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Suppression of plant defence in rhizobia–legume symbiosis

Axel Mithöfer

The symbiosis between rhizobia and legumes is characterized by the formation of dinitrogen-fixing root nodules. Although rhizobia colonize roots in a way that is reminiscent of pathogenic microorganisms, no host plant defence reactions are triggered during successful symbioses. Nevertheless, the plants obviously control the invading bacteria; failure in effective nodule formation or infections with rhizobia defective in surface polysaccharides often result in pathogenic responses. This article focuses on whether and how defence responses in effective symbiosis might be suppressed. Recent results suggest a central role for rhizobial polysaccharides acting as antagonists in the negative regulation of defence induction.

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Plant–microorganism interactions differ strikingly in the nature of the relationships that are finally established. For instance, host–pathogen interactions are detrimental to one of the two organisms involved. In a compatible interaction, plant disease develops. In an incompatible interaction, a resistant host plant establishes a set of different defence mechanisms directed against the pathogen, such as cell wall fortification, the generation and accumulation of reactive oxygen species (ROS) and phenylpropanoids, including phytoalexins, as well as the expression of pathogen-related (PR) proteins [1,2]. By contrast, symbiotic interactions are beneficial to both partners. An ecologically and agronomically important symbiosis occurs between leguminous plants and rhizobia, involving the *de novo* development of a specialized plant organ, the root nodule [3]. In the nodules, rhizobia fix dinitrogen into ammonia, which is assimilated by the host plant, and, in turn, rhizobia are supplied with carbon compounds. Collectively, these soil-borne bacteria, which belong to the genera *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Sinorhizobium* and *Rhizobium*, are called rhizobia.

The nodulation process in rhizobia–legume symbiosis requires a sequence of highly regulated and coordinated events, initiated by an exchange of specific signalling compounds between both partners [4–6]. Subsequently, rhizobia invade the host by means of an infection thread formed from curled root hairs that grows towards an emerging meristematic nodule zone in the root cortex. Enclosed by the host-derived peribacteroid membrane, bacteria are released into the nodule cells and eventually transform into dinitrogen-fixing bacteroids [3].

Symbiosis and defence responses

During nodulation, the colonization of host plant root tissue by homologous rhizobia does not elicit plant defence reactions normally induced by invading microorganisms, although at some stages the infection resembles a pathogenic interaction [7–9]. However, under certain circumstances, various defence reactions might take place in legume–rhizobia interactions. The most extreme of which is the abortion of the infection [10]. For instance, in a ground-breaking study it was shown that during the homologous *Sinorhizobium meliloti*–alfalfa (*Medicago sativa*) interaction, the plant controlled the extent of infection by initiating defence, suggesting that there is a mechanism in the plant that regulates nodule number [10]. This plant response was characterized by a termination of infection in necrotic cells, concomitant with an accumulation of phenolic compounds and PR proteins. In other studies, also using wild-type rhizobia strains for infection, similar but less dramatic results have been obtained: for example, in the *S. meliloti–Medicago truncatula* symbiosis, proteins (MtN1 and MtN13) structurally related to defence proteins are expressed, or in *S. meliloti*–alfalfa relationships ROS is generated [11,12].

Even more pronounced defence reactions have been described in some ineffective (Fix^-) associations of legumes with rhizobia. These are often accompanied by non-developed, non-functional pseudonodules [13–15], supposedly as a result of numerous elicited plant defence responses. Evidence

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obtained by cytological examinations of the infected plant tissue in the *Rhizobium leguminosarum* bv. *viciae* strain 3841–pea (*Pisum sativum*) associations, as well as in *S. meliloti*–alfalfa interactions, suggested defence-related cell wall alterations such as callose deposits and incrustation with phenolic compounds [14,16]. A closer look revealed the induction of mRNA encoding different isoforms of phenylalanine ammonia lyase (PAL) and chalcone synthase (CHS) during the inoculation of soybean with either a Fix^- mutant or a wild-type strain of *Bradyrhizobium japonicum* [17]. Both PAL and CHS are involved in the biosynthesis of phenylpropanoids, including phytoalexins (glyceollin). Moreover, a pronounced accumulation of glyceollins has been found in nodules in response to infection with ineffective *Bradyrhizobium* strains [18–20]. These results indicate that legumes are able to induce defence reactions during associations with rhizobia. Thus, to establish a successful symbiosis, it is generally assumed that rhizobia must suppress or avoid host defence responses [7–9]. Therefore, what are the underlying mechanisms for 'no defence'?

