX/IAN/IAM	oxd	Indoleacetaldoxime dehydratase and Nitrile hydratase		Kato et al. (1999) and Kato et al. (2005)
Rhodococcus sp. N-771	IAOX/IAN/IAM	oxd nha1	Phenylacetaldoxime dehydratase/Indoleacetaldoxime dehydratase and Nitrile hydratase		Kato et al. (2004) and Kato et al. (2005)
Rhodococcus sp. TPU 3458 *Nocardia asteroides* IFO 3423 *Rhodococcus erythropolis* TPU 3201 *Nocardia asteroides* TPU 3036 *Rhodococcus* sp. TPU 3455 *Rhodococcus* erythropolis IAM 1400 *Rhodococcus erythropolis* IAM 1428 *Rhodococcus erythropolis* IAM 1440 *Pseudomonas* sp. TPU 7162	IAOX/IAN/IAM		Phenylacetaldoxime dehydratase and Nitrile hydratase		Kato et al. (2000)
Aureobacterium testaceum IAM 1561 *Rhodococcus erythropolis* IAM 1463	IAOX/IAN		Phenylacetaldoxime dehydratase and nitrilase		Kato et al. (2000)
Kocuria varinus IAM 12146 *Bacillus subtilis* ATCC 21697	IAOX/IAN		Phenylacetaldoxime dehydratase		Kato et al. (2000)
Rhodococcus erythropolis JCM 3201	IAOX/IAN/IAM	oxd nha1	Phenylacetaldoxime dehydratase and Nitrile hydratase		Kato et al. (2005)
Rhodococcus rhodochrous J-1	IAOX/IAN/IAM	nha1	Phenylacetaldoxime dehydratase and nitrile hydratase and Phenylacetonitrilase		Kato et al. (2005)
Rhodococcus sp. NCIBM 11215,	IAOX/IAN	oxd nha1	Phenylacetaldoxime dehydratase and Phenylacetonitrilase		Kato et al. (2005)
Rhodococcus sp. NCIMB 11216	IAOX/IAN	oxd	Phenylacetaldoxime dehydratase and Phenylacetonitrilase		Kato et al. (2005)
Rhodococcus sp. AK32		oxd	Phenylacetonitrilase		Kato et al. (2005)
Brevibacterium butanicum ATCC 21196	IAOX/IAN/IAM	oxd nha1	Phenylacetaldoxime dehydratase and Nitrile hydratase		Kato et al. (2005)

Table 1 continued

Bacterium	Pathway	Genes identified	Enzymatic activity	Intermediates identified	References
Rhodococcus erythropolis BG 13	IAOX/IAN/ IAM	oxd nha1	Phenylacetaldoxime dehydratase and Nitrile hydratase		Kato et al. (2005)
Rhodococcus erythropolis BG 16	IAOX/IAN/ IAM	oxd nha1	Phenylacetaldoxime dehydratase and Nitrile hydratase		Kato et al. (2005)
Corynebacterium sp. C5	IAOX/IAN	oxd	Phenylacetonitrilase		Kato et al. (2005)

The occurrence of each pathway is based on genes that have been identified in the bacterial genome, enzymatic activities that have been experimentally confirmed or intermediates of the respective pathway that have been identified in the bacterial culture filtrate/supernatant

Note that phenylacetoldoxime dehydratase, phenylacetonitrilase and nitrile hydratase activity on PAN was also included in the table. These enzymes were tested with phenyl substrates rather than indole. These enzymes appear to be involved in the production of PAA, an auxin-like compound with activity similar to indoleacetic acid (Somers et al., 2005). Both phenyl and indole substrates (PAOx/IAOX, PAN/IAN) are aryl compounds with the functional group attached to either a phenyl or indole aromatic ring. Therefore it is likely that enzymes acting on phenylacetaldoximes and phenylacetonitriles can convert the indole form as well, however, this remains to be confirmed experimentally

affinity for indolepyruvate; they can also act upon phenylpyruvate, pyruvate and benzoylformate. Thus, their main role may not be the production of IAA, although they can incidentally catalyze that step of the pathway (Patten et al. 2012; Schütz et al. 2003a, b; Phi et al. 2008).

The IPA pathway is common among non-pathogenic plant-associated bacteria and has also been suggested to exist in phytopathogens such as *Agrobacterium tumefaciens, Pseudomonas syringae* subsp. *savastanoi* and *Erwinia herbicola* pv. *gypsophilae* (Table 1) (Kaper and Veldstra 1958; Manulis et al. 1991; Brandl et al. 1996; Brandl and Lindow 1996). The presence of the IPA pathway in some of these phytopathogens was proposed based on the identification of the corresponding intermediates or based on the ability of the bacterial cells to metabolize the indole derivatives of this pathway (Kaper and Veldstra 1958; Manulis et al. 1991). However, it is necessary to identify IPA pathway genes and their respective enzymes in order to experimentally confirm their involvement in IAA biosynthesis (Patten et al. 2012).

Since IAA is the main mode of plant growth promotion by some bacteria, there is great interest in genetic manipulation of IAA biosynthesis to maximize phytostimulation. It is imperative that we understand the ecological impact of bacterial phytostimulators modified for IAA production in order to determine ideal usage conditions for these inoculants (Baudoin et al. 2010). Enhanced IAA production has been obtained in *Az. brasilense* Sp245 by introducing recombinant plasmid-based copies of the *ipdC* gene under the control of the constitutive promoter PnptII or the root exudate- responsive promoter PsbpA. The modified strains maintained high cell densities 1 month after sowing. More importantly, the recombinant plasmids were maintained in all inoculant cells and had no negative effect on the rhizosphere fitness of the bacteria. Winter wheat inoculated with the IAA-overproducing strains displayed enhanced shoot biomass. This is an exemplary demonstration that IAA-overproducing inoculants are ecologically functional as phytostimulators (Baudoin et al. 2010).

The indole-3-acetamide pathway

The indole-3-acetamide (IAM) pathway has been described mainly in phytopathogenic bacteria, although it does occur in phytosymbiotic bacteria as well (Kochar

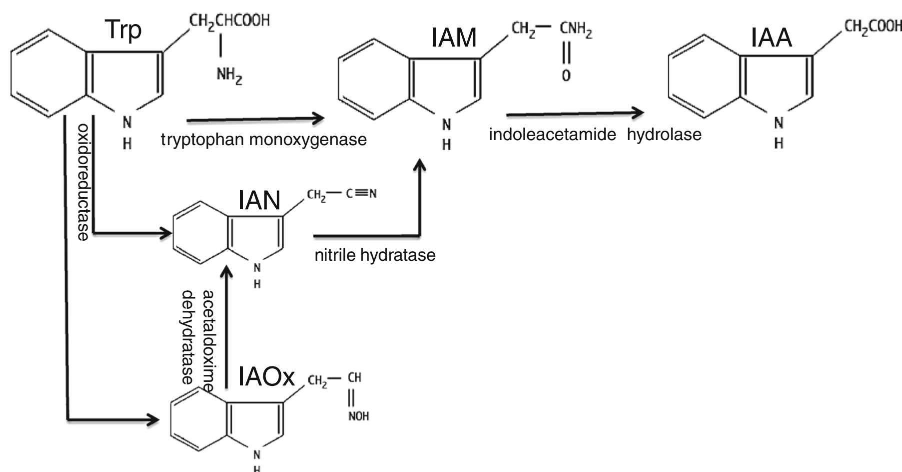

Fig. 2 The indole-3-acetamide pathway. IAM indole-3-acetamide, IAN indole-3-acetonitrile, IAA = indole-3-acetic acid, IAOx = indole-3-acetaldoxime. Figure adapted from (Prinsen et al. 1997)

et al. 2011). In this pathway, IAA is produced in a two-step reaction from a tryptophan precursor (Fig. 2). The first enzyme is a tryptophan 2-monooxygenase, which converts tryptophan to the IAM intermediate (Fig. 2). The second reaction is catalyzed by an IAM-specific hydrolase/amidase, which hydrolyzes IAM to IAA (Fig. 2) (Pollmann et al. 2006). The occurrence of this pathway in different bacteria is based on the chemical identification of the IAM intermediate, the production of IAA following feeding of the bacterial cells with IAM and/or the incorporation of radioactive carbon from tryptophan into IAM and IAA (Manulis et al. 1994).

The main genes driving the IAM pathway are *iaaM/tms-1* encoding tryptophan monooxygenase and *iaaH/tms-2* encoding indoleacetamide hydrolase/amidase enzymes (Comai and Kosuge 1982; Schröder et al. 1984; Thomashow et al. 1984). Lehmann et al. (2010) inferred the evolutionary relationships among amidase related proteins and observed that the annotated bacterial indole-3-acetamide hydrolases (IaaH) group together on the phylogenetic tree and underwent a functional diversification from more general amidases that convert substrates other than IAM. The *tms-2* encoded hydrolase from *Agrobacterium tumefaciens* has been demonstrated to convert a range of substrates besides IAM (Follin et al. 1985); this enzyme is also capable of hydrolyzing phenylacetamide and indole-3-acetonitrile to phenylacetic acid (PAA) and IAA, respectively (Kemper et al. 1985; Patten et al. 2012).

Patten et al. (2012) performed a phylogenetic analysis on the genes of the IAM pathway observing that homologues of the *iaaM* gene exist in few bacterial genera, and the sequences fall into two groups. Group I sequences are known to participate in IAA synthesis, such as those encoding tryptophan 2-monooxygenases in the plant pathogens *A. tumefaciens, P. syringae* pv. *savastanoi, P. agglomerans, and D. dadantii* (Comai and Kosuge 1982; Thomashow et al. 1984; Van Onckelen et al. 1986; Clark et al. 1993; Yang et al. 2007). Homologous *iaaM*-like sequences from *Burkholderia* spp., *Agrobacterium* spp. and a few other *P. syringae* strains also fall into this cluster. The functions of Group II sequences are not well characterized and their involvement in IAA biosynthesis remains to be determined experimentally but homologues exist within more diverse bacteria. It is not known whether tryptophan is a substrate of these group II gene products, however a representative sequence from *P. putida* KT2440 (DavB) has been shown to catalyze lysine degradation (Revelles et al. 2005; Patten et al. 2012). Genes encoding amidases that are homologues of indoleacetamide hydrolase occur in a large number of different bacterial genera. Among the sequences that cluster with known indoleacetamide hydrolases is a mandelamide hydrolase, which converts mandelate and also produces PAA (Gopalakrishna et al. 2004). Other amidases that are closely related to known bacterial indoleacetamide hydrolases include an aspartate/glutamate tRNA-dependent amidotransferase, 5-

aminovaleramide hydrolases and an arylpropionamide hydrolase (Patten et al. 2012).

The *Agrobacterium tms-1/tms-2* genes are not functional inside the bacteria, but are transferred into the plant cell where they are inserted into the plant chromosome and exert their pathogenic effect (Follin et al. 1985). Directly transforming these IAA biosynthesis genes into plant cells results in supra-optimal levels of IAA production, which in turn leads to uncontrollable tumour growth. Conversely the phytopathogenic *P. savastanoi iaaM* and *iaaH* genes are only functional inside the bacterium and are not directly transferred into plant cells. Therefore, the *P. savastanoi* induced plant tumours depend on the continuous production of IAA by the infecting bacteria (Follin et al. 1985). There is a correlation between the amount of IAA produced by *P. savastanoi* and virulence. IAA overproducing mutants that accumulated threefold more IAA than parental strains displayed increased virulence and larger galls on the host plant (Glass and Kosuge 1988). The *iaaM* and *iaaH* genes occur in an operon that is borne on the pIAA plasmid in oleander strains of *P. savastanoi*. Mutants cured of pIAA are less virulent and when the mutant strain is transformed with pIAA, it is restored to full virulence (Yamada et al. 1985).

The production of IAA by plant pathogens may have biological and ecological implications for the bacteria. Manulis et al. (1998) observed that inactivation of the IAM pathway in *E. herbicola* pv. *gypsophilae* reduced gall formation by about 40 %, whereas inactivation of the IPA pathway did not significantly effect gall formation. In contrast, when the IPA pathway was disrupted, the epiphytic bacterial population decreased 14-fold compared to the wild-type strain. No change in epiphytic fitness was observed when the IAM pathway was disrupted. These results suggest that the IAM pathway contributes less to colonization and more to pathogenicity, while the IPA pathway has a strong impact on bacterial fitness (Manulis et al. 1998).

