A NOVEL DEFENCE PATHWAY IN *ARABIDOPSIS* INDUCED BY BIOCONTROL BACTERIA

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SUMMARY

Plants have the ability to acquire an enhanced level of resistance to pathogen attack after being exposed to specific biotic stimuli. In *Arabidopsis thaliana*, non-pathogenic, root-colonising *Pseudomonas fluorescens* bacteria with biological disease control activity trigger an induced systemic resistance (ISR) response against infection by *P. syringae* pv *tomato* (*Pst*), the causal agent of bacterial speck disease. In contrast to classic, pathogen-induced systemic acquired resistance (SAR), this rhizobacteria-mediated ISR is independent of salicylic acid (SA) accumulation and pathogenesis related (PR) gene activation. Using the jasmonate response mutant *jar1*, the ethylene response mutant *etr1*, and the SAR regulatory mutant *npr1* of *Arabidopsis*, it is demonstrated that signal transduction leading to *P. fluorescens* WCS417r-mediated ISR requires responsiveness to jasmonate and ethylene, and is dependent on the SAR regulatory protein NPR1. In addition to WCS417r, methyl jasmonate (MeJA) and the ethylene precursor 1-aminocyclopropane-1-carboxylate (ACC) were effective in inducing resistance against *Pst* in transgenic *Arabidopsis* NahG plants that are unable to accumulate SA. Furthermore, MeJA-induced protection was blocked in *jar1, etr1*, and *npr1* plants, whereas ACC-induced protection was affected in etr1 and npr1 plants, but not in *jar1* plants. Hence, we postulate that rhizobacteria-mediated ISR follows a novel signalling pathway in which components from the jasmonate and ethylene response are successively engaged to trigger a defence reaction that, like SAR, is regulated by NPR1.

INTRODUCTION

Elucidation of plant signalling pathways controlling disease resistance is a major objective in the investigation of plant-pathogen interactions. *Arabidopsis thaliana* has emerged as an ideal model for studying the molecular basis underlying genetically determined as well as induced disease resistance (Kunkel, 1996). Plants of which the roots have been colonised by selected strains of non-pathogenic fluorescent *Pseudomonas* spp. develop an enhanced level of protection against pathogen attack (reviewed by Van Loon, 1997). *P. fluorescens* strain WCS417r is a biological control strain that has been shown to trigger an induced systemic resistance (ISR) response in several plant species including carnation (Van Peer et al., 1991), radish (Leeman et al., 1995), and tomato (Duijff et al., 1996). With the goal to study the molecular basis underlying WCS417r-mediated ISR we developed a model system using *Arabidopsis* as the host plant (Pieterse et al., 1996). To date, WCS417r-mediated ISR in *Arabidopsis* has been demonstrated against the bacterial leaf pathogen *P. syringae* pv. *tomato* (*Pst*), the fungal root pathogen *Fusarium oxysporum* f. sp. *raphani* (Pieterse et al., 1996; Van Wees et al., 1997), and the fungal leaf pathogen *Peronospora parasitica* (J. Ton and C.M.J. Pieterse, unpublished data), indicating that this type of biologically-induced resistance is effective against different types of pathogens.

The ISR pathway clearly differs from the SA-dependent pathway controlling systemic acquired resistance (SAR), a well studied form of biologically-induced disease resistance that is triggered upon pathogen infection (reviewed by Ryals et al., 1996). The state of SAR is characterised by an early increase in endogenously synthesised SA and the concomitant activation of genes encoding pathogenesis-related (PR) proteins. SA-nonaccumulating *Arabidopsis* plants expressing the bacterial salicylate hydroxylase (*nahG*) gene, are incapable of developing SAR and do not show *PR* gene activation upon pathogen infection, indicating that SA is a necessary intermediate in the SAR signalling pathway (Delaney et al., 1994). In contrast to pathogen-induced SAR, rhizobacteria-mediated ISR is not associated with the activation of *PR* genes (Pieterse et al., 1996; Van Wees et al., 1997). Moreover, NahG plants that are unable to express