The basic question that needs to be answered is whether the invading rhizobia are recognized as putative pathogens or not. As suggested by David Smith, enclosing the rhizobia with an infection thread or with the peribacteroid membrane might prevent the rhizobia from being perceived [21]. It is also conceivable that the host recognizes the bacteria as 'self' because the surface determinants might be similar to structures on the plants' cell surface [22]. In both cases, there is no obvious reason for the plant to induce defence. However, the invaders are probably perceived as 'non-self', and a decision is made to tolerate or eliminate the microorganisms. Thus, the presence of specific rhizobia-derived compounds counteracting the elicitation of defence in the plant tissue might allow the host to be colonized.

Role for rhizobial polysaccharides in symbiosis

Bacterial cell surface components, such as oligo- and polysaccharides, are now acknowledged to be crucial signals for many microorganism–host relationships, including those in animals [1,2,5,23]. Symbiotically relevant carbohydrates of rhizobia include exopolysaccharides (EPS), lipopolysaccharides (LPS), and cyclic β-glucans (Fig. 1). A series of studies of different rhizobia–legume associations has shown that mutants defective in the synthesis of any of these carbohydrates are unable to infect the host successfully and/or to form effective dinitrogen-fixing nodules. This was accompanied by plant defence reactions [5,14–16,24–26]. These results indicate that the rhizobial poly- and oligosaccharides might play an important role in the nodulation process, probably as signals to the host plant. Whereas the absence of LPS and cyclic β-glucans, correlating with ineffective, non-functional nodules, supports this hypothesis, the data obtained with EPS are even more dramatic. EPS I, also known as succinoglycan, and EPS II represent the major classes of EPS in wild-type *S. meliloti* Rm1021. EPS I is composed of repeating subunits of octasaccharides modified with one acetyl, one succinyl and one pyruvyl substituent per subunit (Fig. 1a). Both a high molecular weight (HMW) and a low molecular weight (LMW, up to three subunits) form of succinoglycan are produced. The LMW fraction, in particular the trimer, is of particular interest because it has been reported to restore nodule invasion capability of *S. meliloti* mutants defective in exopolysaccharide synthesis [27,28]. This effect relies on the particular EPS structure because non-succinylated EPS I does not repair nodulation deficiency nor does a EPS derivative produced by *Rhizobium* sp. strain NGR234 (Fig. 1a) [27]. Moreover, a LMW fraction of EPS II (Fig. 1a), consisting of 15–20 disaccharide subunits, substitutes succinoglycan when nodulated by *S. meliloti* [29,30]. EPS II was not required if EPS I was present [31].

All these observations suggest an essential role for EPS, LPS and cyclic β-glucans, or molecules derived from these carbohydrates, as signalling compounds, possibly acting as suppressors of plant defence reactions in symbioses. Although the effects seem to be specific and probably restricted to distinct rhizobia–host interactions, this model would imply the existence of specific plant receptors involved in the recognition of the signals.

Agonist–antagonist-based defence suppression

Putative (lipo)oligosaccharide receptors in plants are described for both pathogenic (e.g. β-glucans, oligochitin) and symbiotic relationships (Nod-factors) [32,33]. However, experimental evidence of binding sites or receptors for either EPS or LPS is needed. Interestingly, the cyclic β-(1,3)-β-(1,6)-glucans from the soybean (*Glycine max*) symbiont *B. japonicum* USDA110 share some structural features with non-cyclic β-glucans carrying the hepta-β-(1,3)-β-(1,6)-glucan motif (hepta-β-glucoside, Fig. 1b) derived from the cell wall of the phytopathogenic oomycete *Phytophthora sojae*. The hepta-β-glucoside elicitor is well characterized with regard to its biological activities, such as the induction of early and late cellular defence responses in differentiated plants and cell cultures, and to the biochemical properties of the receptor–ligand interactions [2,32]. Recently, the cloned hepta-β-glucoside-binding proteins from soybean and French bean (*Phaseolus vulgaris*) have been identified as members of a putative receptor family in legumes [34].