Nitrile hydratase enzymes link the IAN and IAM pathways by catalyzing the hydration of indole-3-acetonitrile into IAM, which is then converted to the final product IAA by indoleacetamide hydrolase/amidase (Vega-Hernández et al. 2002). Nitrile hydratases have been reported in different bacteria; however, few studies have explored this enzyme's involvement in IAA biosynthesis (Patek et al. 2009; Coffey et al. 2010; Patten et al. 2012). Some of the earliest reports of this pathway come from Asano et al. (Asano et al. 1982a, b) who purified a nitrile hydratase and an amidase from *Arthrobacter* sp. strain *J-1* (Asano et al. 1982a, b). Nitrile hydratase enzymes can act on different nitrile substrates including aliphatic, aromatic and heterocyclic nitriles, converting them into the corresponding amides (Blakey et al. 1995; O'Mahony et al. 2005). Therefore, the role of these enzymes is not solely in IAA biosynthesis. Amidase genes are often located adjacent to nitrile hydratase genes as they convert the amide product of the nitrile hydratase to the corresponding carboxylic acid (Song et al. 2008). Genes encoding predicted amidases are found in the vicinity of nitrile hydratase genes in the genomes of *Pseudomonas* sp. UW4, *Bacillus* sp. BR449, *R. rhodochrous* J1, *Rhodococcus* sp. N-774, *Pseudomonas chlororaphis* B23, *Brevibacter* sp. 316, *R. jostii* RHA1, *A. radiobacter* K84, *B. japonicum* USDA 110 and *Ruegeria pomeroya* DSS-3 (Duan et al. 2012; Kim and Oriel 2000; Nishiyama et al. 1991; Mayaux et al. 1990; Patten et al. 2012). Nitrile hydratase and co-acting amidase enzyme activities have also been described in *R. rhodochrous* NCIMB 11216, *Rhodococcus equi* TG328, *A. tumefaciens* d3, *P. putida* IP08, *Microbacterium imperiale* CBS 498-74, *Nocardia globerula* NHB-2, *Bacillus subtilis* ZJB-063 and several *R. erythropolis* strains (Tauber et al. 2000; Gilligan et al. 1993; Stolz et al. 1998; Cantarella et al. 2006; Bhalla and Kumar 2005; Zheng et al. 2008; Brandão 2003). However the concerted action of these two enzymes specifically in the conversion of IAN to IAM and then to IAA has not been investigated. Xie et al. (2003) demonstrated a genetic relationship between three enzymes, an aldoxime dehydratase, nitrile hydratase and amidase in *R. globerulus* A-4. In this bacterium, the nitrile hydratase operon contains an aldoxime dehydratase gene upstream of the amidase and nitrile hydratase genes. The aldoxime dehydratase produces the nitrile substrate for the nitrile hydratase, while the nitrile hydratase produces the amide substrate for the amidase. A similar gene arrangement is observed for the nitrile hydratase gene cluster in *P.* sp. UW4 and in *Rhodococcus* sp. N-771 (Duan et al. 2012; Kato et al. 2004). The organization of these genes in a putative operon reflects their concerted action. Aldoxime dehydratase and nitrile hydratase activity has been reported in *Aureobacterium testaceum, Nocardia asteroids, Kocuria varinus, Bacillus subtilis,*

Rhodococcus erythropolis JCM 3201, *Brevibacterium butanicum* ATCC 21196, *Rhodococcus erythropolis* BG 13, BG 16 and *Pseudomonas* sp. K-9 (Kato et al. 2000, 2005). However this activity was tested with phenylacetaldoxime and phenylacetonitrile substrates.

Vega-Hernández et al. (2002) have shown that nitrile hydratase is responsible for the production of IAM in *Bradyrhizobium* cultures rather than tryptophan monooxygenase as in *P. savastanoi* and *A. tumefaciens*. Tryptophan monooxygenase activity was not detected in *Bradyrhizobium* strains, nor was the presence of the *iaaM* gene. Altogether these results suggest the absence of the tryptophan monooxygenase mediated IAM pathway and implicate the presence of the indole-3-acetonitrile pathway in which nitrile hydratase and indoleacetamide hydrolase work together to produce IAA (Vega-Hernández et al. 2002).

Kobayashi et al. (1995) also observed that IAA is produced by the sequential action of nitrile hydratase and amidase in *Agrobacterium tumefaciens* and *Rhizobium spp*.

The indole-acetaldoxime/indole-3-acetonitrile pathway

The IAOx/IAN pathway for IAA biosynthesis has not been as well studied in bacteria as it has in plants. The first step of this pathway is the conversion of tryptophan into indole-3-acetaldoxime (Fig. 3). The microbial enzyme responsible for this conversion is likely an oxidoreductase, however this enzyme has not been identified in bacteria thus far. This speculation is based on plant studies in which cytochrome p450 oxidoreductases catalyze this step (Hull et al. 2000; Mikkelsen et al. 2000).

In the second step, the indole-3-acetaldoxime intermediate is converted into indole-3-acetonitrile by an indoleacetaldoxime dehydratase (Fig. 3). Aldoxime dehydratase enzymes have been identified in several bacteria and are encoded by *oxd* genes (Table 1). However, most of their enzymatic activities have been characterized using phenylacetaldoxime as the substrate. Since phenylacetaldoxime and indoleacetaldoxime have a similar structure, it is possible that enzymes annotated as phenylacetaldoxime dehydratases can also convert the indole-type substrate. Experimental enzyme assays must be performed using both of these arylaldoxime substrates to reveal the substrate specificity of these enzymes. The indole-3-acetonitrile intermediate is consequently converted to IAA by a nitrilase enzyme in a single step (Fig. 3) or by a nitrile hydratase and an amidase in a two-step process (Fig. 2) (Zhao 2012).

In bacteria, nitrilase enzymes may have roles in hormone synthesis, nutrient assimilation and detoxification of exogenous and endogenous nitriles (Howden and Preston 2009). Nitrilase enzymes are part of a large superfamily with over 180 known members classified into 13 branches. Branch I enzymes are known to have true nitrilase activity, converting nitriles into their corresponding carboxylic acids and ammonia (Podar et al. 2005; Pace and Brenner 2001). Although this branch of nitrilase enzymes is implicated in IAA biosynthesis, there are few reports on bacterial nitrilases producing IAA. Numerous bacterial nitrilases have been purified and assayed in vitro for activity on various nitrile substrates, however only a minority have been specifically tested for their ability to convert IAN into IAA.

In some bacteria, nitrilase genes are found near acetaldoxime dehydratase genes and their enzymatic activities are linked. This was observed in *Rhodococcus* sp. NCIMB 11216, *Rhodococcus* sp. AK32 and *Corynebacterium* sp. C5, where the activities of both these enzymes were detected (Kato et al. 2005). The nitrilase gene from *Bacillus* sp. OxB-1 is upstream of the phenylacetaldoxime dehydratase gene, whose product converts phenylacetyldoxime and indoleacetaldoxime into phenylacetonitrile and indole-3-acetonitrile, respectively (Kato et al. 2000). Once the indole-3-acetonitrile intermediate is formed, nitrilase

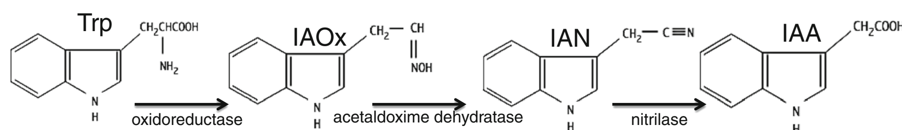

Fig. 3 The indole-3-acetonitrile/indole-3-acetaldoxime pathway. Trp tryptophan, IAOx indole-3-acetaldoxime, IAN indole-3-acetonitrile, IAA indole3-acetic acid (Patten and Glick 1996)

can convert it directly into IAA (Howden et al. 2009). The nitrilase regions of *Pseudomonas syringae* B728a and DC3000 also encode a putative phenylacetaldoxime dehydratase upstream of the nitrilase (Feil et al. 2005). In some instances both nitrilases and nitrile hydratases are associated with the acetaldoxime dehydratase. Both nitrile-hydrolyzing enzymes can act on the same substrate, which is provided by the acetaldoxime dehydratase, but perhaps each is induced under different conditions. All three of these enzymatic activities were detected in *Rhodococcus* sp. NCIBM 11215 and *Rhodococcus rhodochrous* J-1 (Kato et al. 2005). Genes that are involved in a common metabolic process are often clustered together; therefore, analyzing the genetic neighbourhood of nitrilase genes might reveal other IAA-biosynthesis genes involved in the same pathway (Podar et al. 2005; Howden et al. 2009).

Regulation of IAA

Environmental factors involved in IAA production

Determining the factors that control IAA biosynthesis in bacteria may enable a maximization of the beneficial effects of IAA-producing PGPB and a reduction of the virulence of IAA-producing phytopathogens. The IAA secreted by rhizospheric bacteria acts in conjunction with the plant's endogenous IAA supply. Thus, the impact of bacterial IAA on plants can either be positive or negative, depending on the level of IAA produced and secreted and the sensitivity of the plant tissue to IAA (Ali et al. 2010). For instance, the plant root is most sensitive to fluctuations in IAA, and its response to different IAA concentrations ranges from elongation of the primary root, formation of lateral and adventitious roots, to inhibition of growth (Davies 1995). For example, cucumber plants inoculated with a wild-type IAA-producing *Pseudomonas fluorescens* CHA0 displayed enhanced growth, whereas an IAA-overproducing mutant stunted cucumber growth (Beyeler et al. 1999). Plants have regulatory systems that maintain IAA homeostasis. However, high levels of bacterially-derived IAA may override the plant regulation of IAA and have detrimental effects. The phytopathogen *Pseudomonas syringae* pv. *tomato* DC3000 is able to induce the plant biosynthesis of IAA in *Arabidopsis* (Schmelz et al. 2003). Volatile organic compounds released by *Bacillus subtilis* were also observed to induce the expression of IAA biosynthetic enzymes in *Arabidopsis* (Zhang et al. 2007). Likewise, inoculating *Arabidopsis* with *Phyllobacterium brassicacearum* STM196 stimulated IAA biosynthesis pathways in the plant shoots (Contesto et al. 2010). Whether an IAA-producing bacterium will stimulate plant growth or pathogenesis may depend on the overall level of IAA within the plant. If the endogenous levels of IAA in the plant are suboptimal, the addition of bacterial IAA elevates the IAA concentration to plant growth stimulating levels. However, if plant endogenous IAA levels are already optimal, the additional bacterial IAA input can cause the IAA level to surpass the ideal threshold, resulting in pathogenesis and plant senescence (Pilet et al. 1979; Pilet and Saugy 1985; Taiz and Zeiger 1991; Patten and Glick 1996).

Plants must have an IAA regulatory system in place that maintains IAA at nontoxic physiologically appropriate levels (Sitbon et al. 1992). Converting free IAA to conjugated forms is part of this regulatory system. Inside the plant, free IAA can be inactivated through conjugation to sugars, amino acids, or peptides (Fig. 4) (Sitbon et al. 1992). Both ester and amide IAA conjugates have been identified in vivo, as well as after exogenous application of IAA (Sitbon et al. 1991). However it may be that high levels of IAA produced by bacteria such as *A. tumefaciens* exceed the capacity for IAA conjugation by the host plant, leading to the formation of tumours (Sitbon et al. 1992; Patten and Glick 1996). Perhaps some plants are better at regulating endogenous IAA levels than others. Endophytic bacteria such as *P. syringae* pv. *savastanoi* also produce deleteriously high concentrations of IAA. This bacterium colonizes the interior of the plant and secretes IAA directly to plant cells and induces tumorous growths (Smidt and Kosuge 1978; Comai and Kosuge 1980). Conversely, secretion of IAA by rhizobacteria exposes the plant to lower concentrations of IAA as the hormone may be degraded in the rhizosphere before it reaches the plant cell. The population size of the bacteria also reflects the amount of microbial IAA imposed upon the plant and therefore determines whether that bacterium promotes or inhibits plant growth (Loper and Schroth 1986; Morgenstern and Okon 1987; Harari et al. 1988).

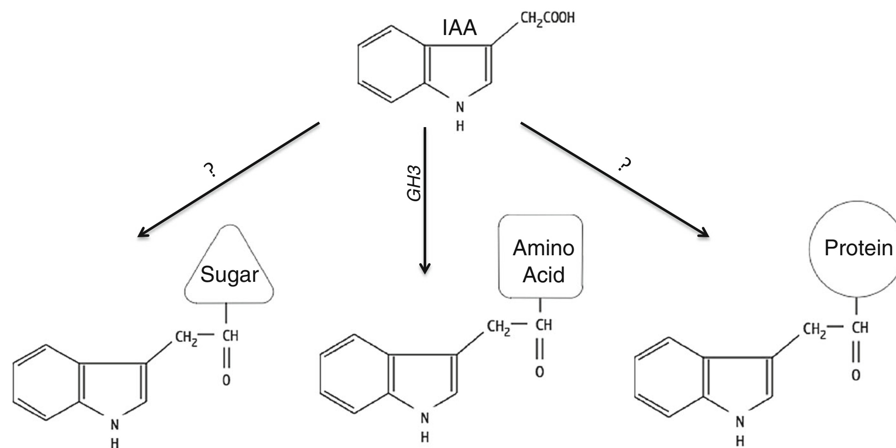

Fig. 4 IAA conjugated to sugars, amino acids and proteins. The GH3 gene family encodes amidosynthetases that conjugate IAA to amino acids

Production of IAA by microbial isolates varies among different species and strains of the same species and is influenced by culture conditions, growth stage, and substrate availability (Shokri and Emtiazi 2010). It appears that IAA biosynthesis is finely tuned to adapt to different environmental conditions and stresses that may be encountered by the bacteria in the environment (Spaepen et al. 2007). The presence of tryptophan, vitamins, salt, oxygen, pH, temperature, carbon source, nitrogen source and growth phase are all contributing factors in the regulation of IAA biosynthesis. Controlling IAA production will require a better understanding of how all these factors can be optimized (Apine and Jadhav 2011).

