

Solanidine in potato (*Solanum tuberosum*) tuber tissue disrupted by *Erwinia atroseptica* and by *Phytophthora infestans*

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Solanidine has been isolated and identified from potato tuber tissue disrupted by *Erwinia atroseptica*, and by *Phytophthora infestans*, in a compatible interaction. Tuber varieties, resistant to *P. infestans* in which cellular disintegration was not apparent, did not produce the aglycone. The evidence supports the view that solanidine is formed in disrupted tissue through the activity of hydrolytic potato enzymes on the glycoalkaloids.

#### INTRODUCTION

Solanidine, the aglycone of solanine and chaconine, has occasionally been found in trace amounts during glycoalkaloid analysis of potato tuber tissue. Its presence has generally been regarded as a product of the hydrolysis of the related glycoalkaloids. This report describes the isolation and identification of solanidine in potato tuber tissue under certain stress conditions where cellular disruption or tissue liquefaction has occurred.

#### MATERIALS AND METHODS

Tubers of *Solanum tuberosum* var. Kennebec, Wauseon, Katahdin and Houma were used.

##### *Treatment*

All tuber slices were 3.5 to 4 mm thick, cut on a food slicer and alcohol sterilized before use. Each slice was washed with a stream of sterile distilled H<sub>2</sub>O before further treatment.

Slices (six) of each of the four varieties were surface inoculated with a 1 ml suspension of *Erwinia atroseptica*. Slices (six) each of cvs Katahdin, Kennebec and Wauseon were surface inoculated with a 1 ml suspension of *Phytophthora infestans* containing approximately 40 000 sporangia per ml.

Katahdin, Kennebec and Houma slices (six) each had 1 ml of sterile  $5 \times 10^{-3}$  M-NaF applied to their surface.

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Each of two sets of Katahdin slices (five) placed in closed sterile Petri dishes were heated by microwave energy in a Litton† microwave oven (Model 500) for 60 and 90 s, respectively, and then surface inoculated with a 1 ml suspension of *E. atroseptica* per slice.

In all treatments, there were accompanying control slices to which 1 ml of sterile distilled H<sub>2</sub>O was surface applied.

Wedges of six Kennebec tubers were ground in a Waring Blendor for 30 s with a small amount of distilled H<sub>2</sub>O under sterile conditions.

All slices and the slurry were incubated in individual Petri dishes at 20 °C for 2 to 8 days as indicated.

α-Solanine (25.7 mg) was dissolved in 100 ml tryptone–glucose extract medium adjusted to pH 6.0. Contact and growth of the pathogen was allowed to proceed for 72 h.

#### *Extraction*

The incubated slices of each treatment were pooled and lyophilized to dryness and ground. The powdered material was extracted twice with 100 ml each of CHCl<sub>3</sub> in a Waring Blendor and evaporated to near-dryness under reduced pressure. The residue was extracted with 5 to 6 ml *n*-hexane and the extract reduced to dryness with a stream of N<sub>2</sub>. The residue was taken up in 0.5 ml MeOH and centrifuged at 0 °C. The clear supernatant was removed for t.l.c. and g.l.c. analysis.

The tryptone–glucose culture medium containing solanine was twice extracted with CHCl<sub>3</sub>, dried over anhyd. MgSO<sub>4</sub>, concentrated to dryness and the residue dissolved in *n*-hexane.

#### *Isolation*

The extracts of the *Erwinia*-treated and NaF-coated slices were combined and separated on t.l.c. silica gel G plates, using cyclohexane : EtOAc (1 : 1). The solanidine band was located and extracted from the silica gel with CHCl<sub>3</sub> and evaporated to dryness. The residue was taken up in a small amount of CHCl<sub>3</sub> and filtered through a small bed of anhyd. MgSO<sub>4</sub> and glass wool. Cochromatography of the isolated potato alkaloid with authentic solanidine on t.l.c. silica gel G plates with either cyclohexane : EtOAc (1 : 1) or benzene : acetone (9 : 1) produced a single red spot when sprayed with saturated SbCl<sub>3</sub> in CHCl<sub>3</sub>. A mixture of the isolated alkaloid and authentic aglycone yielded a single peak on g.l.c. Subsequently, isolation of the aglycone from 100 slices incubated with *E. atroseptica* yielded solanidine as fine needles from acetone after elution of the aglycone from a silica gel column with 19% EtOAc in *n*-hexane.