In contrast to the *P. sojae* β-glucan-elicitor, which induces processes that eventually inhibit microorganism invasion, *B. japonicum* cyclic β-glucans are essential in establishing a symbiotic relationship with soybean plants [15,35]. To

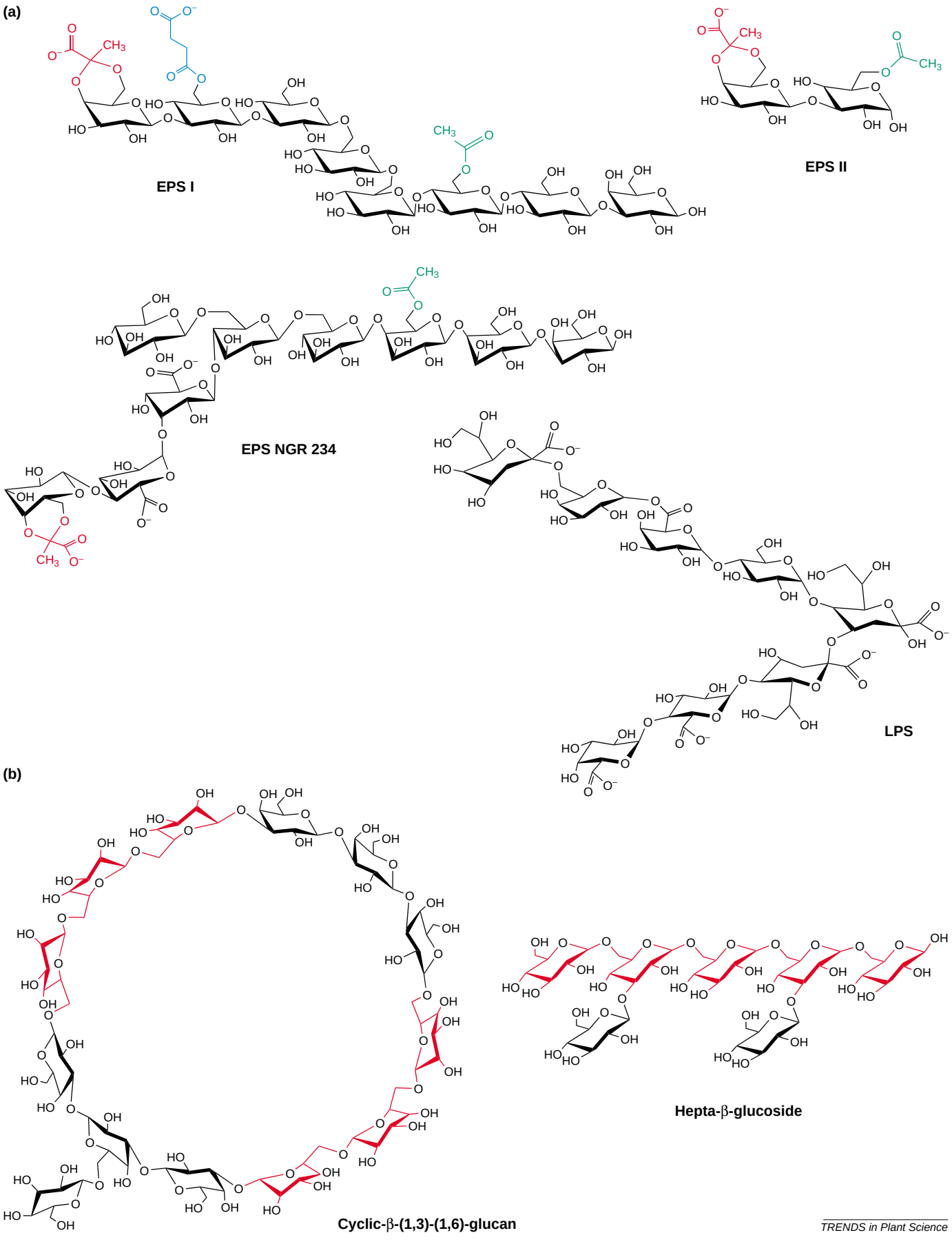
(a)
EPS I
EPS II
EPS NGR 234
LPS
(b)
Cyclic-β-(1,3)-(1,6)-glucan
Hepta-β-glucoside
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Fig. 1. Chemical structures of various relevant oligo- and polysaccharides in rhizobia–legume symbiosis. (a) Repeating units of rhizobial exopolysaccharides from *Sinorhizobium meliloti* Rm1021 (EPS I, EPS II); exopolysaccharide from *Rhizobium* sp. strain NGR234 (EPS NGR234); core structure of the lipopolysaccharide from *Rhizobium etli* (LPS). Substituents are indicated by colours: acetyl (green), pyruvyl (red), and succinyl (blue). (b) Cyclic β-(1,3)-β-(1,6)-glucans from *Bradyrhizobium japonicum* USDA110 (cyclic β-glucan); hepta-β-(1,3)-β-(1,6)-glucan from the oomycete *Phytophthora sojae* (hepta-β-glucoside). The β-(1,3)-bound glucose units are indicated in black, the β-(1,6)-bound glucose units are indicated in red (in the case of the cyclic-β-glucan, only the ring structure has been considered with respect to its biosynthesis).