SAR develop normal levels of ISR after treatment of the roots with ISR-inducing rhizobacteria (Pieterse et al., 1996; Press et al., 1997; Van Wees et al., 1997). This demonstrates that biologically-induced disease resistance in plants is controlled by at least two pathways that diverge in their requirement for SA.
Besides SA, also the plant growth regulators jasmonic acid and ethylene have been implicated in plant defence responses (Boller, 1991; Wasternack and Parthier, 1997), including defence reactions that are induced systemically (Farmer and Ryan, 1992; Penninckx et al., 1996; Van Loon, 1977). However, in many cases their role is still unclear. Several *Arabidopsis* mutants affected in their response to the signalling molecules jasmonate, ethylene, or SA have been characterised in the past years. To gain more insight in the signalling pathway controlling biocontrol bacteria-mediated ISR, we examined whether the jasmonate response mutant *jar1* (Staswick et al., 1992), the ethylene response mutant *etr1* (Bleecker et al., 1988), and the SAR regulatory mutant *npr1* (Cao et al., 1994) are able to express ISR after colonisation of the roots by WCS417r. Mutant *jar1* exhibits reduced sensitivity to methyl jasmonate (MeJA). Mutant *etr1* is altered in its ability to perceive and react to ethylene due to a mutation in the *ETR1* gene, encoding an ethylene receptor (Chang et al., 1993; Schaller and Bleecker, 1995). *Arabidopsis jar1* plants, as well as ethylene-insensitive tobacco plants expressing the mutant *Arabidopsis ETR1* gene, are susceptible to opportunistic micro-organisms (Staswick et al., 1997; Knoester et al., 1998), whereas wild-type plants show a resistant phenotype, indicating that both mutations affect signalling events leading to disease resistance. *Arabidopsis* mutant *npr1* is affected downstream of SA in the SAR signalling pathway and as a result is blocked in the SAR response (Cao et al., 1994). The *NPR1* gene codes for an ankyrin repeat-containing protein with homology to the mammalian signal transduction factor IκB, which is implicated in disease resistance responses in a wide range of higher organisms (Cao et al., 1997; Ryals et al., 1997). Using the *Arabidopsis* mutants *jar1*, *etr1* and *npr1* we demonstrate that WCS417r-mediated ISR follows a novel signalling pathway that is dependent on responsiveness to both jasmonate and ethylene. Moreover, we show that in similarity to pathogen-induced SAR, the regulatory protein NPR1 protein is required for the expression of ISR.

MATERIALS AND METHODS

Cultivation of bacterial strains

ISR-inducing *P. fluorescens* WCS417r bacteria (WCS417r; Van Peer et al., 1991) were grown on King's medium B agar plates (King et al., 1954) for 24 hr at 28°C. The bacterial cells were collected, resuspended in 10 mM $MgSO_4$, and adjusted to a concentration of 10^9 colony-forming units (cfu) per mL (OD_{600} = 1.0) before mixing through the soil.
The avirulent *P. syringae* pv. *tomato* strain DC3000(*avrRpt2*) (*Pst*(*avrRpt2*); Whalen et al., 1991) used for induction of SAR, was cultured overnight at 28°C in liquid King's medium B supplemented with 20 mg/L tetracycline to select for the plasmid carrying the avirulence gene *avrRpt2*. The bacterial cells were collected by centrifugation, resuspended in 10 mM $MgSO_4$, and adjusted to a concentration of 10^7 cfu/mL before pressure infiltration into the leaves.
The virulent *P. syringae* pv. *tomato* strain DC3000 (*Pst*; Whalen et al., 1991) used for challenge inoculations, was grown overnight in liquid King's medium B at 28°C. After centrifugation, bacterial cells were resuspended to a final concentration of $2.5x10^6$ or $2.5x10^7$ cfu/mL in 10 mM $MgSO_4$ containing 0.01% (v/v) of the surfactant Silwet L-77 (Van Meeuwen Chemicals BV, Weesp, The Netherlands).

