MPMI Vol. 10, No. 7, 1997, pp. 926–928. Publication no. M-1997-0710-01N.

Research Note

The Activity of Lipid A and Core Components of Bacterial Lipopolysaccharides in the Prevention of the Hypersensitive Response in Pepper

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Received 28 April 1997. Accepted 16 June 1997.

Pre-treatment of leaves of pepper (*Capsicum annuum*) with lipopolysaccharide (LPS) preparations from enteric bacteria and *Xanthomonas campestris* could prevent the hypersensitive response caused by an avirulent *X. campestris* strain. By use of a range of deep-rough mutants, the minimal structure in *Salmonella* LPS responsible for the elicitation of this effect was determined to be lipid A attached to a disaccharide of 2-keto-3-deoxyoctulosonate; lipid A alone and the free core oligosaccharide from a *Salmonella* Ra mutant were not effective. For *Xanthomonas,* the core oligosaccharide alone had activity although lipid A was not effective. The results suggest that pepper cells can recognize different structures within bacterial LPS to trigger alterations in plant response to avirulent pathogens.

The recognition by plants of extracellular or cell surface components of phytopathogenic bacteria has a fundamental role in the interaction of these bacteria with plants. We are particularly interested in responses of plants to bacterial lipopolysaccharides (LPS) and the role that such responses may play in plant-microbe interactions. It has been known for a number of years that inoculation of tobacco plants with LPS or LPS-protein complexes from a number of bacteria can prevent the hypersensitive response (HR) caused by avirulent plant-pathogenic bacteria. This response, which is light-independent and usually localized to the site of inoculation, requires several hours to become established, suggesting that the protective mechanism depends upon a plant response to LPS. The phenomenon has been interpreted in terms of an LPS-induced resistance response and termed localized induced resistance (reviewed by Sequeira 1983). In contrast, other workers have suggested that LPS induces an increased tolerance of plants to bacteria by mediating a suppression of the HR (Mazzucchi et al. 1979). Although the molecular basis remains obscure, the prevention of HR provides a ready assay of the ability of different LPS forms and different moieties within LPS to trigger alterations in plant response. In this paper, we refer to this effect as the localized induced response (LIR), as this term has no mechanistic implications. Graham et al. (1977) showed that purified LPS from both rough and smooth forms of *Ralstonia solanacearum* and from *Escherichia coli* induced LIR in tobacco at concentrations as low as 50 μg ml^{-1}. Hydrolysis of the core oligosaccharide-lipid A linkage of LPS from *R. solanacearum* or *E. coli* caused a loss of all biological activity. It was suggested that a conserved lipid A–inner core oligosaccharide structure in the LPS from these different bacteria was responsible for the LIR induction in tobacco. The aim of the work described in this paper was to further define the structures within LPS from both enteric bacteria and the plant pathogen *X. campestris* that are required to induce LIR.

We have primarily studied effects of LPS preparations on the HR response in pepper (*Capsicum annuum*) ECW 10R induced by the avirulent *X. campestris* pv. *campestris* strain 8004 (see Table 1). LPS preparations from enteric bacteria were purchased from Sigma Chemical Co. (Poole, U.K.). LPS was prepared from the *X. campestris* strains outlined in Table 1 by the hot phenol method (Carlson et al. 1987) and further purified as described previously (Dow et al. 1995). Inoculation of fully expanded leaves of pepper ECW10R with *X. campestris* pv. *campestris* strain 8004 at 10^7 CFU ml^{-1} leads to the confluent cellular collapse of the HR within 24 h. This collapse was prevented by pre-inoculation of pepper with LPS preparations from *E. coli*, *Salmonella minnesota*, or *X. campestris* pv. *campestris* strain 8004 at 50 μg ml^{-1}. In all cases, a period of 10 to 12 h was required between inoculations to ob-

Table 1. Bacterial strains

Bacterium	Relevant characteristic	Reference
Xanthomonas campestris pv. *campestris*		
8004	Wild type; avirulent on pepper ECW 10R	Daniels et al. 1984
8530	8004::Tn*5* (truncated lipopolysaccharide)	Dow et al. 1995
X. campestris pv. *vesicatoria*		
71-21	Wild type; virulent on pepper ECW 10R	Bonas et al. 1989
X. campestris pv. *raphani*		
1946	Wild type; avirulent on pepper ECW 10R	NCPPB[a]
X. campestris pv. *armoraciae*		
1930	Wild type; avirulent on pepper ECW 10R	NCPPB

[a] National Collection of Plant Pathogenic Bacteria, Harpenden, U.K.

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tain complete prevention of the HR. A standard time of 18 h was used in all subsequent experiments.

