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Research Note

Lipopolysaccharide from *Xanthomonas campestris* Induces Defense-Related Gene Expression in *Brassica campestris*

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Purified lipopolysaccharide (LPS) from *Xanthomonas campestris* pv. *campestris* induced accumulation of transcript for β-1,3-glucanase in turnip at concentrations of 1 μg/ml. The lipid A-inner core structure was required for activity but the O-antigen had no role. We suggest that release of LPS in planta triggers expression of at least some defense-related genes.

Additional keywords: altered lipopolysaccharide, black rot.

There is considerable interest in bacterial products which are responsible for triggering defense-related gene expression in plants during interactions with phytopathogenic bacteria (Davis et al. 1984; Ausubel et al. 1993; Dangl et al. 1993; Jakobek and Lindgren 1993; Palva et al. 1993). Although emphasis has been placed on gene expression during incompatible interactions, defense-related genes are also activated in compatible interactions, usually at later time points. In some pathosystems, activation of certain genes is only seen in the later phases of compatible interactions associated with considerable bacterial growth (Dong et al. 1991). In the compatible interaction between turnip (*Brassica campestris*) and *Xanthomonas campestris* pv. *campestris*, the causal agent of black rot of crucifers, transcripts for β-1,3-glucanase (BGL) accumulate up to a maximum level at 24 h after inoculation (Newman et al. 1994). This is a slower response than that seen in incompatible interactions with *X. campestris* pvs. *armoraciae* or *raphani*. Analysis of BGL transcript levels in response to defined mutants of *X. campestris* pv. *campestris* suggest that transcription is not triggered by the action of extracellular enzymes, other extracellular proteins or *hrp* gene dependent processes (Newman et al. 1994). Rather, the levels of transcript reflect the growth of the different bacterial strains. Two exceptions are responses to mutants carrying lesions in genes involved in the biosynthesis of extracellular polysaccharide (EPS) or which give altered lipopolysaccharide (LPS); here bacterial numbers decline rapidly after inoculation although transcript levels are up to 70% of those seen in the wild type response. In this paper we report that purified LPS from both wild type *X. campestris* pv. *campestris* strain 8004 and the LPS-defective mutant strain 8530 can induce BGL transcript accumulation in turnip. This raises the possibility that in plant-pathogen interactions, expression of at least some defense-related genes occurs as a response to LPS released either as a consequence of normal bacterial growth or rapid bacterial death within the plant.

Two forms of LPS which partition into different phases on hot phenol-water extractions of *X. campestris* pv. *campestris* wild-type strain 8004 have been described (Dow et al. 1995). Phenol-phase LPS carries an O-antigen, whereas water-phase LPS comprises lipid A-core oligosaccharide alone. These LPS forms (as triethylamine salts) were inoculated at 1 and 50 μg/ml into leaves of turnip cultivar Just Right. Total RNA samples were isolated at different times after inoculation and analyzed by Northern blotting with a cDNA for turnip β-1,3-glucanase as described by Newman et al. (1994). We have previously shown that BGL transcript does not accumulate in response to wounding or inoculation with water or salicylic acid (Newman et al. 1994). With 1 μg of wild-type water-phase LPS per ml, BGL transcript accumulation was first detected at 4 h after inoculation, and levels increased steadily up to 24 h (Fig. 1). At 50 μg/ml, transcript accumulation in response to the water-phase LPS was transient, with lower levels at 24 h than at 12 h. Similar results were seen with the phenol-phase LPS forms (not shown).

To determine which moieties in LPS were required for BGL induction, transcript levels were investigated in response

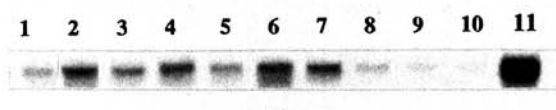

Fig. 1. Northern blot of total RNA extracted from turnip leaves at different times after inoculation with purified water-phase LPS preparations from wild type strain 8004 and *lps* mutant strain 8530 and probed with the β-1,3-glucanase cDNA clone. Lanes 1 and 2: 8530 LPS, 1 μg/ml, 12 and 24 h; lanes 3 and 4: 8530 LPS, 50 μg/ml, 12 and 24 h; lanes 5 and 6: 8004 LPS, 1 μg/ml, 12 and 24 h; lanes 7 and 8: 8004 LPS, 50 μg/ml, 12 and 24 h; lanes 9 and 10: water control, 12 and 24 h; lane 11: 8004 bacteria at 10^7 per ml, 24 h.