Plant extracts or specific compounds present in the rhizosphere or on plant surfaces are among the factors that influence bacterial IAA biosynthesis. Different microbes vary in their preferential carbon and nitrogen sources for IAA production (Table 2). Organic nitrogen sources have been observed to promote IAA production better than inorganic nitrogen sources (Narayana et al. 2009; Malhotra and Srivastava 2009). Perhaps this is due to increased availability of tryptophan from proteins. *Rhizobium* strains isolated from various host plants had different carbon and nitrogen source preferences. Some strains preferred mannitol, while others preferred glucose. Likewise, for the nitrogen source some strains preferred KNO_3 and $NaNO_3$ while others preferred glutamic acid (Table 2) (Sridevi et al. 2008). Perhaps the preferences of each bacterial strain are a reflection of what is most available to them in that specific plant environment and what they have adapted to. Shokri and Emtiazi (2010) report that the most important factor that impacts on IAA production is the nitrogen source. Omay et al. (1993) also observed that the IAA content in the supernatant of *Az. brasilense* Cd was more than 10 times higher in ammonium-containing nutrient media than in nitrogen-free medium (Omay et al. 1993). Similarly, Malhotra and Srivastava (2009) report that *Az. brasilense* SM had lower IAA levels in nitrogen-free conditions (Malhotra and Srivastava 2009). When bacteria are supplied with an exogenous nitrogen source, endogenous tryptophan reserves can be used towards the production of secondary metabolites such as IAA, rather than being prioritized for protein synthesis.

Tryptophan is a major effector of IAA biosynthesis, considering most IAA biosynthesis pathways begin with tryptophan (Apine and Jadhav 2011). Table 2 summarizes the optimal tryptophan concentrations for IAA production by different bacteria. The amount of IAA secreted by *Az. brasilense* increased by up to 100-fold when tryptophan was added to the medium (Baca et al. 1994). Likewise, Akbari et al. (2007) report that IAA production by different *Azospirillum* isolates increased when the culture medium was supplemented with L-tryptophan. Zimmer and Bothe (1988) observed that the addition of tryptophan to the medium resulted in 6–8 times more IAA production by *Az. brasilense* Sp7 than was produced by a culture grown without tryptophan

Table 2 Summary of the optimal factors and conditions for IAA production by different bacteria

Bacterium	[TRP] (mg/mL)	Growth phase	Carbon source	Nitrogen source	pH	Temp. (°C)	Other compounds	References
Rhizobium sp. NGR234							Flavonoids	Prinsen et al. (1991) and Theunis et al. (2004)
Xanthomonas axonopodis							Leaf extracts	Costacurta et al. (1998)
Pantoea agglomerans PVM	1	48 h	Sucrose	Meat extract	7	30		Apine and Jadhav (2011)
Klebsiella K8					8	37		
Rhizobium sp.	2.5-3	72 h	Varies	Varies		30		Sridevi et al. (2008) and Shokri and Emtiazi (2010)
Azospirillum brasilense SM.	1	15 days 30 days			6.2–6.8	37		Malhotra and Srivastava (2009)
Azospirillum lipoferum UAP 06, *Azospirillum brasilense* AZ 30 INTA, *Azospirillum brasilense* H18, *Azospirillum brasilense* UAP 14		96 h		KNO_3				Baca et al. (1994)
Pseudomonas putida UB1	0.2	96 h	Sucrose	$(NH_4)_2SO_4$	7.5			Bharucha and Kamlesh (2013)
Acetobacter diazotrophicus	1.2	6 days		NH_4Cl	6			Patil et al. (2011)
Klebsiella SN1.1	2-9	9–10 days				30		Chaiharn and Lumyong (2011)
Pseudomonas aeruginosa	0.5							Sasirekha and Shivakumar (2012)
Pseudomonas sp.	5		Glycerol	Yeast extract	7			Balaji et al. (2012)
Bacillus subtilis CM5		6 days			7			Swain and Ray (2008)
Pseudomonas fluorescence	0.5	72 h	Lactose	Peptone				Jeyanthi (2013)
Streptomyces atrovirens ASU14	5	>13 days			6	30		Abd-Alla et al. (2013)
Rhizobium sp.					6	37		Sudha et al. (2012)
Bacillus sp.					3	37		Sudha et al. (2012)
Streptomyces viridis CMU-HOO9	2	72 h			7	30		Khamna et al. (2010)
Pantoea rodasii	6	12 h				30		Walpola et al. (2013)
Streptomyces albidoflavus	5	96 h	Glucose	Yeast extract	7	35		Narayana et al. (2009)
Rhizobium sp.	2		Mannitol	KNO_3				Mazumder et al. (2010)

Table 2 continued

Bacterium	[TRP] (mg/mL)	Growth phase	Carbon source	Nitrogen source	pH	Temp. (°C)	Other compounds	References
Azospirillum brasilense Sp245	0.05	18 h						Ona et al. (2005)
Azospirillum brasilense sp7		4–5 days						Zimmer and Bothe (1988)
Azospirillum brasilense CD		72 h						Omay et al. (1993)
Pantoea agglomerans								
2-32	0.50	48 h		Trp				Sergeeva et al. (2007)
2-54	0.25	48 h						
3-117	0.25	48 h						
4-20	0.50	24 h						
5-51	0.50	24 h						
5-105	0.25	48 h						
Pseudomonas putida GR12-2	0.25	48 h		Trp				Sergeeva et al. (2007)
Bacillus pumilus ZED17	3	72 h	Glucose	KNO_3		30		Zaghian et al. (2012)
Rhizobium sp.	5	24 h	Sucrose	KNO_3			Nicotinic acid	De and Basu (1996)
Rhizobium sp.		16 h	Lactose	Glutamic acid			$NiCl_2$, Cetyl pyridinium chloride	Datta and Basu (2000)
Rhizobium sp.	2	80 h	Maltose				Triton-X-100, $MnSO_4$	Roy and Basu (1989) and Bhowmick and Basu (1987)
Rhizobium sp.	2	18 h						Ghosh and Basu (1997)
Bradyrhizobium japonicum	>4	>8 h						Hunter (1989)
Rhizobium sp.	1	72 h	Mannitol					Kumar and Ram (2012)
Azobacter sp. A1		2 days			7	30		El-Mahrouk and Belal (2007)
Pseudomonas fluorescens AK1	0.5							Karnwal (2009)
Pseudomonas aeruginosa AK2	0.5							Karnwal (2009)
Rhizobium sp. VMA301		26 h	Glucose	KNO_3	7.2			Mandal et al. (2007)
Klebsiella pneumoniae								
K8		72 h			8	37	0.5 % NaCl	Sachdev et al. (2009)
K11		72 h			8	37	0.5 % NaCl	
K17		72 h			7	28	1 % NaCl	
K23		72 h			8	37	1 % NaCl	
K38		48 h			8	37	1 % NaCl	
K42		48 h			8	37	0 % NaCl	

Table 2 continued

Bacterium	[TRP] (mg/mL)	Growth phase	Carbon source	Nitrogen source	pH	Temp. (°C)	Other compounds	References
Bacillus megaterium	1		Glucose	$NaNO_3$	8	30		Mohite (2013)
Lactobacillus casei	1		Mannitol	KNO_3	8	30		
Bacillus subtilis	1		Mannitol	Peptone	8	30		
Bacillus cereus	1.5		Glucose	Peptone	7	30		
Lactobacillus acidophilus	0.05		Glucose	$NaNO_3$	9	30		
Pseudomonas sp.	5							Ahmad et al. (2005)
Azobacter sp.	5							Ahmad et al. (2005)
Azospirillum brasilense		72 h						El-Khawas and Adachi (1999)
Klebsiella pneumoniae		36–48 h						El-Khawas and Adachi (1999)
Bacillus sp. MQH-19					6			Acuña et al. (2011)
Paenibacillus sp. SPT-03					5			Acuña et al. (2011)

supplementation (Zimmer and Bothe 1988). Patten and Glick (2002) report that *ipdC* promoter activity and IAA production in *P. putida* GR12-2 increased in response to exogenous tryptophan. Cells grown in the presence of L-tryptophan had fivefold higher *ipdC* promoter activity than those grown in medium without tryptophan (Patten and Glick 2002). Sergeeva et al. (2007) examined six legume root-associated *Pantoea agglomerans* isolates for their ability to produce IAA when supplemented with different tryptophan concentrations. It was observed that the ideal concentration of tryptophan needed for maximum IAA production differed between the strains (Sergeeva et al. 2007). For *Rhizobium* sp., there appears to be no linear correlation between the amount of tryptophan and IAA production. For some strains, higher concentrations (0.5 mg/ml) actually reduced IAA production (Shokri and Emtiazi 2010). Ahmed et al. (2010) isolated the cyanobacterium *Arthrospira platensis* MMG-9 from rice fields and observed that the amount of IAA produced gradually increased with increasing levels of tryptophan supplementation. This cyanobacterium was able to utilize supra high concentrations of tryptophan (1.5 mg/mL) which would normally cause a decrease in IAA production in other bacteria (Ahmed et al. 2010). Agricultural cyanobacteria are often overlooked with respect to IAA biosynthesis, but are certainly worth studying in the future due to their ability to produce much larger amounts of IAA ($>$0.25 mg/mL) than other plant-growth-promoting bacteria. Different bacteria require different amounts of exogenous tryptophan supplementation to obtain maximum IAA production. This likely corresponds to each bacterium's inherent ability to synthesize tryptophan endogenously. Normally, cellular tryptophan levels are 0.004 mg/mL (Patten et al. 2012). Bacteria that can produce more tryptophan rather than depending on exogenous sources have an advantage over other bacteria because they can produce IAA and stimulate plant growth even in a tryptophan-limited rhizosphere (Sergeeva et al. 2007).

Dimkpa et al. (2012) demonstrated that CuO and ZnO nanoparticles affect the amount of IAA produced in vitro by the soil isolate *Pseudomonas chlororaphis* O6. The levels of Cu and Zn from the nanoparticles used in this study were similar to levels found in contaminated soils. At 0.2 mg/mL of Cu, the CuO nanoparticles resulted in higher IAA levels in the culture medium than in control cultures. Conversely, the presence of ZnO

nanoparticles at 0.5 mg/mL of Zn, lowered the levels of IAA in the culture medium compared with controls. To test whether the CuO and ZnO nanoparticles impacted the utilization of tryptophan by *P. chlororaphis* O6, the residual tryptophan present in the cell-free culture medium was quantified by HPLC. The results indicated that more tryptophan was utilized by cultures amended with CuO nanoparticles than those amended with ZnO. A 23 % decrease in tryptophan levels was observed in the presence of CuO nanoparticles compared to the unamended control. The ZnO nanoparticles did not impact the amount of tryptophan utilized. Previous studies have reported that ZnO nanoparticles interact with tryptophan, limiting its natural fluorescence (Mandal et al. 2009; Joshi et al. 2012). In this study, quenching of tryptophan fluorescence by ZnO nanoparticles in the growth medium was also observed. Perhaps the interaction of tryptophan with the ZnO nanoparticle affects its bioavailability, limiting the amount of precursor available for IAA biosynthesis. Neither of the two nanoparticles had an effect on the expression of the IAA biosynthesis genes *iaaM* (tryptophan monooxygenase) and *iaaH* (indole-3-acetamide hydrolase). Bhattacharyya (2006) investigated the effect of Hg, Pb, Cd and Ba metals on IAA production by *Rhizobium* and observed that at 0.001 mg/mL, all metals had an inbibitory effect. Dimkpa et al. (2008) also observed that the presence of Fe, Al, Cd, Cu and Ni metals significantly reduced the levels of IAA produced by *Streptomyces tendae* F4 and *Streptomyces acidiscabies* E13, without affecting the growth of the bacteria. Similarly, Kamnev et al. (2005) observed that adding 0.01 mg/mL Cu or 0.02 mg/mL Cd in the culture medium of *Azospirillum brasilense* Sp245 and Sp7 resulted in significantly lower levels of IAA without affecting the bacterial growth rate. Therefore, heavy-metal-polluted soils may compromise the beneficial effects of IAA-producing plant symbionts (Dimkpa et al. 2012).

Acidic pH, osmotic and oxygen stress and temperature changes are environmental circumstances frequently encountered by bacteria. Each of these abiotic factors alters IAA biosynthesis; therefore it is important to determine the optimal conditions for each bacterium (Table 2). The optimum temperature for IAA production is not necessarily correlated with the optimum temperature for growth of that bacterium. For example, *Az. brasilense* SM grows best at 30 °C; however, maximum IAA production is observed at 37 °C (Narayana et al. 2009). The optimal temperature and pH for IAA production may be a reflection of the niche that the bacterium occupies, be it at various temperatures and slightly acidic conditions of the rhizosphere or at the physiological conditions inside a plant. Neutrophiles have internal pH values of 7.5–8.0 (Booth 1985) and mesophilic bacteria prefer temperatures between 20 and 40 °C (Prasad et al. 2009). The enzyme systems in these bacteria evolve to operate best within these temperature and pH ranges (Booth 1985). Therefore, the optimal temperature and pH for IAA production is likely a function of what is optimal for the enzymes that carry out IAA biosynthesis.