understand this striking pathogenic versus symbiotic dichotomy, the plant responses to these glucans have been investigated. Surprisingly, bradyrhizobial cyclic β-glucans were found not to induce but to suppress typical soybean host defence reactions challenged by *P. sojae* elicitor treatment [36,37], including different elements of the putative signal transduction cascade, as well as phytoalexin accumulation. However, the generation of the ROS hydrogen peroxide was not inhibited but stimulated [38]. By contrast, in alfalfa, LPS from *S. meliloti* suppresses yeast extract-elicited ROS production [39]. However, effective nodules do produce considerable amounts of ROS and overcome this problem by highly efficient antioxidative activities [40]. Thus, ROS have been suggested to be a control element rather than a crucial defence component in rhizobia–legume symbioses [12].

Suppression of receptor-mediated cellular responses is well described in animal cells [41,42]. The molecular basis of this effect ranges from inhibition of signalling cascade elements or transcription factors to a direct interaction with the receptor. In direct interaction with the receptor, the suppressing factor might either block the interaction of the receptor with the subsequent signal transducer or interfere directly with the receptor binding site, substituting the activating compound, the agonist, which initiates the full response. In contrast to the agonist, a suppressing antagonist binds to the receptor without causing any response (Fig. 2).

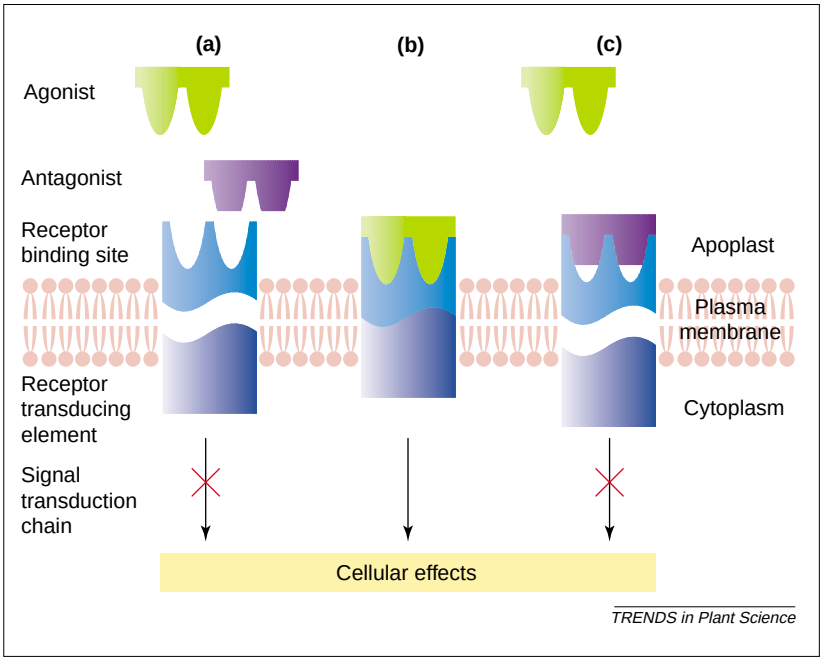

Fig. 2. Model of agonist and antagonist dependent signal transduction at the plasma membrane. (a) Simplified numbers of elements involved in a ligand–receptor-mediated signal transduction cascade leading to specific cellular effects: agonist (depicted in green) and antagonist (depicted in purple) representing specific ligands of a receptor binding site, a receptor transducing element being part of or connected to the receptor, a (cytosolic) signal transduction chain. (b) Effective agonist–receptor interaction leading to specific cellular effects. (c) Ineffective antagonist–receptor interaction blocking the activation of the connected signalling cascade.

The ability of the cyclic β-glucans to compete efficiently with the hepta-β-glucoside for the same receptor binding site (IC_{50} 7.3 μM, [20]) reveals an agonist–antagonist-based mechanism underlying the decision for or against plant defence responses in the *B. japonicum*–soybean symbiosis [20,36]. Thus, whether defence is induced or suppressed might be regulated by the ratio of receptor occupancy by the two signalling compounds involved.

Because cyclic β-glucans are produced even by bacteroids during symbiosis, they could be employed to maintain suppression of plant defence in a functional nodule [43]. This might explain why in nodules formed by wild-type *B. japonicum*, the amount of phytoalexins is significantly lower than in ineffective nodules obtained after infection with *B. japonicum* mutant strains defective in the synthesis of cyclic β-(1,3)-β-(1,6)-glucans [20].