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to LPS isolated from the LPS-defective mutant strain 8530 and subfractions derived from the wild-type LPS by mild acid hydrolysis. Both water-phase and phenol-phase LPS from strain 8530 have a truncated core and the phenol-phase LPS lacks the O-antigen seen in the wild type (Dow et al. 1995). However, these LPS forms were effective in induction of BGL transcript accumulation, with a steady increase in levels from 4 h up to 24 h in response to both 1 and 50 μg/ml (Fig. 1). Mild acid hydrolysis (1% acetic acid, 100°C, 3 h) of the wild type water-phase LPS cleaves the bonds between the lipid A moiety (which is insoluble in water) and the core oligosaccharides (Dow et al. 1995). The lipid A was collected by centrifugation, washed with water and conjugated with bovine serum albumin (BSA) exactly as described by Matsuura et al. (1983). No induction of BGL transcription was seen in response to this preparation up to concentrations corresponding to 5 μg of lipid A per ml. The solution after mild acid hydrolysis, containing the core oligosaccharides, was lyophilized to remove acetic acid. No induction of BGL transcription was seen in response to aqueous solutions of these core oligosaccharides at concentrations up to 10 μg/ml. Overall these results suggest that an intact lipid A-inner core structure is required for the induction of BGL, but that the O-antigen has no role. However it is also possible that other acid-labile residues required for activity are lost during the acetic acid treatment.

LPS from *Escherichia coli* or *Salmonella minnesota* (Sigma) did not induce transcript accumulation at concentrations up to 50 μg/ml. Lipid A from *E. coli* (as a BSA conjugate) was also ineffective at 10 μg/ml as was delipidized *E. coli* LPS derived by alkaline hydrolysis (Sigma). These results are consonant with the lack of induction of BGL in turnip by *E. coli* (Newman et al. 1994).

The role of LPS in bacterial-plant interactions is likely to be complex. As well as providing an indispensible barrier for the bacteria against toxic plant compounds (Nikaido and Vaara 1985), the interaction of LPS with the plant cell may promote pathogenesis or symbiosis (Dazzo et al. 1991) and/or may trigger defense-related responses (see below). There are, however, few reports of induction of specific gene products in response to LPS application to plants. It has been known for some time that inoculation of tobacco plants with purified LPS or LPS-protein complexes from a number of bacteria can prevent the hypersensitive response to incompatible bacteria and reduce symptoms in compatible interactions (Mazzucchi and Pupillo 1976; Graham et al. 1977; Mazzucchi et al. 1979). However nothing is known of the molecular basis of these plant responses. LPS from *Pseudomonas solanacearum* (at 400 μg/ml) induces the synthesis of new polypeptides in tobacco (Leach et al. 1983) and LPS preparations from *Pseudomonas syringae* pv. *syringae* are weak, nonspecific elicitors of phytoalexin biosynthesis in soybean hypocotyls (Barton-Willis et al. 1984).

Extrapolation from results with purified LPS preparations to interactions that occur in planta clearly presents many problems. Nevertheless, the results presented here do suggest an explanation for our previous observations on BGL transcript accumulation in response to mutants of *Xanthomonas campestris* and other bacteria. Release of LPS may occur as a normal process during the growth of phytopathogenic bacteria in plants as has been suggested for symbiotic rhizobia (Dazzo et al. 1991) and shown for bacteria, including *Erwinia*, growing in culture (Crutchley et al. 1967; Rothfield and Pearlman-Kothencz 1969; Mazzucchi and Pupillo 1976). The substantial growth of most mutants of *Xanthomonas* tested in our previous experiment (Newman et al. 1994) may have resulted in release of sufficient LPS to trigger BGL transcript accumulation, whereas heat-killed or antibiotic-killed bacteria or mutant strains that failed to grow in planta were inactive. In the case of the *eps* and *lps* mutants, we postulate that release of LPS caused by the substantial and rapid bacterial death *within* the plant triggers the responses seen. However a role for LPS alone in triggering BGL transcript accumulation during compatible interactions must remain speculative. Release of other bacterial components such as periplasmic glucans and other oligosaccharides, some of which are known to elicit different plant responses (Stefani et al. 1994), could clearly also occur.

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LITERATURE CITED

Ausubel, F. M., Glazebrook, J., Greenberg, J., Mindrinos, M., and Yu, G.-L. 1993. Analysis of the *Arabidopsis* defense response to *Pseudomonas* pathogens. Pages 393-403 in: Advances in Molecular Genetics of Plant-Microbe Interactions. E. W. Nester and D. P. S. Verma, eds. Kluwer Academic Publishers, Dordrecht.

Barton-Willis, P. A., Wang, M. C., Holliday, M. J., Long, M. R., and Keen, N. T. 1984. Purification and composition of lipopolysaccharides from *Pseudomonas syringae* pv. *glycinea*. Physiol. Plant Pathol. 25:387-398.