IAA-production is typically associated with a reduction in growth rate or the entry into the stationary growth phase. Table 2 summarizes the optimal growth phase for IAA production by different bacteria. The sigma factor RpoS (σ^{38}) is effective at the onset of the stationary phase of growth (Latifi et al. 1996). This sigma factor is referred to as a general stress regulator in many bacteria such as *P. aeruginosa, P. fluorescens* and *P. putida*. RpoS enhanced IAA production in *E. cloacae* and *P. putida* by up-regulating the expression of the *ipdC* gene (Saleh and Glick 2001; Patten and Glick 2002). Recombinant *P. putida* GR12-2 that constitutively produced RpoS also produced IAA earlier and continued to produce high levels compared with cells that produced natural levels of RpoS (Patten and Glick 2002). Some bacteria reach stationary phase within 24 h, while others take several days (Table 2). Beyond stationary phase, IAA levels begin to decrease perhaps due to the release of enzymes that degrade or conjugate IAA to amino acids (Shokri and Emtiazi 2010). Similar reports that a reduction in growth rate and entry into stationary phase is necessary for IAA biosynthesis have been made for several *Az. brasilense* strains and *Pantoea agglomerans* (Table 2) (Hartmann et al. 1983; Zimmer and Bothe 1988; Ona et al. 2005; Sergeeva et al. 2007). Likewise, Omay et al. (1993) observed that the IAA concentration produced by *Az. brasilense* Cd increased significantly in the stationary growth phase. During the first 24 h of growth (log phase), the number of *Az. brasilense* Cd cells increased exponentially, yet the IAA concentration remained consistently low (Omay et al. 1993). Malhotra and Srivastava (2009) investigated the ability of *Az.*

brasilense SM to produce IAA throughout its life cycle. The growth curve shows that the number of cells increased during the exponential phase and then gradually dropped and reached death phase by day 15. The level of IAA increased steadily until 15 days and then starting decreasing until 25 days. Following this death phase, a portion of viable cells remained alive up to 30 days and this signified the long-term stationary phase. The highest IAA levels were observed at 30 days when the cells were in long term stationary phase (Malhotra and Srivastava 2009). *Rubrivivax benzoatilyticus* JA2 is an anoxygenic photosynthetic bacterium isolated from the rhizosphere of a flooded rice paddy. Uniquely, this strain produced more IAA during the logarithmic growth phase than in stationary phase. IAA production was not affected by carbon exhaustion, oxygen availability or entry into the stationary phase. This distinguishes *R. benzoatilyticus* JA2 from other commonly studied IAA producers such as *Az. brasilense, E. cloacae and P. putida* (Mujahid et al. 2011).

Other factors that may affect IAA biosynthesis include salt and vitamins (Table 2). For example, very low levels of the B vitamins, especially pyridoxine and nicotinic acid increased production of IAA in *Az. brasilense* (Akbari et al. 2007).

Genetic regulation of IAA biosynthesis

There are still many unanswered questions pertaining to the biosynthesis of IAA. Among these questions are the molecular mechanisms that regulate IAA biosynthesis at the transcriptional and translational level in bacteria.

The bulk of the work on the regulation of IAA biosynthetic pathways is based solely on the IPA pathway. Although this pathway is common and perhaps predominant in some groups of bacteria, there are several alternative pathways that may be equally important in IAA production. Therefore, it is imperative that we develop an understanding of the regulation of all the IAA biosynthetic pathways (IAM, IAN-IAOx, TAM). This is especially important since multiple pathways co-exist within the same organism and may be differentially regulated.

The amount of IAA produced by a bacterium is subject to several means of regulation. The pathway(s) and the location of the IAA biosynthesis genes (on the chromosome or a plasmid) affect the amount of IAA produced by that microbe. Having IAA biosynthesis genes on a high copy number plasmid within the bacterial cell provides more genes to be transcribed as compared with those located on the chromosome (Brandl et al. 1996; Patten and Glick 1996). For example, the phytopathogen *P. savastanoi* pv. *savastanoi* has plasmid-borne IAA biosynthetic genes and produces more IAA than *P. syringae* pv. *syringae,* whose homologous genes are on the chromosome (Mazzola and White 1994; Spaepen et al. 2007). Introducing IAA biosynthesis genes on a low-copy number plasmid into the latter *Pseudomonas* strain increases the IAA levels dramatically (Mazzola and White 1994; Spaepen et al. 2007). Manulis et al. (1998) suggest that different IAA biosynthesis pathways have adapted to be expressed in different microenvironments such as on plant surfaces or inside plant tissues. This assertation came from a study on the plant pathogen, *E. herbicola* pv. *gypsophilae*. In this bacterium, the genes for the IAM pathway are located on the pPATH plasmid. These genes are highly transcribed inside the plant stems as opposed to leaf surfaces (Manulis et al. 1998). Another important factor is the level of expression of IAA genes in the rhizosphere, which is largely unknown.

The *P. savastanoi iaaM* and *iaaH* genes are naturally plasmid borne in strains isolated from oleander and chromosome borne in strains isolated from olive knots. In oleander gall isolates, IAA is further converted to the conjugated form, IAA–lysine (Fig. 5). The *iaaL* gene encoding IAA–lysine synthetase is responsible for this conversion and is located near the IAA operon on plasmid pIAA1 (Glass and Kosuge 1988). The amount of free IAA in cells of *P. savastanoi* is dependent upon the rate of IAA production and conversion to IAA–lysine. An *iaaL* mutant was constructed via transposon mutagenesis. This mutant had undetectable IAA–lysine synthetase activity, consequently accumulating five-fold more IAA in culture than the wildtype strain (Glass and Kosuge 1988). On the other hand, olive gall isolates do not convert IAA to IAA–lysine, naturally producing twice as much IAA as oleander isolates do. However, when the IAA–lysine synthetase gene (*iaaL*) was introduced into the olive gall isolate, IAA–lysine accumulated in culture, reducing IAA levels by 30 % (Glass and Kosuge 1988).

The presence of the *iaaL* gene within a bacterium's genome is presumed to regulate the amount of free

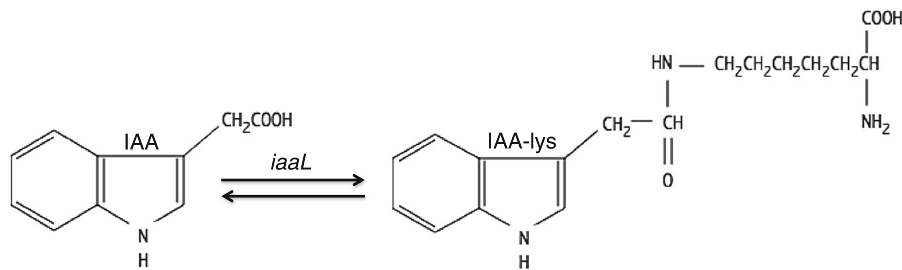

Fig. 5 The conversion of IAA to the IAA–lysine conjugate

IAA. Researchers screened 55 strains of *P. syringae* for the presence of the *iaa*L gene. Remarkably, 78 % of the assayed pseudomonads possessed sequences similar to the *iaaL* probe (Glickmann et al. 1998). Interestingly, the presence of *iaaL*-related sequences is not correlated with the presence of the *iaaM/iaaH* homologues in the assayed strains. While the *iaaL* gene appears to be common in *P. syringae* strains, other bacteria such as *E. herbicola*, *A. tumefaciens*, *A. faecalis*, *R. meliloti*, *P. fluorescens, P. putida, P. corrugata* and *P. stutzeri* did not harbor *iaaL*-related sequences within their genomes (Glickmann et al. 1998). Perhaps the *iaaL* gene was not detected in these latter strains due to a lack of sufficient complementarity with the *iaaL*-probe that was used.

Blumer et al. (1999) and Kitten et al. (1998) report a two component regulatory system composed of a GacS sensory kinase and a GacA response regulator, which may be involved in controlling the synthesis of IAA (Blumer et al. 1999). This system has been known to regulate an array of genes involved in the production of secondary metabolites (Kang et al. 2006). Saleh and Glick (2001) observed that when *En. cloacae* CAL2 and *P.* sp. UW4 were transformed with a plasmid harboring either *RpoS* or *GacS* genes from *P. fluorescens*, there was a 10- and 2-fold increase in IAA levels, respectively. This finding implies that both RpoS and GacS work together in the regulation of IAA synthesis. Whistler et al. (1998) report that the GacS system actually up-regulates RpoS, therefore perhaps the observed increase in IAA production is due to RpoS accumulation (Whistler et al. 1998). Kang et al. (2006) report the opposite effect, the GacS system down-regulated IAA synthesis in *P. chlororaphis* O6. A GacS mutant produced 10-fold more IAA than the wild-type when supplemented with tryptophan in stationary phase (Kang et al. 2006). The level of RpoS was not measured in this study, therefore, it is not known whether in this particular bacterium, GacS regulates IAA levels via RpoS or acts together with other regulators.

Wild-type cultures of *En. cloacae* did not produce detectable amounts of IAA in log-phase growth with tryptophan or in stationary phase in the absence of tryptophan. However, *ipdC* expression was still detectable under both conditions, albeit at lower levels than bacterial cells in stationary phase cultures supplemented with tryptophan (Ryu and Patten 2008). This indicates that *ipdC* is transcribed constitutively at a basal level but is inducible by tryptophan. In the rhizosphere, bacteria can obtain tryptophan from plant root exudates, thus to some extent, the host plant controls microbial IAA production by supplying this root-exudate inducer (Ryu and Patten 2008). However, this may not always be the case, for example the *ipdC* promoter of *E. herbicola* 299R is not influenced by exogenous tryptophan (Ona et al. 2005).

The end product IAA is also responsible for the induction of *ipdC* gene expression in a positive feedback manner (Vande Broek et al. 1999; Malhotra and Srivastava 2008). In *Az. brasilense* Sp245, exogenous IAA induced expression of a plasmid-borne *ipdC*::*gus* fusion. However, *Az. brasilense* Sp7 with a chromosomal *ipdC::lacZ* fusion showed no increase in expression upon addition of IAA. Differences in regulation of *ipdC* expression between the two strains may be due to regulatory elements in the promoter regions of the gene, which remain to be investigated. PAA a weak auxin with antimicrobial activity, similar plant growth promoting properties to IAA (Somers et al. 2005) and a structure similar to IAA can also induce *ipdC* gene expression in a concentration range similar to that of IAA.

Az. brasilense inhabits areas of low oxygen and uses O_2 as the terminal electron acceptor in aerobic or microaerobic conditions. Studies have reported that

the IPA pathway for IAA biosynthesis is enhanced under anaerobic conditions (Ona et al. 2005). When *Az. brasilense* was cultured under aerobic conditions, IAA production and *ipdC* gene expression were greatly reduced compared to microaerobic conditions, while the bacterial biomass remained relatively unaffected (Ona et al. 2005). Moreover, IAA biosynthesis and *ipdC* gene expression were impeded during exponential growth in fed-batch cultures when the malate carbon source was plentiful. Conversely, in batch cultures the carbon source was rapidly depleted and consequently these had the smallest bacterial cell concentration, yet still produced the most IAA and had the highest *ipdC* expression (Ona et al. 2005). These results suggest that carbon limitation and a reduction in growth rate promote *ipdC* gene expression in *Az. brasilense*. These conditions are characteristic of the stationary phase of growth (Ona et al. 2005). The inability of *Azospirillum* to produce IAA when carbon is abundant may explain its failure to promote plant growth in rich or highly fertilized soils (Ona et al. 2005).

In order to understand the regulation of IAA biosynthesis genes at the transcriptional level, the promoters and regulatory sequences of these genes must be investigated. Ryu and Patten (2008) discovered that the promoter region of the *ipdC* gene of *En. cloacae* UW5 contained a sequence (TGTAAA-N_6-TTTACA) that is highly similar to the TyrR recognition sequence of *E. coli*. The *tyrR* gene encodes a regulatory protein that is both an activator and a repressor of genes involved in aromatic amino acid transport and metabolism. To test whether TyrR regulates IAA biosynthesis, an *En. cloacae tyrR* insertion mutant was constructed. In contrast to the wild-type strain, the *tyrR* mutant did not produce detectable IAA in the presence of tryptophan in the stationary phase. Similarly, an *ipdC* mutant also could not synthesize IAA under these conditions. These results indicate that TyrR and IPDC are required for IAA production. Real-time qRT-PCR revealed that *ipdC* transcript levels in the *tyrR* mutant were very low in the stationary phase even in tryptophan-supplemented cultures. Electrophoretic mobility shift assays confirmed that TyrR binds to the predicted TyrR box in the *ipdC* promoter region and induces *ipdC* expression (Ryu and Patten 2008).

