A bacterial extracellular polysaccharide which enhances the attachment of *Agrobacterium tumefaciens* to the plant cell surface

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Summary. Intensive screening of soil microbial cultures for agglutinating activity of *Agrobacterium tumefaciens* resulted in the discovery of a bacterium, *Xanthobacter* sp. KB-3001, which produced an agglutinin of *A. tumefaciens*. This compound is an acidic polysaccharide consisting of glucose and galacturonic acid in the ratio 4:1. This compound assists the attachment of *A. tumefaciens* to plant cells and promotes crown gall formation, owing to its affinity to both *A. tumefaciens* and plant cells.
Key words. Agrobacterium tumefaciens; agglutination; extracellular polysaccharide; *Xanthobacter* sp.; cell attachment.

The tumor-inducing (Ti) plasmids in *Agrobacterium tumefaciens* are causative agents for crown gall formation of many dicotyledonous plants. Derivatives of these plasmids have been used as cloning vectors for plant genetic engineering. With the root-inducing (Ri) plasmids of *Agrobacterium rhizogenes*, the Ti plasmids have been for a long time the only vectors used to insert foreign DNA into the genome of higher plants. However, improvement of the efficiency of plant cell transformation is required because of the poor expression of foreign genes.

The first step in tumor formation in vivo is the site-specific attachment of *A. tumefaciens* to the plant cell surface [2]. It has been suggested that the tumor formation could be enhanced by concanavalin A [3], soybean lectin [3], and poly-L-lysine [4] facilitating the attachment of *A. tumefaciens* to plant cell surfaces.

Assuming that a compound which assists the attachment of *A. tumefaciens* to plant cells also agglutinates the cells of the bacterium, we have employed the agglutinating activity as a guide in searching for compounds capable of increasing the frequency of transformation. The activity of the culture filtrates of microorganisms was assayed by a decrease in turbidity (absorbance at 660 nm) of a cell suspension of *A. tumefaciens* (IFO13263). Intensive screening of soil microorganisms resulted in the discovery of a bacterium, designated as KB-3001, which produces an agglutinin tentatively named PS-1. Taxonomic study revealed that this bacterium is a species of *Xanthobacter*.

Guided by the agglutinating activity, production and purification of PS-1 were achieved. *Xanthobacter* sp. KB-3001 was grown in 500 ml shaking flasks, each containing 150 ml of potato-sucrose-malt extract medium. The bacterial cells were removed from the fermentation liquor by centrifugation and the supernatant was dialysed and lyophilised. The lyophilisate was purified by DEAE-Cellulofine (AM) column chromatography (linear gradient elution with 0–2 M NaCl, 1000 ml). Because PS-1 was retained by an anion exchanger and was positive to the phenol-sulfuric acid color reaction [5] test, it seemed to be an acidic polysaccharide. Its molecular weight was determined to be 3.5×10^6 by GPC-HPLC analysis. Quantitative analyses by GLC of the alditol acetates derived from the hydrolysates of the original and carboxyl reduced [6] PS-1 showed that the original PS-1 comprised glucose and galacturonic acid in a ratio 4:1.

The minimum effective concentration of PS-1 was 500 µg/ml, and at this concentration the agglutinated bacteria sedimented within 30 min. To investigate the interaction between PS-1 and the plant cell surface, PS-1 adherence to the surface of tobacco protoplasts was observed under a fluorescence microscope, using the fluorescent stain Calcoflour White ST [7–9]. One hundred µl of 0.05% PS-1 solution in 0.7 M mannitol and 100 µl of tobacco protoplast suspension (10^4 cell/ml) were mixed and incubated at 27 °C for 3 h. The protoplasts were stained for 5 min with 0.05% Calcoflour White ST in 0.7 M mannitol. The stained protoplasts were washed with 0.7 M mannitol and measured under a fluorescence microscope (excitation wavelength: 380 nm, measuring wavelength: 430 nm). Since regeneration of protoplasts had not yet commenced, PS-1 adhering to the protoplast surface could be measured by this method. The stronger fluorescence intensity of treated protoplasts (fig. 1) shows that PS-1 has an affinity for the surfaces of tobacco protoplasts. It was apparent that PS-1 has an affinity for the surfaces of both plant and *A. tumefaciens* cells.

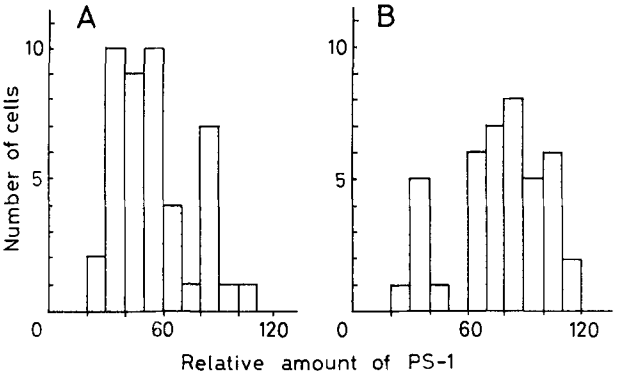

Figure 1. Histograms of relative amounts of PS-1 adhering to the surface of tobacco protoplasts (*Nicotiana tabacum* cv. 38 Havana). *A* control; *B* treated with 0.05% PS-1.