#### *G.l.c.*

G.l.c. separations were carried out on a Varian 2100 using flame ionization detection. The column was 3% OV1 on 100/120 mesh Gas Chrom Q, 0.2 × 122 cm, injector temperature 250 °C, starting temperature 110 °C, programmed 2 °C per min, He 55 ml/min, H<sub>2</sub> 30 ml/min, air 450 ml/min. Retention temperature for solanidine

† Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

was 207 °C. Quantitation was by triangulation of the solanidine peak and comparison with a standard curve.

#### Mass spectrometry

The mass spectrometry was carried out on a DuPont 492 mass spectrometer using a direct probe inlet. The spectrum of the isolated alkaloid was identical with authentic solanidine.

### RESULTS

When slices of the tubers of four cultivars of potato had been exposed to *E. atroseptica* for a period of 5 days at 20 °C, the tissues were virtually liquefied and solanidine appeared in relatively large amounts. In the case of Kennebec slices, the solanidine was present at 52 µg/g dry weight. After 2 days exposure to the pathogen, rotting occurred but solanidine was not detected.

When tuber slices of the late blight susceptible cv. Katahdin were exposed to *P. infestans* race 4, solanidine (19 µg/g dry weight) was found in the liquescent tissue after 8 days. On the other hand, slices of the late blight resistant cvs, Kennebec and Wauseon, exposed to this same organism failed to yield detectable quantities of the aglycone. During the hypersensitive interaction, cellular structure of the host tissue remained largely intact.

Small amounts of the aglycone (2.2 µg/g dry weight) were found in tuber tissue ground under sterile conditions and allowed to stand for 5 days at 20 °C. None was detected in intact slices maintained under the same conditions. The detectable level for solanidine by g.l.c. was 25 ng. This would indicate that those slices where the aglycone could not be detected had below 1.75 µg solanidine/g dry weight.

Incorporation of solanine into tryptone–glucose culture medium and subsequent incubation with *E. atroseptica* did not produce detectable amounts of the aglycone. Katahdin slices exposed to microwave treatment for 90 s failed to produce solanidine when incubated with *E. atroseptica* although tissue liquefaction occurred. Slices that

TABLE 1  
*Solanine development in potato tuber slices after infection with E. atroseptica and P. infestans*

Cultivar	Treatment for 5 days	Alkaloid (µg/g dry wt)	
		Solanidine	Solanine and chaconine
Kennebec	None; initial slices	<1.75	450
	<i>E. atroseptica</i>	52	—
	<i>P. infestans</i> R-4	<1.75	—
	Water	<1.75	—
	Ground in water	2.2	—
Katahdin	None; initial slices	<1.75	290
	<i>E. atroseptica</i>	23	—
	<i>P. infestans</i>	19	—
	Water	<1.75	—
	Microwave 90 s, then <i>E. atroseptica</i>	<1.75	—
Wauseon	None; initial slices	<1.75	425
	<i>P. infestans</i>	<1.75	—

were not exposed to the microwave treatment or those treated for 60 s followed by incubation with this same organism yielded detectable amounts of solanidine.

Solanidine analyses for the above treatments are shown in Table 1. When NaF was applied to slices of Kennebec, Houma and Katahdin tissue, liquefaction with the large accumulation of solanidine ( $> 52 \mu\text{g/g}$  dry weight) was recorded.

## DISCUSSION

Although Metlitskii *et al.* [5] had reported the induction of sesquiterpenoid phytoalexins in potato slices treated with NaF, in this study  $5 \times 10^{-3}$  M-NaF only produced large accumulations of solanidine accompanying tissue liquefaction. Since tissue-rotting organisms were detected in this liquefied tissue, conceivably NaF mediates the activity of endophytic organisms which normally remain quiescent.

Although a glycosidase has been described in potato sprouts [2, 6] and potato leaves [6] capable of splitting the sugar moieties from solanine and chaconine as well as tomatine and demissine, the enzyme has not been reported in the tuber, nor has solanidine been generally observed in sprouts or tubers. Whenever found, presence of the aglycone has been attributed to enzymatic hydrolysis, the result of acid extraction of the tissue [8] or the result of an exhausted sugar supply required for incorporation under conditions where glycoalkaloid synthesis is readily induced. The preparation of our extracts did not entail the use of acid or base, hence non-enzymatic hydrolysis can be ruled out as the mechanism of aglycone production. Zitnak [10], in observing free solanidine in the cv. Netted Gem under conditions of high solar energy and near-freezing temperatures with unprotected tubers in the field, regarded the latter to be the causal factor. Under the conditions of cellular destruction reported here, the appearance of "solaninase" activity seems to be stimulated by the degradative processes.