The 5′ upstream region of the *ipdC* gene of *Az. brasilense* SM, Sp45 and Sp7 has been analyzed to determine regulatory elements in the *ipdC* promoter. A cis element similar to the auxin responsive elements in plants (TGTCNC) was identified in the promoters of the IAA-induced genes and was completely conserved between the three *Az. brasilense* sequences analyzed (Vande Broek et al. 2005; Malhotra and Srivastava 2009). Vande Broek et al. (2005) produced progressive deletions of the 5′ flanking region of the *Az. brasilense ipdC* to determine the cis-acting elements required for promoter activation and for the IAA-mediated upregulation of *ipdC*. Their results indicated that this promoter region extends to position −81 relative to the *ipdC* transcription initiation site. Within this upstream region, located between positions −2 and −23 is the plant-like TGTCNC element, which is essential for *ipdC* expression, as point mutations within this region completely abolished expression (Lambrecht et al. 2000). Perhaps the −10 RNA polymerase binding site falls within this region and mutations affect polymerase binding. Between positions −58 and −38 lies a two 8-bp inverted repeat separated by a 4-bp spacer. Site-directed mutagenesis of this dyadic sequence as well as deletion mutations demonstrated that this element is also required for full activation of the *ipdC* promoter and for its IAA inducibility (Vande Broek et al. 2005).

Vande Broek et al. (2005) also identified an open reading frame immediately (99 bp) downstream of the *Az. brasilense ipdC* that is co-transcribed in an operon with *ipdC* and appears to reduce IAA production. This open reading frame is known as *iaaC*. There is no bacterial promoter sequence or transcriptional regulatory element in the short intergenic region between *ipdC* and *iaaC*. Moreover, a putative mRNA stem-loop structure that may function as a transcription terminator was found in the region downstream of *iaaC*. Vande Broek et al. (2005) then analyzed the role of *iaaC* in *ipdC* transcription by transferring an *ipdC-gusA* fusion plasmid into an *iaaC* insertion mutant. GusA activity was assayed when these cells were grown under different conditions, including stationary phase IAA-induced and uninduced. The growth rates and biomass of the *iaaC* mutant were similar to those of the parent strain Sp245 under all the conditions tested. Furthermore, there was no difference in *ipdC* expression in the parent strain Sp245 and the *iaaC* insertion mutant. However, the IAA levels in the

iaaC insertion mutant were dramatically increased. IAA concentrations were up to sixfold higher in the *iaaC* mutant in the stationary growth phase as compared to the parent strain Sp245. These findings suggest that the protein encoded by *iaaC* inhibits IAA production but not by repressing *ipdC* expression (Vande Broek et al. 2005).

Malhotra and Srivastava (2008) compared levels of IAA produced by *Az. brasilense* Sp245, SM and Sp7. Their findings revealed that Sp245 with *iaaC* produces 40 % less IAA than strains Sp7 and SM which both lack *iaaC*. Since *A. brasilense* SM lacks *iaaC*, the effect of introducing this gene into strain SM from strain Sp245 was investigated. Heterologous over-expression of *iaaC* in strain SM resulted in a ~50 % decrease in IAA. This finding reinforces earlier speculations that the *iaaC* gene encodes a regulatory protein that down-regulates IAA biosynthesis, and in *Az. brasilense* Sp245 IAA biosynthesis is more strictly controlled than in strains Sp7 and SM (Malhotra and Srivastava 2008).

An AraC-type transcriptional regulator (*nitR*) has been identified downstream of the nitrilase gene of *Rhodococcus rhodochrous* J1 and upstream of the nitrilase genes of *Bacillus* sp. OxB-1, *P. syringae* B728a and DC3000. The *Rhodococcus nitR* gene is essential for nitrilase expression and may provide insight into the regulation of the IAN pathway (Komeda et al. 1996a, b, c; Kato et al. 2000). In *P.* sp. UW4, a predicted LysR transcriptional regulator is located upstream of the nitrilase gene that is involved in IAA biosynthesis and a predicted AraC transcriptional regulator is directly upstream of a gene cluster containing amidase, aldoxime dehydratase and nitrile hydratase (Duan et al. 2012). This nitrile hydratase can convert IAN into IAM and is likely involved in IAA biosynthesis (Duca et al. unpublished results). It is possible that these transcriptional regulators regulate this IAN/IAOx pathway by controlling the expression of these IAA-enzymes.

IAA production by Gram-positive bacteria

The ability to synthesize IAA is a common trait of both plant-pathogens and plant growth-promoters and is not restricted to Gram-negative bacteria (Vandeputte et al. 2005). Gram-positive bacteria are established IAA producers, however the biosynthetic pathways utilized by these bacteria have been less extensively investigated. The benefit of utilizing IAA-producing Gram-positive spore forming bacteria as agricultural inoculants comes from the capacity of their spores to survive in soil for long periods of time and under adverse environmental conditions. One study isolated sixteen *Bacillus* strains from the rhizosphere, histoplane and phyllosphere of various plant species and evaluated them for in vitro IAA production. The rhizospheric isolates produced more IAA than histoplane and phyllosphere isolates (Ali et al. 2009).

A study by Ahmed and Hasnain (2010) looked at the growth promotion bestowed upon plants inoculated with two Gram-positive *Bacillus* strains; *B. flexus* P4 and *B.* sp. S6. These strains were observed to produce 59 and 86 μg/mL of IAA, respectively (Ahmed and Hasnain 2010). *B. flexus* P4 increased shoot length by 40 % while *B.* S6 increased it by 35 %. Furthermore, root length increased by 40 and 50 %, respectively, compared to non-inoculated treatments. Both isolates increased the number of leaves on the plant. Inoculation of plants with these strains increased the overall IAA content of the plants up to 71 % and inoculation of plant sprouts enhanced the number of roots by 100 and 130 %, respectively (Ahmed and Hasnain 2010). Such experiments demonstrate that IAA-producing Gram-positive bacteria can be used as effective plant growth promoting inoculants. However, to prove that IAA is the mechanism behind the stimulatory effect on plant growth, mutants deficient in IAA biosynthesis should be utilized.

Bacillus amyloliquefaciens FZB42 is another Gram-positive plant growth-promoting rhizobacterium with the ability to produce IAA. When the bacterial culture filtrate was applied to duckweed plants, there was an increase in fresh weight compared with the control plants. Plant growth promotion was even more pronounced when bacterial cells were directly applied to the plant at the appropriate concentration. Exceeding this concentration of cells resulted in a significant reduction of plant fresh weight (Idris et al. 2007). Three candidate genes with homology to genes previously reported to be involved in IAA biosynthesis were identified and disrupted to assess their effect on IAA levels. Of the three mutants, only two displayed significantly reduced amounts of IAA in culture filtrates. The strain carrying a mutation in the putative IAA acetyltransferase gene produced only 28 % of the amount of IAA produced by the wild type. While the

strain with a mutation in the putative nitrilase gene produced 50 % of the amount of the wild type. Direct application of these mutants resulted in only a slight increase of plant fresh weight compared with the untreated control, while their culture filtrates did not result in any plant growth promotion (Idris et al. 2007).

Rhodococcus fascians is a Gram-positive phytopathogen that inhibits plant growth, causes deformations of leaves and forms galls on its host plants. Vandeputte et al. (2005) investigated the role of IAA in the *R. fascians*-plant interaction. Higher levels of IAA were detected in infected plant tissues compared to non-infected tissues. The addition of exogenous tryptophan to the minimal growth medium did not significantly impact IAA production. However, IAA secretion was highly induced upon the addition of infected-plant extracts and tryptophan simultaneously. Compounds produced during the formation of the leafy gall stimulate the IAA biosynthetic pathways in *R. fascians*. This leads to an excessive amount of IAA, i.e. 10 times the concentration found in non-infected plant tissues (Vandeputte et al. 2005). Interestingly, the progressive increase in IAA levels coincided with a decrease in indole-3-ethanol; substrate-feeding assays demonstrated that *R. fascians* could use indole-3-ethanol as a precursor to synthesize IAA (Vandeputte et al. 2005). The conversion of indole-3-ethanol into IAA has also been reported in *Streptomyces* spp. and *Rhizobium lupini* (Manulis et al. 1994; Frankenberger and Arshad 1995). This side step in the IPA pathway is speculated to be a regulatory reaction for IAA biosynthesis in bacteria (Fig. 6) (Vandeputte et al. 2005). Narumiya et al (1979) identified indole-3-ethanol as a metabolite of L-tryptophan in *P. fluorescens* ATCC29574 in late-stationary phase. These researchers observed that when IAAld was added to cells, it was metabolized to IAA and indole-3-ethanol (Fig. 6) (Narumiya et al. 1979). Spaepen et al. (2007) suggest that indole-3-ethanol and indole-3-lactic are storage compounds formed by the reduction of intermediates of IAA biosynthesis such as IPA and IAAld, respectively (Fig. 6). It has been suggested that these storage compounds may be converted into IAA when needed by the bacterium (Spaepen et al. 2007).

Abd-Alla et al. (2013) screened 210 Gram-positive actinomycetes isolates from 100 different rhizospheric soil samples for their ability to produce IAA. More than half (65 %) of those isolates produced this phytohormone. However, the study focused on 12 particular strains of *Streptomyces* isolated from rhizospheric soil of wheat and corn plants in Egypt. These strains produced the highest levels of IAA (3.5–22.5 μg/mL). Khamna et al. (2010) also investigated 270 *Streptomyces* isolates from 14 different medicinal plant rhizospheres and observed that only 11 % of the isolates were able to produce between 11 and 144 μg/mL of IAA. Different plants secrete different root exudates, which in turn promote the growth and IAA-producing activity of some bacterial species better than others (Frankenberger and Arshad 1995). Khamna et al. (2010) focused on one particularly effective *Streptomyces* sp. (i.e. strain CMUH009) isolated from a Thai medicinal plant rhizosphere. The culture filtrates of *Streptomyces* CMUH009 promoted maize seed germination by 20 % compared to control seeds treated with sterile water. Moreover, the maize root length increased from 50 mm (control) to 185 mm. Other IAA-producing species such as *S. olivaceoviridis, S. rimosus*, *S. rochei* and *Streptomyces* spp. from the tomato rhizosphere have been reported to improve seed germination, root elongation and root dry weight (Aldesuquy et al. 1998; Tokala et al. 2002; El-Tarabily 2008). These *Streptomyces* can serve as Gram positive microbial inoculants used to facilitate plant growth.

Degradation of IAA by bacteria

Many bacteria have the ability to produce IAA and some can also actively degrade it (Mino 1970; Claus and Kutzner 1983; Gieg et al. 1996; Jarabo-Lorenzo et al. 1998; Leveau and Lindow 2005; Leveau and Gerards 2008; Faure et al. 2009; Scott et al. 2013; Zúñiga et al. 2013). IAA is an ideal food source for bacteria as it provides both the carbon and nitrogen needed to survive. Being able to degrade IAA may give some bacteria a selective advantage in IAA-rich environments. It may also be that IAA is degraded because it can be toxic to bacterial cells. It is a weak acid, which becomes deprotonated in the neutral cytoplasm and may inhibit bacterial growth by acidifying the cytoplasm (Tromas and Perrot-Rechenmann 2010). Microbial IAA-degraders can also use this feature to manipulate plant physiology in a way that benefits their survival or colonization (Scott et al. 2013). Zúñiga et al. (2013) report that

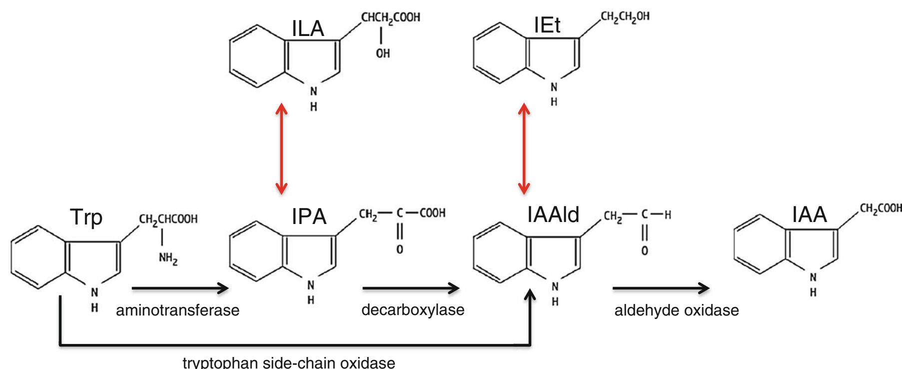

Fig. 6 Proposed regulatory/storage compounds of the IPA pathway. ILA indole-3-lactic, IEt indole-3-ethanol

degradation of IAA by *Burkholderia phytofirmans* plays a key role in plant-growth-promotion and is necessary for efficient rhizosphere colonization. They observed that wild-type IAA-degrading *B. phytofirmans* promotes the growth of *A. thaliana roots* in the presence of exogenously added IAA, however a mutation in the IAA-degradative gene *iacC* abolishes this growth promotion effect (Zúñiga et al. 2013).