The effect of *X. campestris* LPS was not dependent on the strain or on its interaction with pepper ECW10R. Purified LPS from *X. campestris* pv. *vesicatoria* strain 71-21 (which is virulent on pepper ECW10R) and *X. campestris* pvs. *raphani* and *armoraciae* (which are both avirulent on pepper ECW10R) all prevented the HR induced by *X. campestris* pv. *campestris* strain 8004 at 50 μg ml^{-1}. The structural requirements of *Xanthomonas* LPS for induction of LIR in pepper were assessed by the use of LPS preparations from the wild-type *X. campestris* pv. *campestris* strain 8004, from an LPS-defective mutant derivative, strain 8530 (Table 1), and fractions derived from these preparations by mild acid hydrolysis. On extraction of LPS from *X. campestris* pv. *campestris* strain 8004 with hot phenol, a fraction of the LPS partitions into the phenol phase (Dow et al. 1995). This phenol-phase LPS carries the O-antigen, while water-phase LPS preparations (which were used in the experiments described above) lack the O-antigen and consist of lipid A attached to core oligosaccharides. *X. campestris* pv. *campestris* strain 8530 has a truncated core oligosaccharide with no O-antigen. The effects of these LPS preparations on the induction of LIR are shown in Figure 1A. At 50 μg ml^{-1}, all these preparations were effective in preventing HR. However, at concentrations lower than 50 μg ml^{-1} none of these preparations were effective (data not shown). The lipid A and the core oligosaccharide components of the water-phase LPS from *X. campestris* pv. *campestris* strain 8004 were separated by mild acid hydrolysis (1% acetic acid, 100°C, 3 h), which cleaves the bond between the two moieties. The core oligosaccharide induced LIR in pepper leaves at concentrations as low as 5 μg ml^{-1} (Fig. 1A). As lipid A is not soluble in water, it was solubilized by complexing to bovine serum albumin (BSA) exactly as described by Matsuura et al. (1983). The lipid A moiety (as a noncovalent conjugate to BSA) was totally ineffective at both 5 and 50 μg ml^{-1} (Fig. 1A). As a control, BSA (1 mg ml^{-1}) was inoculated into leaf and found not to be an inducer of LIR. In addition, *E. coli* LPS suspended in a 1 mg ml^{-1} solution of BSA were still effective in inducing LIR, suggesting that BSA did not prevent the LIR reaction.

In the case of the LPS from enteric bacteria, the availability of a series of LPS variants of *S. minnesota* (Rc to Re mutants) with progressive truncations of the core oligosaccharide allowed identification of the minimal lipid A–core structure responsible for the elicitation of LIR. As shown in Figure 1B, purified LPS from *S. minnesota* Rc and Rd mutants at 50 μg ml^{-1} completely prevented HR. LPS from the Re mutant, which only contains the lipid A attached to a disaccharide of 2-keto-3-deoxyoctulosonate (KDO), gave partial inhibition. Lipid A alone was ineffective (Fig. 1B). At lower concentrations, LPS from the *Salmonella* Rc, Rd, or Re mutants did not prevent HR. When used at higher concentration, LPS from the *S. minnesota* Re mutant still gave the partial inhibition seen at 50 μg ml^{-1}. The core oligosaccharide derived from LPS from the *S. minnesota* Ra mutant by mild acid hydrolysis was to-

