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Uptake of Galacturonic Acid in *Erwinia chrysanthemi* EC16

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Uptake of [^{14}C]galacturonic acid in *Erwinia chrysanthemi* was found to be stimulated during growth on pectin and its degradation products, saturated digalacturonic acid and galacturonic acid. Cells isolated from macerated potato tissue also showed increased levels of uptake activity for this molecule compared with those showed by glycerol-grown cells. Uptake was found to be an active process, and it displayed saturation kinetics. An *Escherichia coli* galacturonic acid transport mutant harboring the *E. chrysanthemi exuT* gene(s) for galacturonic acid uptake was able to transport galacturonic acid but unable to take up the dimer [^{3}H]digalacturonic acid.

The production of enzymes that breach the plant cell wall is often a necessary prerequisite for a successful pathogenic infection. Many members of the plant pathogenic genus *Erwinia* produce pectin-and pectate-degrading lyases and hydrolases (1, 2, 7). Products of pectin degradation (monomers of galacturonic acid [GA] and saturated and unsaturated dimers and oligomers of GA and 2-keto-3-deoxygluconate) are known to be catabolized within cells of *Erwinia* spp. (6, 7, 11). Intracellular catabolism necessitates uptake of the compounds being catabolized. This study focuses on the mechanism of GA uptake in *Erwinia chrysanthemi* EC16 as well as in vitro and in vivo stimulation of the transport system.

With [^{14}C]GA (58 mCi/mmol; Sigma Chemical Co.) or [^{3}H]digalacturonic acid (dGA) (15.5 mCi/mmol) as substrate, uptake in cells grown to exponential phase (8 h) in M9 salts medium with a carbon concentration of 0.1% at 30°C was monitored (8). dGA was radiolabeled essentially as described by Evans et al. (4) with tritium gas in the presence of a metal catalyst. The resulting [1-^{3}H]dGA was purified by ion-exchange chromatography essentially as described by Donner et al. (3) with AG-MP-1 resin and a linear gradient of 0 to 100% 1 M sodium formate, pH 4.7. The carbon substrates used for growth were either glycerol, GA, dGA, or pectin plus poly(GA). Uptake was measured at 30°C in M9 salts containing 5 mM $MgCl_2$ and 0.3 mM dithiothreitol with a substrate concentration of 0.5 mM. The final assay volume of 0.5 ml contained 50 μl of an exponentially grown cell suspension. The cell suspension was preincubated for 2 min in the transport assay buffer at the assay temperature prior to the addition of radioactive substrate (250 nmol of 0.058 mCi of [^{14}C]GA per mmol or 250 nmol of 0.015 mCi of [^{3}H]dGA per mmol). Uptake was terminated at various times by filtration through a nitrocellulose filter (0.45-μm pore size; Millipore). All inhibitor and osmotic shock experiments were carried out with GA-grown cells. The transport system concentrated the substrate approximately 1,000-fold within cells (1 mg [dry weight] of cells has a cell volume of approximately 2.7 μl [5, 12]). Uptake of GA was saturable with increasing substrate concentrations between 0.0002 and 0.1 mM, with an apparent K_m of 25 μM and V_{max} of 38 nmol/min/mg of protein (Fig. 1). The addition of a 10-fold excess of nonradioactive GA resulted in efflux of approximately 65% of the radioactive analog over 10 min. This suggests that over the duration of the assay, the compound is not significantly incorporated into cellular material. Efflux measurements were made by the addition of 5 mM GA 2.5 min after the addition of the [^{14}C]GA. Aliquots were removed at 1-min intervals.

As a first step toward an understanding of the regulation of the uptake systems for plant cell wall molecules into phytopathogenic bacteria, the influence of the carbon substrate used for growth on the activity of the GA uptake system was studied. Glycerol-grown cultures possessed measurable levels of [^{14}C]GA uptake. The inclusion of GA, dGA, or a mixture of pectin and poly(GA) into the growth medium increased the activity of the uptake system two- to threefold (Fig. 2). These induction values are slightly lower than the fivefold increase in uptake observed by Hugovieux-Cotte-Pattat et al. (5) for uptake of the aldohexuronate, glucuronic acid, in *E. chrysanthemi* B374. To study the effects on the GA transport system in bacteria grown in plant tissue, surface-sterilized potatoes were inoculated at two or three positions to a depth of 0.5 cm with approximately 10^5 cells and incubated at 30°C for 48 h in a moist, sterile environment

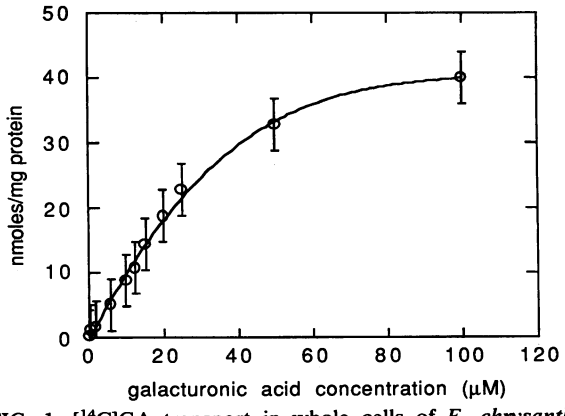

FIG. 1. [^{14}C]GA transport in whole cells of *E. chrysanthemi* EC16. Uptake measurements were made 15 s after the addition of radioactive substrate. Values represent averages of four independent experiments. The bars represent standard errors.