Crutchley, M. J., Marsh, D. G., and Cameron, J. 1967. Free endotoxin. Nature 214:1052.

Dangl, J., Debener, T., Gerwin, M., Kiedrowski, S., Ritter, C., Bendahmane, A., Liedgens, H., and Lewald, J. 1993. Genetic approaches to an understanding of specific resistance responses of *Arabidopsis thaliana* against phytopathogenic pseudomonads. Pages 405-415 in: Advances in Molecular Genetics of Plant-Microbe Interactions. E. W. Nester and D. P. S. Verma, eds. Kluwer Academic Publishers, Dordrecht.

Davis, K. R., Lyon, G. D., Darvill, A. G., and Albersheim, P. 1984. Host-pathogen interactions. XXV. Endopolygalacturonic acid lyase from *Erwinia carotovora* elicits phytoalexin accumulation by releasing plant cell wall fragments. Plant Physiol. 74:1286-1290.

Dazzo, F. B., Truchet, G. L., Hollingsworth, R. I., Hrabak, E. M., Pankratz, H. S., Philip-Hollingsworth, S., Salzwedel, J. L., Chapman, K., Appenzeller, L., Squartini, A., Gerhold, D., and Orgambide, G. 1991. *Rhizobium* lipopolysaccharide modulates infection thread development in white clover root hairs. J. Bacteriol. 173:5371-5384.

Dong, X., Mindrinos, M., Davis, K. R., and Ausubel, F. M. 1991. Induction of *Arabidopsis* defense genes by virulent and avirulent *Pseudomonas syringae* strains and by a cloned avirulence gene. Plant Cell 3:61-72.

Dow, J. M., Osbourn, A. E., Wilson, T. J. G., and Daniels, M. J. 1995. A locus determining pathogenicity of *Xanthomonas campestris* is involved in lipopolysaccharide biosynthesis Mol. Plant-Microbe Interact. 8:768-777.

Graham, T. L., Sequeira, L., and Huang, T.-S. R. 1977. Bacterial lipopolysaccharides as inducers of disease resistance in tobacco. Appl. Environ. Microbiol. 34:424-432.

Jakobek, J. L., and Lindgren, P. B. 1993. Generalized induction of defense responses in bean is not correlated with the induction of the hypersensitive response. Plant Cell 5:49-56.

Matsuura, M., Kojima,Y., Hamma, F. Y., Kubota, Y., Shibukawa, N., Shibata, M., Inage, M., Kusumota, S., and Shiba, T. 1983. Interferon-

inducing, pyrogenic and proclotting enzyme of horseshoe crab. Activation activities of chemically synthesised lipid A analogues. Eur. J.Biochem. 137:639-642.

Mazzucchi, U., and Pupillo, P. 1976. Prevention of confluent hypersensitive necrosis in tobacco leaves by a bacterial protein-lipopolysaccharide complex. Physiol. Plant Pathol. 9:101-112.

Mazzucchi, U., Bazzi, C., and Pupillo, P. 1979. The inhibition of susceptible and hypersensistive reactions by protein-lipopolysaccharide complexes from phytopathogenic pseudomonads: Relationship to polysaccharide antigenic determinants. Physiol. Plant Pathol. 14:19-30.

Newman, M.-A., Conrads-Strauch, J., Scofield, G., Daniels, M. J., and Dow, J. M. 1994. Defense-related gene induction in *Brassica campestris* in response to defined mutants of *Xanthomonas campestris* with altered pathogenicity. Mol. Plant-Microbe Interact. 7:553-563.

Leach, J. E., Sherwood, J., Fulton, R. W., and Sequeira, L. 1983. Comparison of soluble proteins associated with disease resistance induced by bacterial lipopolysaccharide and by viral necrosis. Physiol. Plant Pathol. 23:377-385.

Nikaido, H., and Vaara, M. 1985. Molecular basis of bacterial outer membrane permeability. Microbiol. Rev. 49:1-32.

Palva, T., Holmstrom, K.-O., Heino, P., and Palvio, E. T. 1993. Induction of plant defense response by exoenzymes of *Erwinia carotovora* subsp. *carotovora*. Mol. Plant-Microbe Interact. 6:190-196.

Rothfield, L., and Pearlman-Kothencz, M. 1969. Synthesis and assembly of bacterial membrane components A lipopolysaccharide-phospholipid-protein complex excreted by living bacteria. J. Mol. Biol. 44:477-492.

Stefani, E., Bazzi, C., and Mazzucchi, U. 1994. Modification of the *Pseudomonas syringae* pv. *tabaci*-tobacco leaf interaction by bacterial oligosaccharides. Physiol. Mol. Plant Pathol. 45:397-406.