There are several different pathways for bacterial transformation of IAA. Decarboxylation of IAA to skatole has been reported for *Lactobacillus* sp. (Yokoyama and Carlson 1981; Deslandes et al. 2001). A *Pseudomonas* species from soil was observed to further convert skatole to catechol (Proctor 1958). Catechol is also a confirmed intermediate in the transformation of IAA by *Pseudomonas putida* 1290, *Pseudomonas* sp. LD2 and *Arthrobacter* sp. (Leveau and Lindow 2005; Gieg et al. 1996; Mino 1970). *Bradyrhizobium japonicum* metabolizes IAA via dioxindole-3-acetic acid, dioxindole, isatin, isatinic acid and anthranilic acid (Jensen et al. 1995) while *Alcaligenes* sp. does this via isatin, anthranilic acid and gentisate (Claus and Kutzner 1983). *Rhizobium phaseoli* transforms IAA into indole-3-methanol (Ernstsen et al.1987) and *Azoarcus evansii* metabolizes IAA to 2-aminobenzoyl-coenzyme A (CoA) or benzoyl-CoA derived from the pyrrole ring and the side chain (Ebenau-Jehle et al. 2012).

Pseudomonas putida 1290 is a model strain for the identification and characterization of IAA-degradative genes. A DNA fragment consisting of ten *iac* genes, mediates IAA degradation in this bacterium. Homologs of the *iac* genes have been identified in the genomes of α-, β-, and γ-Proteobacteria (Leveau and Gerards 2008). *P. putida* GB-1, *Marinomonas* sp. MWYL1, *Burkholderia* sp. 383, *Sphingomonas wittichii* RW1, *Rhodococcus* sp. RHA and *Acinetobacter baumannii* ATCC 19606 have been experimentally confirmed to have the ability to grow solely on IAA (Leveau and Gerards 2008; Lin et al. 2012). However, the role of each individual *iac* gene in the IAA degradation pathway remains to be determined experimentally. Collectively, the *iac* genes in *P. putida* 1290 are presumed to carry out the conversion of IAA to the final product catechol. The *iacA, iacB, iacC, iacD, iacE, iacF* and *iacG* genes occur in one operon, *iacH* and *iacI* in another and *iacR* as a single gene. The same gene arrangement is observed in *P. putida* GB-1 (Leveau and Gerards 2008). The *iacA* gene product is proposed to mediate the conversion of IAA into 2-OH-IAA/OxIAA, which can dimerize to form the blue pigment indigo (Alemayehu et al. 2004). Orthologs from *Pseudomonas alcaligenes* PA-10 (*idoA*) and *Acinetobacter baumannii* ATCC 19606 (*iacA*) have similar activity (Alemayehu et al. 2004; Lin et al. 2012). Recombinant *E. coli* that was transformed with vectors carrying functional *P. putida* 1290 *iacAB* and disrupted *iacR*, developed a blue color on nutrient agar. When the disrupted *iacR* construct was introduced into a low-indole-producing *E. coli* strain, the blue colour did not develop, but could be restored by adding exogenous indole. On the other hand, an *E. coli* variant that overproduced indole, developed the blue colour when carrying the disrupted *iacR* construct. These results suggest that the blue color (indigo) is produced from indole by an *iacAB*-encoded activity and that this activity is repressed by *iacR* (Scott et al. 2013). Earlier, Leveau and Gerards (2008) observed four library clones of the *iac*-gene cluster that produced a blue color and the common denominator between the four clones was that they all contained the *iacA* gene insert. Therefore, it appears that only the *iacA*

gene product is responsible for pigment formation and that *iacB* is not needed (Scott et al. 2013). No specific function has been predicted for *iacB* and *iacI*, however *iacI* is essential for IAA degradation. Also, a transposon insertion into the *iacH* gene abolished IAA degradation by *P. putida* 1290 (Leveau and Gerards 2008). This ability could be restored only by complementation with both *iacH* and *iacI* genes (Leveau and Gerards 2008).

The *iac* gene expression appears to be inducible. In *A. baumannii* ATCC 19606, the IacA protein was detected in cells cultured on IAA but not in cells cultured on pyruvate (Lin et al. 2012). *P. putida* 1290 grown solely on IAA, had higher levels of catechol and 1,2-dioxygenase activity which is encoded by the *catA* gene of the *cat* operon (Leveau and Lindow 2005). The *catBCA* gene cluster is responsible for the transformation of catechol (the product of the *iac* gene cluster) to 3-oxoadipate enollactone (Harwood and Parales 1996). Disrupting the *catBCA* operon results in the accumulation of catechol. Perhaps the *iac* genes and downstream *cat* genes are regulated together. IAA stimulates *iac* genes, which results in more catechol production, which in turn stimulates the cat operon to transform the catechol. Therefore, catechol can be maintained at appropriate levels. *IacR* is not in an operon and is predicted to encode a MarR-type transcriptional regulator (Leveau and Gerards 2008). These types of regulators have been reported to be repressors of the degradative operons for aminophenol and of the operon for 3- and 4-hydroxy- and 3,4-dihydroxyphenylacetic acid in *E. coli* (Park and Kim 2001; Roper et al. 1993; Galán et al. 2003). Another MarR-type regulator represses the operon for 3-chlorobenzoate degradation in *Comamonas testosteroni* BR60 (Providenti and Wyndham 2001). On the other hand, some MarR-type regulators act as inducers (Egland and Harwood 1999). Therefore IacR may serve as a transcriptional regulator, repressing or inducing the IAA-degradative operon. Scott et al. (2013) also demonstrated the inducibility of the *P. putida* 1290 *iac* gene expression in response to IAA by quantitative PCR analysis. When this bacterium was grown solely on IAA, the expression of *iacA* and *iacC* was twofold higher than when it was grown with glucose. Elevated expression following growth on IAA was also observed for *iacH* and *iacR,* although not to the same extent. Egebo et al. (1991) have demonstrated that IAA and 5´OH-IAA both induce IAA degradative activity in *Bradyrhizobium japonicum*. Likewise, Ebenau-Jehle et al. (2012) observed that only IAA-grown cells of *Azoarcus evansii* were actively degrading IAA, suggesting that the enzymes of the degradative pathway were induced by IAA.

Scott et al. (2013) assessed the individual role of the *P. putida* 1290 *iac* genes in the degradation of IAA by transforming non-IAA-degrading *P. putida* KT2440 or *E. coli* with vectors carrying functional or disrupted *iac* genes and testing the resulting phenotypes. Recombinant *E. coli* carrying a vector with the complete *iac* gene cluster was able to degrade IAA when grown on glucose plus IAA, but not solely on IAA. This ability was abolished when *iacA* was disrupted but not any one of the other *iac* genes. These results suggest that the *iacA* gene is responsible for conferring IAA-degrading ability, likely mediating the first step in the IAA degradation pathway (IAA → 2-OH-IAA). The compound 5-OH-IAA was observed in the supernatants of *E. coli* carrying disrupted *iacE* or functional *iacAB*. The *iacAB* gene products are proposed to hydroxylate the indole ring of IAA yielding either 3-oxindole with release of the acetic acid side chain, or 2-OH-IAA, a tautomer of 5-OH-IAA. *E. coli* carrying the complete *iac* cluster grown on glucose and IAA, developed a brown colour, assumed to be the accumulation of catechol. This was also evident for *E. coli* strains carrying disrupted *iacF, iacG*, and *iacR*. The supernatants of *E. coli* cells carrying disrupted *iacC* or the full *iac* cluster revealed a compound putatively identified as dioxindole-3-acetic acid (diOxIAA). This compound could be the result of hydroxylation of 2-OH-IAA at position 3 of the indole ring. Perhaps diOxIAA is the substrate for the *iacC* gene product and inactivating this gene leads to accumulation of this substrate. The conversion of diOxIAA to catechol involves multiple steps that require the products of *iacC, iacF* and *iacG*.

It is important to note that not all microbial IAA-degraders contain *iac*-like genes. For example, *B. japonicum* USDA 110 contains an *iacA* homolog but lacks the rest of the *iac* genes (Jarabo-Lorenzo et al. 1998). Therefore, this strain must posses other genes that mediate IAA degradation (Leveau and Gerards 2008). The denitrifying betaproteobacterium *A. evansii* contains a gene cluster comprised of 14 genes arranged in a predicted operon. The first gene (*iaaR*) in this operon codes for a GntR transcriptional regulator, which may be responsible for the IAA-induced expression of

the degradative genes. The next seven genes are putatively involved in IAA degrading activity and consist of a beta-oxoacyl-CoA thiolase (*iaaA*), a CoA ligase acting on aromatic acids (*iaaB*), a hydantoinor 5-oxoproline hydrolase (*iaaCE*), an acyl-CoA dehydrogenase (*iaaF*), a coenzyme B12-dependent mutase (*iaaGH*), a molybdenum enzyme of the xanthine dehydrogenase family (*iaaIJK*), a CoA transferase of family III (*iaaL*) and a periplasmic binding protein for an ABC transporter system (*iaaM*). The *iaaD* gene does not have an annoted function but contains a 3' end highly similar to that of the *iaaC* gene found in *P. putida* 1290. In addition to the *iaaRABCDEFGHIJKLM* operon, two genes coding for a TetR transcriptional regulator (*iaaQ*), and a fusion protein of an L-specific enoyl-CoA hydratase and a 3-hydroxyacyl-CoA dehydrogenase (*iaaP*) are also suspected to be involved in IAA metabolism in this bacterium. Protein databases were queried for homologs of the *A. evansii iaa*-genes and gene clusters coding for the key enzymes of the degradative pathway were identified in the betaproteobacterium *A. aromaticum*, the alphaproteobacterium *Rhodopseudomonas palustris* BisA3, the deltaproteobacterial strain NaphS2 and the euryarchaeum *Ferroglobus placidus* (Ebenau-Jehle et al. 2012).

Egebo et al. (1991) observed that the metabolism of IAA by *Bradyrhizobium japonicum* is oxygen dependent. When IAA-degrading *Bradyrhizobium japonicum* cultures were induced by exogenous IAA and incubated either in a normal atmosphere or in an N_2 atmosphere, IAA was degraded only from oxygenated culture. In contrast, Ebenau-Jehle et al. (2012) report the anaerobic metabolism of IAA in the denitrifying beta-proteobacterium *Azoarcus evansii*. The transformation of IAA did not require molecular oxygen but required electron acceptors like NAD^+ or artificial dyes. A similar bacterium, *Aromatoleum aromaticum* EbN1 also demonstrated highly similar IAA metabolic capacities with IAA as the sole substrate under anaerobic conditions.

The role of IAA in bacteria

Overcoming stress

Besides mediating plant-microbe interaction, what role does IAA play within the bacteria that produce it? Most profoundly, IAA promotes better bacterial adaptation to stress conditions leading to improved survival and persistence in the plant environment (Bianco et al. 2006). The majority of bacteria cultured from plants are IAA-producers. Therefore, from an evolutionary perspective, having the ability to produce IAA must give the bacteria a selective advantage in that environment (Kim et al. 2011a, b).

Recent studies have shown that IAA confers protection against biotic stresses such as UV, salt and acidity. Wild-type *Sinorhizobium meliloti* 1021 and its IAA overproducing mutant (RD64) were used to inoculate *Medicago truncantula* plants (Bianco et al. 2009). IAA production was increased 78-fold in RD64 cells by introducing the IAM pathway on a plasmid. Various stress conditions such as acidity, osmotic shock, UV-irradiation and heat shock were then simulated. Viable bacterial cell counts were higher in plants treated with exogenous IAA or with the IAA-over producing strain (RD64) compared with the wild-type strain under all of the stress conditions tested. The RD64 mutant had 40 % more viable cells when incubated under osmotic shock conditions in 0.5 M NaCl for 3 h. It also had better survival rates than wild-type *S. meliloti* 1021 when exposed to UV irradiation with a germicidal lamp (254 nm) at 100 J/m^2 and heat shock at 55 °C for 10 min (Bianco et al. 2009)

Another study investigated the effects of IAA on the central metabolism of *S. meliloti*. The key enzymes of the tricarboxylic acid cycle in *S. meliloti* were assayed in an attempt to verify whether IAA is able to stimulate this energy metabolism cycle. Results showed an activation of this cycle by exogenous IAA application or by endogenously over-producing IAA (RD64 mutant) (Imperlini et al. 2009). Polyhydroxybutyrate (PHB) biosynthesis was also activated, and both exogenous IAA treatment and endogenous overproduction by the mutant increased PHB accumulation. PHBs provide the carbon and energy sources required to convert free-living bacteria into the differentiated bacteroids that carry out nitrogen fixation. As a result, nitrogen fixation in the nodules of *M. truncantula* plants inoculated with the IAA-overproducer (RD64) was also enhanced (Imperlini et al. 2009).

The IAA-overproducing *S. meliloti* RD64 is less competitive than the wild-type strain under normal conditions, but is more competitive under stress conditions (Bianco et al. 2009). To investigate the general protection systems activated under stress, the

production of polymeric structures such as lipopolysaccharide (LPS), exopolysaccharide (EPS) and biofilm was evaluated. It was observed that IAA-overproducing RD64 cells contained higher levels of LPS molecules and produced more biofilm as compared to wild-type *S. meliloti* 1021 cells (Bianco et al. 2006). Quantitative analysis of EPS, a major component of biofilm, revealed the same trend, with higher levels produced by RD64 than the control strain. Increased LPS in the outer membrane of RD64 cells may confer protection against the innate plant defense system. Likewise, the increased formation of biofilm likely serves as an important survival factor, enabling rhizobia to adapt to changing environmental conditions. In addition, the higher amount of EPS released by RD64 cells might improve their initial attachment to plant roots (Bianco et al. 2009).