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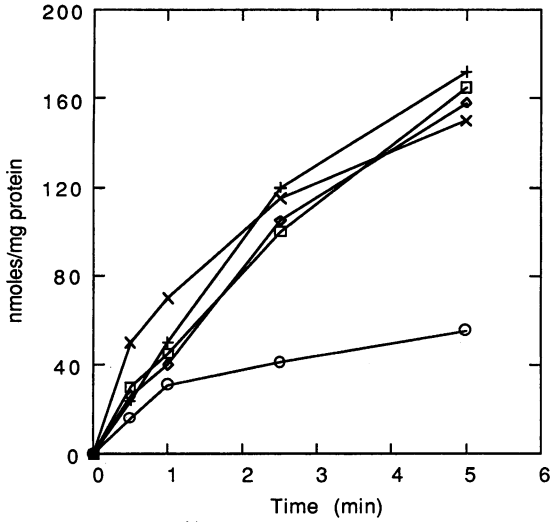

FIG. 2. Uptake of [^{14}C]GA by cells of *E. chrysanthemi* EC16 grown in vitro or in vivo. Cells were grown on the carbon source (0.1%) indicated and assayed for [^{14}C]GA uptake. The carbon sources used for growth were glycerol (○), GA (◇), dGA (□), and a pectin-poly(GA) mixture (+). For the cells grown in vivo (x), the inoculum consisted of glycerol-grown cells. Values represent averages of at least four independent experiments.

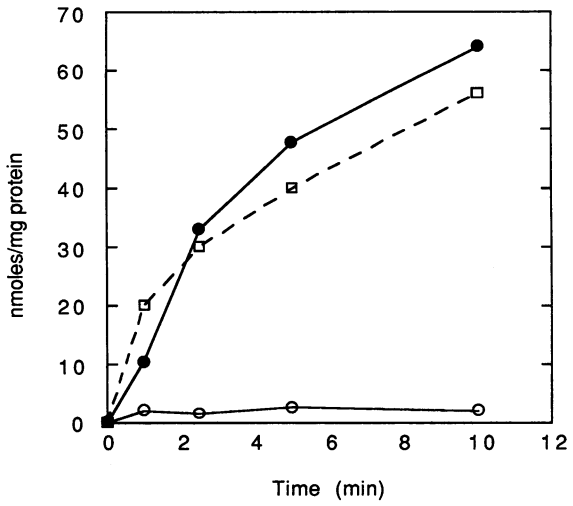

FIG. 3. Uptake of [^{14}C]GA (closed symbols) and [^{3}H]dGA (open symbols) by *E. coli* NT2:R53 (○) and *E. chrysanthemi* EC16 (□). Values represent averages of four independent experiments.

as previously described (7). In the experiments described in this paper, bacteria were grown on the noninducing carbon substrate glycerol prior to inoculation. Bacteria isolated from macerated potato tissue were able to take up [^{14}C]GA, indicating that the uptake activity can be enhanced in vivo. The initial rate of uptake was slightly higher than that of cells grown on pectin and its degradation products in vitro (Fig. 2). This increase may be attributed to the presence of multiple inducers from the degradation of potato cell walls as well as some compound produced in the potato that is not present in the in vitro growth medium. It is well documented that enzymes for pectin degradation are inducible; thus, our observation of the increased activity of the uptake system(s) for molecules produced by pectin degradation into *E. chrysanthemi* is not unexpected. The dependence of GA uptake on a source of metabolic energy was investigated by observing the effects of a variety of metabolic inhibitors on uptake activity. Cyanide (5 mM) and 2,4-dinitrophenol (1 mM) inhibited uptake activity, while arsenate (5 mM) did not (Table 1). Cyanide and 2,4-dinitrophenol inhibited uptake activity 85 and 90%, respectively, suggesting that GA transport is an energy-requiring process. The utilization of periplasmic binding proteins in uptake was investigated by subjecting the cells to cold osmotic shock essentially as previously described for *Escherichia coli* (10). Uptake of GA was reduced approximately 60% in these experiments. The addition of 10 mM lactate to the shocked cells, however, was sufficient to restore transport activity to 85% of the wild-type levels, suggesting that the shock procedure was depleting the cells of energy stores (Table 1). These results do not rule out the involvement of periplasmic binding proteins in uptake of GA.

TABLE 1. Effect of inhibitors and osmotic shock on uptake of [^{14}C]GA by *E. chrysanthemi* EC16[a]

Addition and/or treatment	Concn (mM)	% Uptake
None		100
Arsenate	5	100
2,4-Dinitrophenol	1	10
Cyanide	5	15
Osmotic shock		39
Osmotic shock + lactate	1	85

[a] The control uptake rate was 30 nmol/min/mg of protein. Details of inhibitor and osmotic shock experiments are presented in the text. Values represent averages of three independent experiments.

Degradation of pectin by pectolytic enzymes releases a number of different molecules, among them saturated monomers and dimers of GA. In order to investigate whether the *exuT* gene product (for GA uptake) could facilitate the uptake of more than one of these compounds, a derivative of *E. coli* NT2 (a GA transport mutant [6]) was used. With *E. coli* NT2:R53, which contains a segment of the *E. chrysanthemi* B374 genome on an R′ plasmid to complement the defect (6), both growth on and uptake of GA as well as those of dGA were studied. Although the activity is markedly lower than that of *E. chrysanthemi* EC16, this *E. coli* strain with the appropriate R plasmid can grow on GA as well as take up the radioactive analog. It is, however, unable to either utilize or transport dGA, which is readily taken up by *E. chrysanthemi* EC16 (Fig. 3). This indicates that the two molecules, GA and dGA, do not share the same uptake system. These studies represent the first direct measurement of GA transport in bacteria, and they extend previous studies on the aldohexuronate transport system (5, 9). Future studies will identify and characterize the *E. chrysanthemi* genes and proteins responsible for the uptake of plant cell wall molecules as well as assess their contribution to the phytopathogenic potential of this microorganism.

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NT2:R53. We thank Barry Rosen for a critical reading of the manuscript.

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