Cultivation of plants

Seeds of wild-type *A. thaliana* ecotype Col-0 plants, transgenic NahG plants harbouring the bacterial *nahG* gene (Delaney et al., 1994), and mutant *jar1* (Staswick et al., 1992), *etr1* (Bleecker et al., 1988), and *npr1* plants (Cao et al., 1994) were sown in quartz sand. Two-week-old seedlings were transferred to 60-mL pots containing a sand-potting soil mixture that had been autoclaved twice for 1 hr. Plants were cultivated in a growth chamber with a 9-hr day (200 $\mu E\ m^{-2}\ sec^{-1}$ at 24°C) and 15-hr night (20°C) cycle and 70% relative humidity.

Induction treatments

Plants were treated with non-pathogenic, ISR-inducing rhizobacteria by mixing a suspension of WCS417r bacteria through the soil to a final density of $5x10^7$ cfu/kg just before planting of the seedlings as described by Pieterse et al. (1996).
SAR was induced 3 days before challenge inoculation by pressure infiltrating three lower leaves per plant with the avirulent pathogen *Pst*(*avrRpt2*) at 10^7 cfu/mL in 10 mM $MgSO_4$ using a 1-mL syringe without a needle.
Chemical treatments were performed 3 days before challenge inoculation by dipping the leaves of 5-week-old plants in a solution containing 0.01% (v/v) Silwet L-77 and either H_2O, methyl jasmonate (MeJA; 100 µM) or 1-aminocyclopropane-1-carboxylate (ACC; 1 mM).

Challenge inoculation

Challenge inoculations were performed by dipping the leaves of 5-week-old plants in a bacterial suspension of the virulent pathogen *Pst* at $2.5x10^7$ cfu/mL in 10 mM $MgSO_4$, 0.01% (v/v) Silwet L-77. Because NahG plants are highly susceptible to *Pst* infection, a 10-fold lower inoculum density was used for these plants. Four days after challenge, disease severity was scored by determining the percentage of leaves with symptoms per plant (20 plants per treatment), as described by Pieterse et al. (1996).

Assessment of bacterial population levels

Colonisation of the rhizosphere of wild-type, transgenic and mutant plants by rifampicin-resistant WCS417r bacteria was examined at the end of each bioassay. In duplicate, roots of six plants per treatment were harvested, weighed, and shaken vigorously for 1 min in 5 mL of 10 mM $MgSO_4$ containing 0.5 g of glass beads (0.17 mm). Appropriate dilutions were plated onto King's medium B agar supplemented with cycloheximide (100 mg/L), ampicillin (50 mg/L), chloramphenicol (13 mg/L), and rifampicin (150 mg/L). After overnight incubation at 28°C, the number of rifampicin-resistant colony-forming units per gram of root fresh weight was determined.
Growth of *Pst* in inoculated leaves was assessed in control-, WCS417r-, and *Pst*(*avrRpt2*)-treated plants 4 days after challenge. Two sets of 20 randomly selected leaves per treatment were weighed, rinsed thoroughly in sterile water, and homogenised in 10 mM $MgSO_4$. Subsequently, appropriate dilutions were plated onto King's medium B agar supplemented with 50 mg/L rifampicin and 100 mg/L cycloheximide. After incubation for 48 hr at 28°C, the number of rifampicin-resistant colony-forming units per gram of infected leaf tissue was determined.

Competitive RT-PCR

Analysis of *PR-1* gene expression was performed using the competitive reverse transcriptase-polymerase chain reaction (RT-PCR) as described by Siebert and Larrick (1992). A *PR-1*-specific primer pair

(5'-GTAGGTG-CTCTTGTTCTTCC-3' and 5'-TTCACATAATTCCCACGAGG-3'), yielding RT-PCR products of 422 bp, was prepared based on the *Arabidopsis PR-1* cDNA sequence described by Uknes et al. (1992). A 900-bp heterologous competitor DNA fragment, competing for the same set of primers, was obtained as described by Siebert and Larrick (1992). Fifty nanograms of poly(A)$^+$ RNA, isolated from frozen leaves was converted into first-strand cDNA. Subsequently, equal portions of cDNA were amplified in the presence of 500 pg of competitor DNA using the *PR-1*-specific primer pair as described previously (Pieterse et al., 1996). The products were then resolved on an agarose gel stained with ethidium bromide.