Bianco et al. (2009) analyzed intracellular trehalose levels in the *S. meliloti* strain 1021 and observed that exogenous IAA treatment promoted trehalose accumulation. The IAA over-producer *S. meliloti* RD64 had about 3 times more trehalose than the wild-type strain. Bacteria can use trehalose as a source of carbon and as an osmolyte that confers protection against freezing and desiccation. IAA-treated and RD64 cells survived longer at 4 °C and as dry pellets as compared to wild-type cells. Altogether, these studies suggest that IAA not only acts as a phytohormone but also plays a key role in affecting bacterial metabolism (Imperlini et al. 2009). The increased levels of trehalose, LPS, EPS and biofilm confer upon the bacteria enhanced resistance against environmental stress (Bianco et al. 2009).

Donati et al. (2013) examined the effects of IAA pre-treatment on the tolerance of *Bradyrhizobium japonicum* to oxidative stress (10 mM H_2O_2 for 20 min), heat shock (42 or 50 °C for 10 min.), cold shock (4 °C for 24 h), osmotic stress (0.5 M NaCl for 4 h) and desiccation (27 % relative humidity for 72 h). The results show that under heat shock conditions, 84 % of IAA-treated cells survived, whereas only 47 % of non-treated cells survived. Similarly, under cold-shock conditions 98 % of IAA-treated cells survived, compared to 72 % survival for non-treated cells. IAA-treated cells exposed to oxidative, osmotic and desiccation stress had 96, 86 and 89 % survival, respectively, whereas the non-treated cells had 67, 49, 84 % survival, respectively. Altogether, these cell viability assays indicate that pre-treating *Bradyrhizobium japonicum* with exogenous IAA enhances its ability to tolerate stresses. Moreover, this study revealed that the total EPS production and biofilm formation increased significantly (1.7- to 2.5-fold for biofilm) in response to IAA treatment.

Scott et al. (2013) were the first to observe bacterial chemotaxis towards IAA by the IAA-degrader *P. putida* 1290. Chemotaxis is mediated by methyl-accepting proteins (MCPs), which recognize a specific attractant and transmit a signal to the flagellar machinery to stimulate movement (Krell et al. 2011). It is possible that *P. putida* 1290 possesses specific MCPs that draw the cells towards IAA, however, these have not yet been identified. The genome of the non-IAA-degrading *P. putida* KT2440 contains 27 MCPs (Parales et al. 2013), however, none of them induce chemotactic behavior toward IAA when this bacterium is transformed with the full IAA-degradative (*iac*) gene cluster. Many chemotactic behaviors are linked to metabolism of the attractant (Krell et al. 2011) therefore, bacteria that can actively chemotaxi toward IAA and degrade it, would have a competitive advantage over IAA-degrading bacteria that lack chemotactic ability. IAA can serve as a carbon and nitrogen source for bacteria, as well as conferring protection against environmental stresses, thus having the ability to chemotaxi towards IAA offers a selective advantage to their survival. In nature, bacteria coexist in multispecies communities and compete for resources and space (Drogue et al. 2012). Plants are biological sources of IAA and perhaps bacteria can also use this attractant to better colonize plants, outcompeting other bacteria (Monier and Lindow 2005).

Matsukawa et al. (2007) suggest that IAA plays a role in regulating cellular differentiation and secondary metabolism in *Streptomyces* sp. These researchers investigated the effect of exogenously applied IAA on aerial mycelium formation and antimicrobial activity in several *Streptomyces* species. Low concentrations of IAA (1–50 µg/mL) were observed to significantly stimulate aerial mycelium formation and antimicrobial activity against various bacteria in more than half of the *Streptomyces* species tested. IAA has also been demonstrated to stimulate spore germination and mycelial elongation in *S. atroolivaceus* (El-Shanshoury 1991). Thus, IAA may act as a regulating agent for sporulation and secondary

metabolism in streptomycetes (Matsukawa et al. 2007). Plant-associated *Streptomyces* spp. are well known for their ability to produce antibiotics, which can be used to protect plant roots from fungal and bacterial phytopathogens. IAA can be prevalent in the rhizosphere as it produced and secreted by both plants and bacteria, therefore it may act as a signal for these rhizospheric streptomycetes to enhance antibiotic production and inhibit the growth of other competing microbes, while simultaneously offering protection to plants (Matsukawa et al. 2007).

IAA as a signaling molecule in bacteria

IAA has also been suggested to behave as a signaling molecule for different cellular processes within bacterial cells. High throughput gene expression analyses were performed on wild-type *Az. brasilense* Sp245 and its corresponding *ipdC* mutant to determine overall changes as a result of IAA biosynthesis (Van Puyvelde et al. 2011). In the mutant, 39 genes encoding ribosomal proteins showed decreased expression levels. Therefore, interfering with IAA biosynthesis in *A. brasilense* affects protein synthesis. Genes belonging to a nitrate reducing system show a 1.4- to 1.9-fold increase in expression in the *ipdC* mutant and 1.3- to 1.7-fold decreased expression when this mutant was treated with exogenous IAA. This system plays a role in aerobic denitrification and adaptation to an anaerobic metabolism. The IAA mutant also affects cell respiration by decreasing the expression of members of the NADH dehydrogenase complex, while exogenous IAA treatment increased their expression. There was also increased expression of ATP-binding cassette transporters and tripartite ATP-independent periplasmic (TRAP) transporters. These transporters are implicated in carbohydrate uptake and may be turned on to accommodate the different carbohydrate composition in an environment rich in root exudates. Furthermore, exogenous IAA treatment promotes the expression of a type VI secretion system (T6SS). In this system, different structural components form an injection tube through which proteins can be transported into a plant cell. Perhaps *Az. brasilense* uses this mechanism to directly interact with plant signaling pathways (Van Puyvelde et al. 2011). Another example of IAA acting as a signaling molecule can be seen in *E. chrysanthemi*, where IAA is required for the expression of effectors secreted by the type III secretion system (Yang et al. 2007). The type III secretion systems are used to inject bacterial virulence “effector” proteins into host cells to facilitate pathogencity (Alfano and Collmer 2004). Altogether, these results show that IAA acts as a signaling molecule for a variety of different genes that play a role in the plant–microbe interaction (Van Puyvelde et al. 2011).

A recent study reports that IAA inhibits the expression of the *Agrobacterium* vir operon as a feedback control of vir gene expression and T-DNA transfer (Liu and Nester 2006). Yuan et al. (2008) studied the global transcriptional responses of *A. tumefaciens* to IAA plant signals under growth conditions that mimic the rhizosphere. *A. tumefaciens* C58 was grown in virulence induction medium containing IAA, and its transcription profile was compared with that of cells grown in induction medium only. In response to IAA treatment, 51 genes were up-regulated and 5 were down-regulated. Among the up-regulated genes were those involved in amino acid synthesis and carbon metabolism. Among the repressed genes were biotin synthase, putative zinc ABC transporters and a bacA homologue. Biotin is essential for *S. meliloti* growth on the plant root surface and for the expression of quorum-sensing signals while bacA is essential for the formation of nitrogen-fixing bacteroids (Ichige and Walker 1997; Heinz and Streit 2003). These findings demonstrate that IAA acts as a signal that governs *A. tumefaciens*’ responses to plant hosts (Yuan et al. 2008).

Donati et al. (2013) examined the genome-wide transcriptional profile of *Bradyrhizobium japonicum* in response to exogenous IAA treatment. The gene expression data revealed the up-regulation of heat shock, cold shock, EPS biosynthetic and molecular chaperone proteins. Induction of these genes may serve as a strategy to survive stressful conditions. On the other hand, genes involved in amino acid biosynthesis, cellular processes, energy metabolism, translation, transport and binding proteins were repressed, perhaps as a means of energy conservation.

Matsukawa et al. (2007) investigated the effect of exogenous IAA treatment on the transcription of antibiotic genes in a streptomycete. Quantitative PCR was used to monitor the effect of IAA on the transcription of several genes involved in the biosynthesis of rhodomycin by *S. purpurascens* NBRC

13077. The transcription levels in *S. purpurascens* NBRC 13077 cultivated in media supplemented with 20 μg/mL IAA were higher than those of the same strain cultured without IAA. This report indicates that IAA acts as a signal, which induces antibiotic production in this streptomycete by upregulating antibiotic biosynthesis genes. Enhanced antibiotic production can be used as a weapon against other microbes competing for the same resources, enhancing the streptomycetes chances of surviving in the microbial jungle.

The role of IAA in the plant response to pathogens

Plants employ several mechanisms to defend themselves against phytopathogens. One of these mechanisms is a basal defense reaction that occurs when plant pathogen-recognition-receptors (PRRs) recognize pathogen-associated-molecular-patterns (PAMPs) such as bacterial LPS of the invading microbe (Navarro et al. 2006). Another mechanism is the hypersensitive response (HR), a rapid plant cell death localized at the site of infection. It is triggered by pathogen virulence factors and mediated by plant disease resistance genes (He 1996). Auxin signaling plays a central role in the plant's resistance to bacterial infection (Navarro et al. 2006). The link between auxin signaling within the plant and increased resistance to phytopathogens stems from the ability of bacterial PAMPs such as flg22 to elicit miRNAs which target auxin receptor mRNAs such as TIR1, AFB2 and AFB3. Down-regulation of the TIR1 receptor means that Aux-IAA repressor proteins are not targeted for degradation and can repress auxin-response genes. When auxin-response genes are repressed, plant defense genes such as pathogenesis-related (PR) genes and HR genes are up-regulated and the plant is better equipped to deal with a phytopathogenic attack (Fig. 7) (Navarro et al. 2006).

Arabidopsis plants challenged with the phytopathogen *P. syringae* display high levels of miR393 and are more resistant to infection (Sunkar et al. 2012). Kazan and Manners (2009) reports that conserved bacterial flagellin (flg22) from *P. syringae* activates the expression of miR393, which then leads to the suppression of auxin-response genes and reduced disease development. Similarly, a *tir1* mutant shows

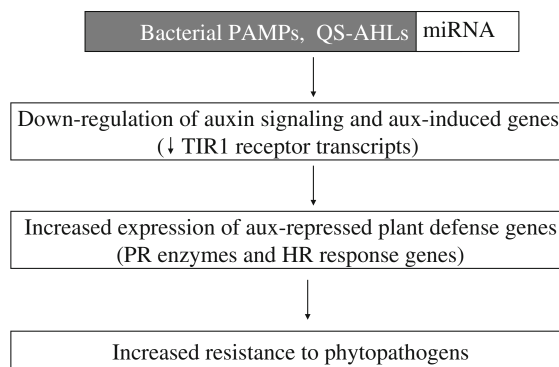

Fig. 7 The auxin response and plant defense. Signaling within the plant is indicated by green boxes

increased resistance to *P. syringae* (Kazan and Manners 2009).

Wang et al. (2007) suggest that salicylic acid (SA) inhibits the auxin-signaling pathway as part of the plant defense mechanism. Their study analyzed transcriptome changes in *Arabidopsis* in response to an SA analog to determine which auxin genes were differentially expressed. Auxin signal transduction genes AUX1 and PIN7 encoding an auxin importer and exporter, auxin receptors TIR1 and AFB1, auxin-inducible SAUR genes and Aux/IAA-repressor genes were repressed by the analog. Conversely, there is an up-regulation of IAA-amidosynthase GH3 genes, which conjugates free IAA to amino acids (Wang et al. 2007). Perhaps by hindering auxin signaling within the plant, the effectiveness of pathogen-derived IAA is encumbered and consequently the plant is more resistant to the phytopathogen. SA treatment of DR5::GUS plants represses the GUS reporter gene expression driven by the auxin-responsive DR5 promoter. Wang et al. (2007) introduced the DR5::GUS reporter into *Arabidopsis* mutants that accumulate endogenous SA and quantified the auxin sensitivity of these plants by measuring the activity of the reporter. An auxin analog was used to induce the DR5 promoter, however, there was no reporter activity in these mutants. This indicates that SA-accumulating plants are less sensitive to exogenous auxin (Wang et al. 2007).

IAA causes plant cells to elongate, thus an auxin-signaling mutant would be hindered in such a process. These defects may cripple a pathogen's ability to infect via the cell wall and colonize extracellular spaces (Kazan and Manners 2009).

IAA signaling has also been reported to contribute to the opening of stomata (Acharya and Assmann 2009). Many phytopathogens use open stomata as gateways to enter into the plant, thus by limiting stomatal opening, pathogen entry is also encumbered (Melotto et al. 2006; Kazan and Manners 2009). Also, plants with mutations in the IAA biosynthesis pathways have defects in lateral root, root hair and vascular tissue development. Many soil pathogens infect the plant through root tips and lateral root initials. Therefore, changes in plant root architecture or vascular tissue could restrict pathogen entry into the roots and colonization within the plant (Kazan and Manners 2009).