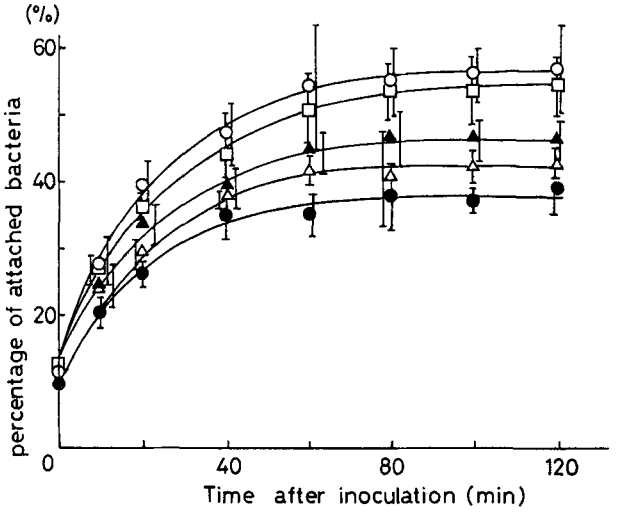

Figure 2. Effect of PS-1 in attachment of *Agrobacterium tumefaciens* onto cultured tobacco cells (*Nicotiana tabacum* cv. 38 Havana). PS-1 concentrations were 500 (○), 250 (□), 125 (▲), 63 (△), 0 µg/ml (●). Bars indicate standard deviation of a minimum of five experiments.

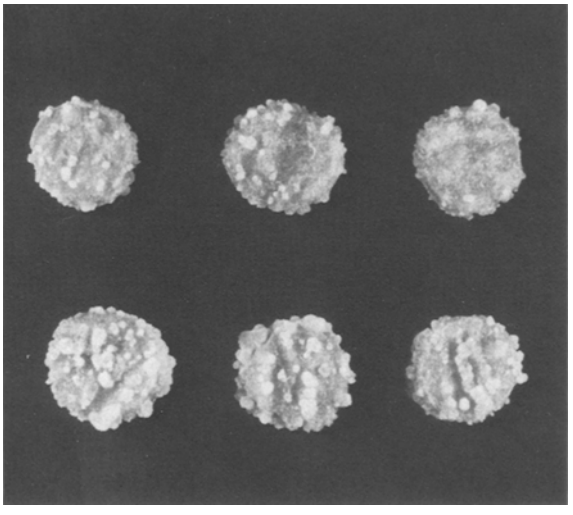

Figure 3. Effect of PS-1 on tumor formation of potato tuber disc (*Solanum tuberosum*). *A* control; *B* treated with PS-1.

The findings above suggest that PS-1 assists the attachment of *A. tumefaciens* to plant cultured cells. To confirm this assumption, the effect of PS-1 on attachment of the bacterium to the tobacco cell surface was investigated. To a suspension of cultured tobacco cells in 2 ml of Murashige-Skoog medium were added 1 ml of suspension of *A. tumefaciens* and 1 ml of PS-1 solution at various concentrations. Two hundred µl of this mixture was taken at regular time intervals and filtered through JK wipes (TM) to separate the attached and the free bacterial cells. The numbers of attached and free bacterial cells were measured by a viable cell count method, and the percentage of bacterial inoculum attached to tobacco cells was calculated. Regardless of the addition of PS-1, the percentage of bacterial inoculum attached to tobacco cells reached a plateau within 80 min and the percentage at the plateau increased from 35% to 55%, depending on the concentration of PS-1 (fig. 2).

The effect of PS-1 on plant cell transformation using *A. tumefaciens* was examined by the potato tuber disc assay first described by Anand and Heberlein[10]. This assay promises a semi-quantitative examination of the frequency of plant transformation by *A. tumefaciens*[11,12]. Tumors with a diameter bigger than 2 mm were counted (fig. 3). On average, 15.6 tumors were formed on a treated potato disc compared to 9.0 in the control. PS-1 apparently enhanced the crown gall formation.

It was concluded that owing to its affinity for both *A. tumefaciens* and plant cells, PS-1 increased the frequency of transformation of plant cells by *A. tumefaciens* by assisting the attachment of the bacterium to the plant cell surface. This compound would be useful for use with *A. tumefaciens* cells as vectors in gene recombination of plant cells.

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Application of decision theory in understanding food choice behavior of hatchling loggerhead sea turtles and chemosensory imprinting in juvenile loggerhead sea turtles[1]

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Summary. Food choice behavior of hatchling loggerhead sea turtles, *Caretta caretta,* and chemosensory choice behavior of juvenile loggerhead sea turtles artificially imprinted prior to emergence from the nest were examined using models derived from choice threshold and set releasing value theories of decision making. Modelling results indicate that food choice behavior of hatchlings is better described by a model based on set releasing value theory and that choice behavior of chemically imprinted juveniles is better described by a model based on choice threshold theory.
Key words. Feeding behavior; food choice; sea turtles; decision making; imprinting; chemoreception.

There is little information on the ontogeny of feeding behavior in turtles, generally, and relatively little experimental evidence to support or refute chemosensory imprinting in sea turtles[2]. Understanding the early feeding behavior and food preference development of sea turtles and chemosensory imprinting in sea turtles is important for the conservation of these endangered animals[4]. The purpose of this study was to use theoretical mathematical models based on ethology and decision theory to examine the underlying mechanism/s of food choice behavior in hatchling loggerhead, *Caretta caretta,* sea turtles and of chemosensory choice behavior in juvenile animals, behaviors which have been previously described ethologically[3,4].

Feeding experiments. It has been proposed that early feeding experience in hatchlings may affect later feeding behavior and may constitute an important component of early