Ethylene determination

Thirty minutes after application of the chemicals, leaves were detached, weighed, and placed in 25-ml gas-tight serum flasks that subsequently were incubated for 24 hr under climate chamber conditions. Ethylene accumulation was measured for three independent plants per treatment by gas chromatography as described by De Laat and Van Loon (1982).

RESULTS

Expression of rhizobacteria-mediated ISR requires responsiveness to both jasmonate and ethylene

To investigate whether jasmonate and ethylene play a role in biocontrol bacteria-mediated ISR, the jasmonate response mutant *jar1* and the ethylene response mutant *etr1* were tested for their ability to develop biologically-induced resistance against infection by *Pst*. Wild-type Col-0 plants, transgenic SA-non-accumulating NahG plants, and mutant *jar1* and *etr1* plants were grown in soil containing ISR-inducing WCS417r bacteria. Another subset of plants received a SAR treatment by inoculating three lower leaves with the avirulent pathogen *Pst*(*avrRpt2*), three days before challenge inoculation with *Pst*. Control plants received no treatment before challenge. Table 1 shows that in Col-0 plants, colonisation of the roots by WCS417r and predisposing infection with *Pst*(*avrRpt2*) resulted in a significant reduction of symptoms 4 days after challenge. Moreover, Col-0 plants pre-treated with WCS417r or *Pst*(*avrRpt2*) showed a strong inhibition of pathogen growth (Table 1), indicating that WCS417r-mediated ISR or pathogen-induced SAR was triggered in these plants. SA-non-accumulating NahG plants mounted resistance against *Pst* infection after WCS417r treatment but not after pre-infection with *Pst*(*avrRpt2*). Furthermore, only plants expressing SAR showed accumulation of *PR-1* transcripts (Table 1). Plants expressing ISR did not, confirming that ISR and SAR are controlled by distinct signalling pathways that diverge in their requirement for SA. Both *jar1* and *etr1* developed SAR and showed activation of *PR-1* gene expression after pre-inoculation with *Pst*(*avrRpt2*), supporting previous findings (Lawton et al., 1995, 1996) that SAR signal transduction in *Arabidopsis* does not require components of the jasmonate or ethylene response. However, neither *jar1* nor *etr1* developed ISR upon colonisation of the roots with WCS417r, indicating that responsiveness to both jasmonate and ethylene is required for the development of rhizobacteria-mediated ISR. Evidently, not SA but both jasmonate and ethylene play a crucial role in the ISR defence pathway.

Rhizobacteria-mediated ISR is dependent on NPR1

NPR1 has been shown to be an important regulatory intermediate in the SA-dependent SAR response (Cao et al., 1994). To investigate whether NPR1 is involved in the SA-independent ISR response as well, *Arabidopsis* mutant *npr1* was tested. Table 1 shows that mutant *npr1* plants failed to develop SAR and did not show *PR-1* gene activation after predisposal infection with *Pst*(*avrRpt2*), confirming that the SAR-

response was effectively blocked in these plants. Surprisingly, *npr1* plants were also affected in the expression of WCS417r-mediated ISR, indicating that both types of biologically-induced disease resistance are dependent on NPR1, in spite of the fact that ISR is not associated with the production of PR proteins.

Table 1. Quantification of rhizobacteria-mediated ISR and pathogen-induced SAR against *Pst* infection, and analysis of *PR-1* gene expression in *Arabidopsis* Col-0, NahG, *jar1*, *etr1*, and *npr1* plants after colonisation of the roots with *P. fluorescens* WCS417r or pre-infection with the avirulent pathogen *Pst*(*avrRpt2*).

