Role of Bacterial Lipopolysaccharide in Attachment of Agrobacterium to Moss

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ABSTRACT

Gametophore induction in moss by Agrobacterium tumefaciens was inhibited by addition of lipopolysaccharide (LPS) from A. tumefaciens. The LPS did not affect bacterial viability or appear to bind to bacterial cells. LPS from nonbinding Agrobacterium radiobacter was not effective in reducing gametophore formation. A. tumefaciens LPS, if added 24 hours after addition of viable bacterial cells, had no effect in reducing gametophore formation. The polysaccharide portion of the LPS was identified as the binding component necessary for attachment of agrobacteria for induction of gametophores in moss and tumors in higher plants.

Agrobacterium tumefaciens strains B6 and ATCC 15955, which are tumorigenic on higher plants, have been shown to promote gametophore formation in the moss Physaliaziella selwynii (2, 5), while nontumorigenic strains have no effect on the moss (5). Crown gall tumor induction by A. tumefaciens requires a specific complementary binding between the bacterium and a plant host wound site (1). Spiess et al. (4) showed that a similar attachment process, but without woundness, is necessary for moss gametophore induction by bacteria. This paper describes experiments demonstrating that the bacterial LPS, the bacterial binding component in tumor induction (8), is also involved in bacterial attachment to moss.

MATERIALS AND METHODS

Cultures of A. tumefaciens (Smith and Town.) Conn, strains B6 and ATCC 15955, and Agrobacterium radiobacter (Beijerinck and van Delden) Conn, strains S1005 and ATCC 6467, were grown to stationary phase (48 hr) as previously described (1). For addition to the moss, cultures were washed twice by centrifugation at 10,000 g for 30 min with resuspension in moss medium each time. Concentrations of bacteria in experiments were from 4 \times 10^8 to 10^9 cells/ml.

For LPS preparations, bacteria were harvested by centrifugation at 10,000 g for 30 min and resuspended in 0.9% (w/v) NaCl and the process repeated. LPS was obtained by treatment of lyophilized cells with 45% phenol (v/v) at 65 C as described by Westphal and Jann (7). The LPS was further purified, dialyzed, and lyophilized as previously described (8). LPS to be used with the moss was lyophilized, weighed, suspended in distilled H_2O at 55 C for 5 min, and then filter-sterilized with a 0.22- \mu m Millipore filter. LPS was used in each experiment at a concentration of 40 \mu g/ml, 0.5 ml being added to each culture dish containing a total volume of 4.5 ml of moss medium with approximately 25 plants.

Polysaccharide from LPS was extracted according to the method described by Weckesser et al. (6). LPS (10 mg/ml) was hydrolyzed in 1% (v/v) acetic acid for 3 hr at 100 C. The lipid portion was removed by centrifugation at 4,000 g for 60 min and the polysaccharide was obtained by lyophilizing the supernatant. For use with the moss, the polysaccharide was treated in the same manner as the LPS.

The moss, P. selwynii, was cultured as described by Spiess et al. (3), treated with bacteria 17 days after sowing, and maintained in a constant temperature cabinet at 25 C with a 16:8-hr light-dark cycle until examined with a dissecting microscope at day 34.

RESULTS AND DISCUSSION

Increase in moss gametophore induction is dependent upon the number of viable B6 cells added (2). LPS, derived from either of the two tumorigenic strains, B6 or ATCC 15955, and added to the moss with the viable B6, reduced the number of gametophores produced in five different experiments by up to 76% (Table I). Although the gametophore response of moss plants to Agrobacterium varied in each of five replicate experiments, the per cent reduction by addition of 40 \mu g/ml of LPS to each culture was quite uniform, indicating reproducibility of the results. LPS alone had no effect on moss development. The same magnitude of effect, 70% reduction in number of tumors, was shown when B6-LPS was mixed with viable B6 and inoculated on pinto bean leaves (8).

It is possible that the LPS binds to the moss preventing bacterial binding, or the LPS may bind to the Agrobacterium cell preventing attachment to the moss. To test these hypotheses, B6-LPS was mixed with viable B6 and incubated at room temperature for 15 min. The bacteria were then harvested by centrifugation and washed once. Control bacteria were treated in the same manner but without LPS. When added to the moss, the untreated B6 induced 56 gametophores on 75 plants, and the LPS-treated B6 gave similar results, 53 gametophores on 75 plants, indicating that the LPS was probably not binding to the bacteria. For a more rigorous control, bacteria and LPS should be incubated for longer periods of time. It had previously been shown that treatment of B6 with LPS had no effect on bacterial viability as estimated by subsequent dilution and plating (8).

B6-LPS was tested for inhibition of moss gametophore formation by A. tumefaciens B6 by adding LPS before or after the viable B6 cells with a 6- or 24-hr interval between additions (Table II). Procedures were the same as described by Spiess et al. (4) for time-sequence experiments with viable and heat-killed bacteria.

LPS inhibited gametophore formation by B6 when they were added at the same time. LPS added 6 hr before or after B6 was...
equally effective in inhibiting gametophore induction, suggesting that the binding process of either bacteria or LPS to the moss was not complete at this time. When LPS was added 24 hr after B6, there was no reduction in the number of gametophores compared to gametophore induction by B6 alone, indicating that binding of B6 had been completed. Addition of bacteria 24 hr after LPS showed that the binding of the LPS to the moss had been completed, almost completely preventing gametophore formation. The LPS functioned in this system as if it inhibited gametophore formation by preventing bacterial binding. Once the attachment process of the B6 bacteria was complete, LPS addition had no inhibitory effect. When whole, heat-killed cells were similarly used to reduce gametophore formation induced by viable cells, the Agrobacterium-moss binding appeared to be complete within 6 hr (4). LPS appears to be more effective than heat-inactivated B6 in interfering with the binding of active B6 to moss.

The polysaccharide obtained after hydrolytic removal of the lipid from purified LPS from a binding strain also significantly reduced gametophore formation by B6 bacteria when added with the bacteria to moss (Table III). The inhibitory effect of the polysaccharide was as great as that of the LPS and, therefore, the polysaccharide appears to be the portion of the LPS responsible for binding. Replicate experiments gave similar results. Polysaccharide alone had no effect on moss gametophore induction. Comparable results were obtained for tumor induction on pinto bean leaves (9).

When A. radiobacter strains S1005 and ATCC 6467 were added to the moss, they did not stimulate gametophore formation and when added with B6, there was no reduction in gametophore formation as compared to the B6 alone (5). The LPS derived from strains S1005 and ATCC 6467 also had no effect on the B6 induction of gametophores on moss, giving, respectively, 94 and 108% of the number of gametophores induced by B6 alone. This supports the conclusion that the LPS is the binding component since it showed the same binding specificity as the strain from which it was derived. The lack of inhibitory effect by these two avirulent strains and their LPS is similar to their failure to inhibit tumor formation on bean leaves (8).

The LPS fraction, specifically the polysaccharide portion, from A. tumefaciens appears to function as the portion of the bacterium responsible for the binding of Agrobacterium to P. selwynii. This bacterial binding is the initial step of an interaction between moss and agrobacteria that results in gametophore development on the moss, P. selwynii. Whether this binding is permanent or temporary cannot be established by these data. Agrobacterium binding with moss has similarities to that of Agrobacterium to higher plants. Further studies of moss-Agrobacterium binding may help clarify other bacterial-plant interactions.

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LITERATURE CITED