Bacterial IAA in deleterious plant interactions

Many studies focus on the beneficial aspects of bacterially derived IAA on plant growth and development. Paradoxically, both plant-growth-promoting bacteria and phytopathogenic bacteria produce IAA. Bacterial IAA plays a major role in plant disease and bacterial pathogenicity (Fu et al. 2011). High levels of IAA and enhanced IAA-signaling lead to disease susceptibility. For example, high levels of IAA produced by phytopathogenic bacteria such as *A. tumefaciens*, *E. herbicola pv*. *gypsophilae*, *P. syringae pv*. *savastanoi* and *P. syringae pv*. *syringae* cause necrotic lesions and gall tumour formation on host plants. The phytopathogens benefit by receiving gall-derived compounds and plant metabolites, which are utilized as a nutrition source. Losing the ability to produce IAA by mutagenesis reduces the virulence of these pathogens (Comai and Kosuge 1982; Surico et al. 1985; Manulis et al. 1998). Native plant IAA biosynthesis genes are induced upon infection by an IAA-producing pathogen. The microbial IAA augments native plant IAA production, thereby amplifying the virulence effect caused by high IAA concentrations (Fu et al. 2011). Plant pathogenic bacteria use a type III secretion system to deliver effector proteins directly into the plant host cells (Alfano and Collmer 2004). One such bacterial effector is AvrRpt2 produced by *P. syringae*. When this effector protein is delivered into plant cells it induces native IAA biosynthesis. This overproduction of IAA may be one of the mechanisms used by the pathogen to colonize its host (Chen et al. 2007). Transgenic *Arabidopsis* plants that overexpress AvrRpt2 have elevated levels of free IAA and are more susceptible to *P. syringae* compared with wild-type plants (Chen et al. 2007).

IAA has been shown to increase the susceptibility of *Arabidopsis*, tobacco, sweet orange and rice to biotrophic, hemibiotrophic or necrotrophic bacterial and fungal infection (Navarro et al. 2006; Ding et al. 2008; Ferrari et al. 2008; Cernadas and Benedetti 2009; Fu et al. 2011). *Arabidopsis* plants treated with the IAA analogs 2,4-dichloro phenoxy acetic acid (2,4-D) or 1-naphthal acetic acid (NAA), had more severe disease symptoms when infected by *P. syringae pv. Tomato* DC3000 than untreated plants. Pretreating *Arabidopsis* with NAA also increased its susceptibility to *P. syringae pv*. *Maculicola* and promoted the phytopathogen's growth (Fu et al. 2011). Moreover, pre-treatment of rice plants with IAA or 2,4-D increased the disease symptoms when inoculated with the phytopathogens *Xanthomonas oryzae pv*. *oryzae*, *Xanthomonasoryzae pv*. *oryzicola* and *Magnaporthe oryzae*. Fu et al. (2011) observed that exogenous IAA treatment of rice plants induced plant IAA biosynthesis-related genes such as those coding for indole-3-acetaldehyde oxidase and nitrilase enzymes. This study did not determine whether *X. oryzae pv*. *oryzae*, *Xa. Pv*. *oryzicola* and *M. grisea* use IAA as a virulence factor to invade rice but the IAA secreted by these pathogens contributes to the accumulation of endogenous IAA in rice plants, which then leads to disease symptoms (Fu et al. 2011). Exogenous treatment of tobacco with oligogalacturonide defense compounds increased the plant's resistance to the necrotrophic fungal pathogen *Botrytis cinerea*, but pretreatment with IAA recovers the susceptibility to the pathogen (Fu et al. 2011). It's important to note that many soil bacteria, including *Pseudomonas* spp., can use auxin analogs such as 2,4-D, as nutrients (Evans et al. 1971). It is possible that pre-treating plants with these compounds prior to phytopathogen inoculation leads to increased bacterial biomass, which in turn aggravates plant disease symptoms. Therefore, it is important to measure the number of infecting cells.

High IAA concentrations obstruct the SA pathway in plants (Robert-Seilaniantz et al. 2011). Studies revealed that an IAA-producing pathogenic *P. syringae* strain increases disease in *Arabidopsis* by suppressing PR gene expression and SA-dependent

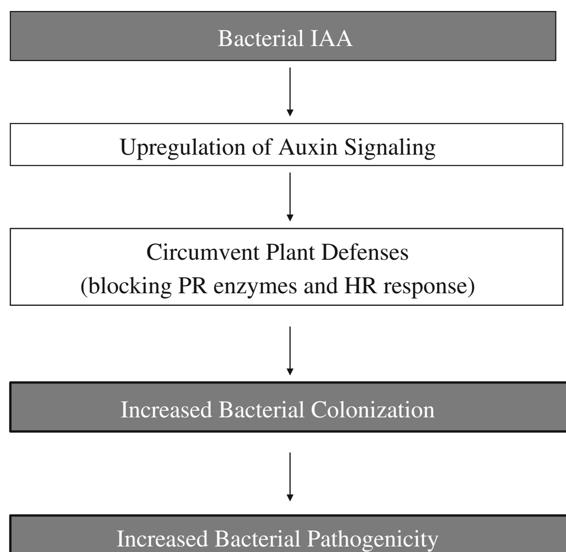

Fig. 8 IAA in pathogenic bacteria–plant interactions. Signaling that takes place within the plant is indicated by green boxes

defense (Fig. 8). This suppression is paralleled by an increase in IAA levels (Fu et al. 2011). Studies by Park et al. (2007) and Wang et al. (2007) also observed that plants co-treated with exogenous IAA and SA had reduced expression of the pathogenesis related gene 1 (PR1), a marker gene of SA-mediated defense, compared with plants treated with SA alone. Moreover, plants deficient in SA also show increased IAA levels (Kazan and Manners 2009). Over-expression of the AFB1 auxin receptor in plants, led to the suppression of SA levels and to a higher susceptibility to biotrophic pathogens (Robert-Seilaniantz et al. 2011).

Mutka et al. (2013) observed that plants over-expressing IAA-biosynthesis genes leading to elevated IAA levels, are more susceptible to the phytopathogen *P. syringae* DC3000. However, this study suggests that the increased susceptibility is not primarily due to suppression of SA-mediated defenses. Instead, high IAA levels promote pathogenesis via a different mechanism. To investigate plant defense responses as a result of elevated IAA levels, wild-type and IAA-overproducing plants were inoculated with *P. syringae* DC3000 expressing the type-III effector protein AvrRpm1. Bacterial growth was higher in IAA-overproducing plants treated with the bacteria compared with wild-type plants. This implies that elevated IAA levels promote pathogen growth. High IAA levels produced by the IAA-overproducing plants were also observed to have an effect on the HR. When wild-type plants were inoculated with a non-pathogenic strain *P. syringae pv. phaseolicola*, expressing AvrRpm1, they display a strong HR with the majority of leaves (70 %) exhibiting tissue collapse. Conversely, when IAA-overproducing plants were inoculated with the same strain, only 23 % of inoculated leaves exhibited tissue collapse. Thus, it appears that elevated IAA levels impede the progression of the programmed cell death. It had been reported earlier by Gopalan (2008) that the hypersenstitive cell death response, induced by a bacterial elicitor, could be reversed by IAA. Gopalan (2008) examined the effect of IAA on the HR cell death by coinfiltrating a purified bacterial elicitor and IAA into tobacco plant leaves. Treatment of the plants with the bacterial elicitor alone, induced plant cell death, however co-infiltrating with IAA prevented the elicited cell death. The researchers then examined whether the expression of a gene induced locally at the site of HR cell death and a systemic acquired resistance marker gene was affected by the HR cell death inhibition caused by IAA. Results showed that the induction of either of the genes was not affected (Gopalan 2008).

To investigate whether IAA-overproducing plants are affected in their SA biosynthesis, the expression of a gene responsible for most SA synthesis during infection was monitored. A similar increased expression was observed in both wild-type and IAA-overproducing plants infected with DC3000. These results suggest that high IAA levels do not significantly impact expression of the SA biosynthesis gene. Moreover, SA levels were not significantly altered in infected IAA-overproducing plants relative to the wild-type plant (Mutka et al. 2013).

In plants, IAA is typically conjugated to amino acids such as IAA-Asp. This conjugate has been reported to promote DC3000 pathogenesis during plant infection (González-Lamothe et al. 2012). In *Arabidopsis*, the GH3.2 gene, which plays a role in synthesizing IAA-Asp, was induced during pathogen infection. When the gene was mutated, the amount of IAA-Asp decreased and consequently the plant was less susceptible to infection (González-Lamothe et al. 2012). Mutka et al. (2013) observed that both infected wild-type and infected IAA-overproducing plants had induced GH3.2 expression to similar levels. Thus, the

induction of GH3.2 seems to be a response to infection and not due to elevated IAA (Mutka et al. 2013).

Bacterial IAA compromises the integrity of the plant cell wall, the first barrier of defense against pathogens. IAA enhances the release of hydrogen ions into the cell wall and this acidification promotes the activities of cell wall glycosidases that hydrolyze polysaccharides in the cell wall leading to cell wall loosening (Johnson et al. 1974; Cleland 1981, 2010; Masuda 1990; Cosgrove 2005). This offers pathogens easier access to host cells for the delivery of type III secreted effectors and other virulence factors (Mutka et al. 2013). Moreover, IAA also induces the expression of proteins responsible for decreasing cell wall rigidity. For example, IAA is reported to induce the expression of expansins, which extend the cell wall while allowing phytopathogens to intrude (Fry et al. 1992; Brummell et al. 1994; Catalá et al. 2000; Kochar et al. 2011; Zhao et al. 2012). Overexpression of expansin in rice has been shown to increase its susceptibility to the bacterial pathogen *Xanthomonas oryzae* (Ding et al. 2008).

Conclusion

IAA is a fundamental phytohormone with the capability to control many aspects of plant growth and development. Its effects in plants have been extensively studied, however the roles it plays in bacteria remain elusive. Different types of bacteria, including Gram-positive and -negative, photosynthetic, methylotrophic and cyanobacteria have the ability to synthesize IAA. Not only does IAA mediate a plant-microbe relationship but it also has a physiological role in the bacteria themselves. IAA can protect bacteria against environmental stresses. This has been demonstrated in nitrogen-fixing bacteria that intimately associate with the root nodules of plants but needs to be further studied in rhizospheric isolates. Moreover, IAA acts as a signaling molecule controlling the expression of various bacterial genes. Among the differentially expressed genes are those associated with virulence, stress responses, metabolism, bacterial adaptation and amino acid synthesis. Studies on how IAA affects the gene expression profile in different bacteria reveal some of the elaborately interconnected roles that IAA plays. Additional studies, utilizing transcriptomic and proteomic approaches are required to better understand how IAA affects changes in IAA producing bacteria, other soil bacteria and in different plants.

This phytohormone can be synthesized via several pathways, including the IPA, IAM and IAN pathways. These pathways are often regulated by tryptophan, carbon and nitrogen availability, a reduction in growth rate and abiotic factors such as temperature, pH and oxygen. The ideal mix of these factors appears to be unique to each bacterium. Therefore, it is important to determine the optimal IAA-producing conditions in order to develop effective bacterial inoculants. Bacterial IAA can have both beneficial and deleterious effects on host plants, depending on the level of IAA produced and the sensitivity of the plant to IAA. Although the IAA system is far from straightforward, it is crucial that we develop a more profound understanding of how these pathways work. Several of the genes involved in IAA biosynthesis and their respective enzymes have been identified, however their regulatory mechanisms are less understood at the genetic level; this is especially true for the IAM and IAN pathways. In addition, future studies need to focus on the IAA signaling cascades and the various auxin receptors and transcription factors that are involved in bacteria as well as plants.

Some bacteria have the dual ability to produce and degrade IAA, yet the focus of studies to date has been on the former. Only recently, progress has been made in deciphering the IAA-degradation pathways in bacteria. The traditional view has been that bacteria stimulate plant growth by providing IAA to the plant. However, it is possible that bacteria may also stimulate plant growth by degrading plant-derived IAA when it has reached detrimentally high levels. Conversely, it may be the case that bacteria degrade IAA for their own benefit, irrespective of the plant. Bacterial IAA degradation is a newer topic that requires further investigations, but it is clear that having this ability can benefit both the plant and the bacteria. While some IAA-degradative genes have been identified, the individual role carried out by each of the encoded enzymes remains to be elucidated.

As the world's population continues to increase, enhanced plant productivity will be needed in order to sustain the rapidly increasing population. Current trends of environmental destruction exacerbated by

the effects of climate change will reduce the availability of suitable land for agriculture, horticulture and silviculture in the imminent future. Thus, it is imperative that the available land is used in as efficient a manner as possible. This could include the deliberate use of IAA-producing plant growth promoting bacteria in an ecologically responsible manner (Chaiharn and Lumyong 2011). Inoculating plants with IAA-producing bacteria to facilitate biofertilization, phytostimulation and biocontrol is a potential alternative to the use of chemical fertilizers and herbicides, which often cause environmental pollution (Ali et al. 2010). This can only be achieved by fully understanding the complex IAA system.

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