Plant type [treatment]	Disease index (%)[1]	Relative growth of *Pst* in the leaves (%)[2]	*PR-1* gene expression[3]
Col-0 [control]	100[a]	100	-
Col-0 [WCS417r]	51[b]	19	-
Col-0 [*Pst*(*avrRpt2*)]	38[c]	13	+
NahG [control]	100[a]	100	-
NahG [WCS417r]	56[b]	46	-
NahG [*Pst*(*avrRpt2*)]	101[a]	113	-
jar1 [control]	100[a]	100	-
jar1 [WCS417r]	94[a]	83	-
jar1 [*Pst*(*avrRpt2*)]	27[b]	5	+
etr1 [control]	100[a]	100	-
etr1 [WCS417r]	122[a]	151	-
etr1 [*Pst*(*avrRpt2*)]	49[b]	51	+
npr1 [control]	100[a]	100	-
npr1 [WCS417r]	95[a]	80	-
npr1 [*Pst*(*avrRpt2*)]	88[a]	106	-

[1] Proportion of leaves with symptoms compared to the control (100%), 4 days after challenge with *Pst*. Different letters within the data of one plant type indicate statistically significant differences (Fisher's LSD test, α=0.05). Plants were treated as described in Materials and Methods.

[2] Relative growth of *Pst* in leaves compared to the control (100%), 4 days after challenge.

[3] *PR-1* mRNA levels were detected in leaves harvested just prior to challenge inoculation by competitive RT-PCR using *A. thaliana PR-1*-specific primers.

Colonisation of the rhizosphere by *P. fluorescens* WCS417r

To investigate whether the inability to express ISR in the mutants was caused by insufficient colonisation of the rhizosphere by WCS417r, the number of rifampicin-resistant WCS417r bacteria per gram of root fresh weight was determined at the end of each bioassay. Table 2 shows that WCS417r colonises the rhizosphere of Col-0, NahG, *jar1*, *etr1*, and *npr1* plants with comparable efficiency. Thus, the loss of the capacity to express WCS417r-mediated ISR in *jar1*, *etr1*, and *npr1* plants is not caused by changes in bacterial root colonisation but must be the result of alterations in the ability to express ISR in these mutants.

Table 2. Colonisation of the rhizosphere of *Arabidopsis* Col-0, NahG, *jar1*, *etr1*, and *npr1* plants by *P. fluorescens* WCS417r.

Plant type	cfu/g fresh weight ($\times 10^6$)[a]
Col-0	8.4±0.4
NahG	7.5±1.1
jar1	9.7±0.8
etr1	6.6±0.6
npr1	7.3±1.1

[a] The values presented are average population densities ± SE at the end of the bioassays. On non-treated roots, no rifampicin-resistant bacteria were detected (detection limit=10^3 cfu/g).

Sequence of signalling events

To elucidate the sequence of signalling events involved in the jasmonate-, ethylene-, and NPR1-dependent ISR response, the resistance-inducing ability of MeJA and ACC, the natural precursor of ethylene, was tested in Col-0, NahG, *jar1*, *etr1* and *npr1* plants. H_2O- and MeJA-treated plants showed basal levels of ethylene production, whereas in ACC-treated plants a 10- to 25-fold increase in ethylene production was observed (Table 3).

As shown in Table 4, pre-treatment of Col-0 and NahG plants with MeJA or ACC resulted in a 30-50% reduction of the symptoms. In *jar1* plants, application of MeJA did not elicit a resistance response, whereas application of ACC resulted in wild-type levels of protection. Mutant *etr1* plants were non-responsive to ACC treatment but also failed to respond to MeJA treatment, indicating that components of the ethylene response act downstream of jasmonate in the signalling pathway leading to protection against *Pst*. In *npr1* plants, responsiveness to applied MeJA or ACC was blocked or highly diminished, respectively, suggesting that components of the jasmonate and ethylene response act upstream of NPR1 in regulating the expression of resistance against *Pst*. The observation that ACC-mediated protection was not completely blocked in *npr1* plants suggests the existence of a parallel ethylene-inducible defensive pathway that does not require NPR1.