Conclusion and perspectives

At present, we do not understand the mechanisms underlying the discrimination between friend and foe in plants. Because it is risky for the host plant to let invaders go unperceived and unaffected, the existence of various receptor–ligand-based recognition systems that initiate plant defence responses is likely. In symbiosis, suppressor-mediated masking of the receptor to achieve its downregulation is one way to avoid defence activation. To date, naturally occurring soybean–pathogen and soybean–symbiont associations are the only example for which, on the molecular level, all protagonists are known: the chemically defined structures of the agonist (hepta-β-glucoside elicitor) and the antagonist (cyclic β-(1,3)-β-(1,6)-glucan suppressor), and their corresponding receptor binding site. In soybean signal transduction, which is elicited by the hepta-β-glucoside motif, cyclic β-glucans of *B. japonicum* play a key role as negative regulators. However, an important question remains: what is the role of the cyclic β-glucans in *B. japonicum*–soybean associations, where no *P. sojae*-derived elicitor is present? The presence of such suppressor activity in *B. japonicum* only makes sense if defence responses induced by unknown signalling compounds from *B. japonicum* acting as elicitor(s) must be suppressed. At present, there is a lack of experimental evidence for the existence of rhizobial elicitors. Thus, the isolation and identification of such compounds, including appropriate agonists of other putative

suppressor molecules, such as LPS and EPS, is an important goal for the future. Moreover, biochemical studies on putative binding-sites for EPS and LPS could substantiate their role as signalling molecules.

The main goal must be the identification of receptors, as well as the elucidation of subsequent signal transduction events involved in the onset of plant defence for both rhizobial elicitors and suppressors. This is a prerequisite for understanding the molecular mechanisms underlying the control of defence in symbioses in general. The whole succession to develop and maintain a functional symbiosis probably does not depend on just one mechanism responsible for defence suppression. Different suppressor molecules might act by the same and/or distinct modes. Because of the ongoing genome projects and the highly developed techniques for genetic analyses of the model legumes *M. truncatula* and *Lotus japonicus*, associations of these plants with microorganisms should be valuable systems to work on. It will be interesting to discover the impact of agonist–antagonist-based defence regulation in the finely tuned balance of a relationship between host plants and symbionts and to analyse whether this represents a common mechanistic concept to establish symbioses.

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