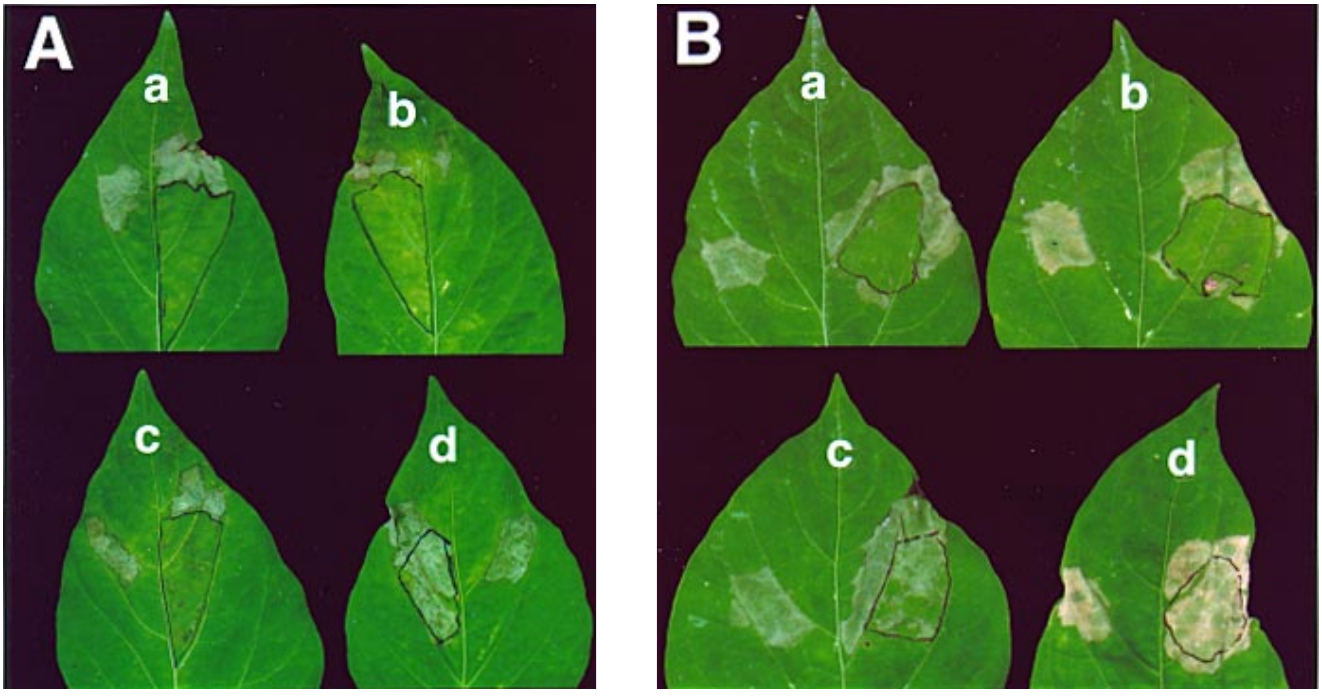

Fig. 1. Effects of inoculation of pepper ECW10R with lipopolysaccharides (LPS) or LPS derivatives from *Xanthomonas campestris* pv. *campestris* (*Xcc*) or from rough mutants of *Salmonella minnesota* (*Sm*) on the development of the hypersensitive response (HR). A pepper leaf area was inoculated with LPS or LPS derivatives and marked; 18 h later, *X. campestris* pv. *campestris* (10^7 CFU ml^{-1}) was inoculated into the marked area, the immediately adjoining area, and in a panel on the other side of the midrib of the leaf (control). **A,** (a) water-phase LPS from *Xcc* at 50 μg ml^{-1}; (b) phenol-phase LPS from *Xcc* at 50 μg ml^{-1}; (c) core oligosaccharide of *Xcc* LPS at 5 μg ml^{-1}; (d) lipid A from *Xcc* at 50 μg ml^{-1}. The phenol-phase LPS, which carries the O-antigen, water-phase LPS (which has no O-antigen), and the core oligosaccharide could prevent the HR response but lipid A was not active. **B,** (a) LPS from *Sm* Rc mutant at 50 μg ml^{-1}; (b) LPS from *Sm* Rd mutant at 50 μg ml^{-1}; (c) LPS from *Sm* Re mutant at 50 μg ml^{-1}; (d) lipid A from *Sm* Ra mutant at 50 μg ml^{-1}. LPS from the Re mutant, which contains lipid A attached to $(KDO)_2$, partially prevents HR whereas lipid A is inactive.

tally ineffective at inducing LIR (data not shown). These results suggest that LIR induction in pepper by enteric bacterial LPS minimally requires the lipid A-KDO-KDO structure.

These results with enteric bacterial LPS are entirely consistent with the previous contention by Graham et al. (1977) that the active portion of the LPS molecule for induction of LIR is the intact lipid A–inner core oligosaccharide. However, in contrast to these previous observations, we have shown that the core oligosaccharide of LPS of *X. campestris* was effective in inducing LIR, an effect not seen with the core oligosaccharides of either *R. solanacearum* or the enteric bacteria. The differences in monosaccharide composition between these various oligosaccharides (Ojanen et al. 1993; Drigues et al. 1985) suggest substantial differences in the structure that may account for these observations. It is also possible that the oligosaccharide domain within LPS from other bacteria has LIR-inducing activity but that the activity depends upon an acid-labile moiety that is lost as a consequence of the procedures used to derive the core oligosaccharide. This finding does not necessarily mean that the core oligosaccharide is the only structure in *Xanthomonas* LPS capable of inducing LIR. A lipid A–disaccharide core derivative (such as the LPS from the *S. minnesota* Re mutant) could also be active, although the lack of an appropriate LPS mutant in *Xanthomonas* means that we are not able to test this directly. However, the results show that the ability to induce LIR is not restricted to lipid A derivatives and can reside in different domains within LPS. As it is likely that the structure of the core oligosaccharide of *Xanthomonas* LPS differs significantly from that of the lipid A-KDO_2 of the enteric bacteria (Ojanen et al. 1993; Dow et al. 1995), we infer the existence of multiple pathways for LIR induction in pepper.

ACKNOWLEDGMENTS

The Sainsbury Laboratory is supported by a grant from the Gatsby Charitable Foundation. This work was carried out according to the provisions of the Ministry of Agriculture, Fisheries and Food license PHF1185/8(48) issued under the Plant Health (Great Britain) Order 1987 (statutory instrument 1758).

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