Table 3. Ethylene production in *Arabidopsis* Col-0, NahG, *jar1*, *etr1*, and *npr1* plants treated with H_2O, MeJA, or ACC[1]

Plant type	Ethylene production (nl/24 hr/g FW)		
	H_2O	MeJA	ACC
Col-0	103	99	1139
NahG	107	133	1178
jar1	102	83	1052
etr1	86	92	958
npr1	128	130	2667

[1] Ethylene production was measured in leaves of 5-week-old plants over the first 24 hr after application of the chemicals. Treatments were performed as described in the Materials and Methods section. Presented are the means (nl of ethylene produced per gram of leaf tissue per 24 hr) of three independent samples that received the same treatment.

Table 4. Quantification of induced resistance against *Pst* infection in *Arabidopsis* Col-0, NahG, *jar1*, *etr1*, and *npr1* plants treated with H_2O, MeJA, or ACC

Plant type	Disease index[1]		
	H_2O	MeJA	ACC
Col-0	100[a]	46[b]	44[b]
NahG	100[a]	63[b]	71[b]
jar1	100[a]	92[a]	43[b]
etr1	100[a]	109[a]	93[a]
npr1	100[a]	93[a]	77[b]

[1] Proportion of leaves with symptoms compared to H_2O-treated control plants (100%), 4 days after challenge inoculation with *Pst*. Different letters within the data of one plant type indicate statistically significant differences (Fisher's LSD test, α=0.05). Plants were treated as described in the Materials and Methods section.

DISCUSSION

We previously demonstrated that plants expressing ISR triggered by biocontrol bacteria, or pathogen-induced SAR, develop significantly less symptoms compared to non-induced plants, and show a strong inhibition of growth of the challenging pathogen *Pst* in the leaves (Pieterse et al., 1996; Van Wees et al., 1997). Despite these phenotypical similarities, the signalling pathways leading to these biologically-induced resistance responses diverge in their requirement for SA. Moreover, the expression of SAR is accompanied by the activation of *PR* genes, whereas this response is lacking during expression of ISR (Pieterse et al., 1996). In this study we used well-characterised *Arabidopsis* mutants to elucidate the steps involved in the SA-independent signalling pathway controlling rhizobacteria-mediated ISR. Systemic resistance induced by biocontrol bacteria was blocked in the *Arabidopsis* mutants *jar1*, *etr1*, and *npr1* (Table 1), indicating that both components of the jasmonate and ethylene response, and NPR1 play a crucial role in the ISR signalling pathway. Consistent with our observations, Lawton et al. (1995, 1996) previously demonstrated that both *jar1* and *etr1* are not impaired in their ability to develop SAR. Thus, the pathogen-induced SAR and the rhizobacteria-mediated ISR signalling pathways diverge in their requirement for SA, on the one hand, and for jasmonate and ethylene, on the other hand.

Several lines of evidence indicate that MeJA- and ACC-induced protection follow the same signalling pathway as WCS417r-mediated ISR. WCS417r, MeJA, and ACC induce resistance against *Pst* in *NahG* plants (Tables 1 and 4), indicating that these agents activate a SA-independent resistance mechanism. This is supported by the fact that WCS417r-, MeJA-, and ACC-treated plants do not show an increase in SA-inducible *PR-1* gene expression (data not shown). Moreover, resistance induced by WCS417r, MeJA, and ACC requires responsiveness to ethylene and is dependent on NPR1 to be fully expressed. All together, this strongly suggests that WCS417r, MeJA and ACC trigger the same signalling pathway controlling induced resistance against *Pst*. Because ACC still induced resistance in *jar1* plants, we postulate that during signal transduction leading to WCS417r-mediated ISR, the JA- and ethylene-response are successively engaged in triggering a defence response that is regulated by NPR1 (Fig. 1).