In this regard, evidence has been presented by Pitt & Coombes [7] that infection of potato tuber tissue and callus with a number of tuber-rotting fungi resulted in swelling and disruption of host spherosomes containing hydrolytic enzymes. Since the onset of cellular destruction, followed by the detection of solanidine, occurred after a relatively long period, it could not be ascertained whether the appearance of the aglycone is attributable to the activity of the glycosidase specific for glycoalkaloids [2, 6] or rather to the slower action of other less specific glycosidases in the tuber.

It would appear that the 90 s exposure to microwave energy inactivated the potato hydrolase responsible for solanidine production. Moreover, conditions were not propitious for *de novo* synthesis of the aglycone following the 60 s exposure to microwave energy.

The possibility of hydrolysis by soil bacteria [9] of solanine and chaconine occurring *in situ* has not been completely ruled out. "Healthy" potato tubers have been shown to contain numerous nonpathogenic bacteria [1, 3] which may enter the tuber tissue through developing secondary roots [3]. Our findings of endophytic organisms with NaF-treated slices supports the contention that "healthy" tubers are not necessarily sterile. Nevertheless, the evidence supports the view that the appearance of solanidine in disrupted potato tuber tissue results from the activity of hydrolytic potato enzymes on the glycoalkaloids.

Although solanidine is a stress metabolite resulting from tissue disruption, it appears to be a product of the decomposition process rather than a phytoalexin. When a tuber resistant to a race of *P. infestans* was capable of controlling the infection, the host failed to produce the aglycone, notwithstanding the mechanism of formation, yet accumulated an array of sesquiterpenoid compounds. On the other hand, the susceptible tuber varieties developed the aglycone while undergoing tissue destruction. Both sesquiterpenes, rishitin and phytuberin, arising in the incompatible interaction between potato tissue and *P. infestans*, have been found in even larger quantities following penetration and disruption by *E. atroseptica* [4]. However, neither the sesquiterpenes nor solanidine were capable of limiting the soft-rot infection.

Chemical compounds (rishitin and/or phytuberin) produced by the host under one set of conditions (e.g. the interaction of potato and *P. infestans*) and defined as phytoalexins in that particular situation, may not conform to that definition when produced under another set of conditions (e.g. the interaction of potato and *E. atroseptica*).

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#### REFERENCES

1. DE BOER, S. H. & COPEMAN, R. J. (1974). Endophytic bacterial flora in *Solanum tuberosum* and its significance in bacterial ring rot diagnosis. *Canadian Journal of Plant Science* **54**, 115-122.
2. GUSEVA, A. R. & PASESHNICHENKO, V. A. (1957). Enzymatic degradation of potato glycoalkaloids. *Biochemistry (USSR)* **22**, 792-799.
3. HOLLIS, J. P. (1951). Bacteria in healthy potato tissue. *Phytopathology* **41**, 350-366.
4. LYON, G. D. (1972). Occurrence of rishitin and phytuberin in potato tubers inoculated with *Erwinia carotovora* var. *atroseptica*. *Physiological Plant Pathology* **2**, 411-416.
5. METLITSKII, L. V., D'YAKOV, YU. T., OZERETSKOVSKAYA, O. L., YURGANOVA, L. A., CHALOVA, L. I. & VASYUKOVA, N. I. (1971). Induction of potato phytoalexins. *Izvestiya Akademii Nauk SSSR, Ser. Biol.*, 399-407.
6. PETROCHENKO, E. I. (1953). Solaninase in potato sprouts. *Doklady Akademii Nauk SSSR* **90**, 1091-1093.
7. PITT, D. & COOMBES, C. (1969). Release of hydrolytic enzymes from cytoplasmic particles of *Solanum* tuber tissues during infection by tuber-rotting fungi. *Journal of General Microbiology* **56**, 321-329.
8. SCHREIBER, K. (1954). The glycoalkaloids of the Solanaceae. *Chemische Technik (Berlin)* **6**, 648-658.
9. YOSIOKA, I., FUJIO, M., OSAMURA, M. & KITAGAWA, I. (1966). A novel cleavage method of saponin with soil bacteria, intending to the genuine sapogenin: on senega and panax saponins. *Tetrahedron Letters* No. 50, 6303-6308.
10. ZITNAK, A. (1961). The occurrence and distribution of free alkaloid solanidine in Netted Gem potatoes. *Canadian Journal of Biochemistry and Physiology* **39**, 1257-1265.