Elicitation of a similar SA-independent defence pathway against *Pst* infection by WCS417r, MeJA and ACC leads one to expect that ISR is associated with an increase in the production of jasmonate or ethylene. However, WCS417r-mediated ISR is not associated with jasmonate- or ethylene-responsive gene expression (data not shown), suggesting that the production of jasmonate and ethylene is not strongly stimulated. Recently, Schweizer et al. (1997) demonstrated that during infection of rice with the fungal pathogen *Magnaporthe grisea*, jasmonate-inducible genes are activated without an increase in endogenous jasmonate levels. Moreover, Tsai et al. (1996) provided evidence that an increase in ethylene sensitivity rather than ethylene production is the initial event in triggering jasmonate-enhanced senescence in

detached rice leaves. Thus, ethylene-, and jasmonate-dependent plant responses can be triggered without a concomitant increase in the levels of these phytohormones. Whether enhanced sensitivity to either jasmonate or ethylene plays a role in rhizobacteria-mediated ISR needs to be elucidated.

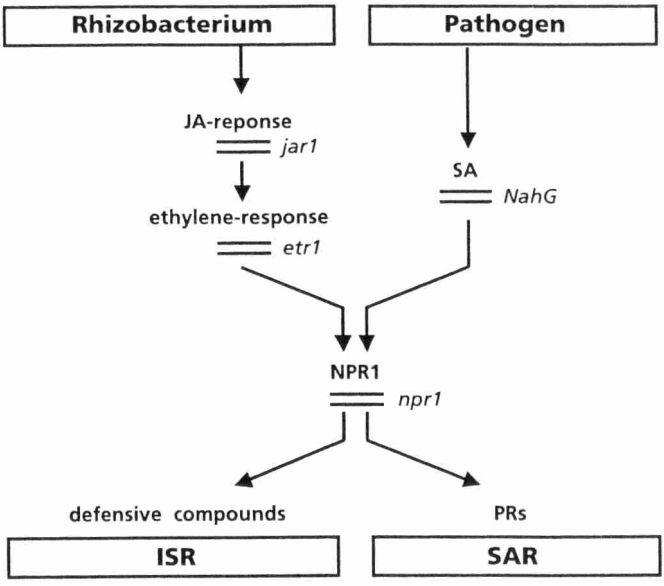

Fig. 1. Proposed model for the biocontrol bacteria-mediated ISR signalling pathway in *Arabidopsis*.

P. fluorescens WCS417r bacteria trigger a SA-independent pathway in which components from the jasmonate and ethylene response act in sequence to activate a systemic resistance response that is dependent on the regulatory protein NPR1. The ISR pathway shares signalling events that are initiated upon pathogen infection, but is not associated with *PR* gene expression. This indicates that WCS417r bacteria trigger a novel defence pathway and that resistance induced by these biological control bacteria involves the production of so far unidentified defensive compounds that are active against *Pst*. The NPR1-dependent pathway controlling *PR* gene expression is according to Ryals et al. (1996).

Pathogen-induced systemic activation of the Arabidopsis plant defensin gene *Pdf1.2*, encoding a small anti-fungal protein, was reported be independent of SA and requires components of both the jasmonate- and the ethylene-response pathway (Penninckx et al., 1996). Therefore, this defence reaction seems to share specific signalling events with WCS417r-mediated ISR. However, signal transduction leading to *Pdf1.2* gene activation appears to be independent of NPR1 (Penninckx et al., 1996), whereas WCS417r-mediated ISR requires NPR1 (Table 1). Thus, the corresponding signalling pathways must be dissimilar. Recently, analysis of the SAR signal transduction mutant *cpr5* revealed that the signalling pathways controlling NPR1-dependent SAR and NPR1-independent *Pdf1.2* gene expression are connected in early signal transduction steps, but branch upstream of SA (Bowling et al., 1997). Here we show that the ISR pathway is connected with that of SAR at a late step, since both require NPR1. Apparently, biologically-induced systemic resistance responses in plants are regulated by a complex network of signalling pathways in which not only SA, but also a concerted action of jasmonate and ethylene is involved.

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