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Tracing Some Enzymatic Activities in Three Virulent Pathogenic Erwiniae

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Abstract□The absence, in the biomass, of glutamic-oxalacetic and glutamic-pyruvic transaminases and the prevalance of gamma-glutamic transpeptidase were taken as basic criteria to differentiate between *Erwinia carotovora* var. *carotovora* from *E. carotovora* var. *citrullis* and *E. toxica*. For further identification, detection of cholesterol in the biomass confirmed the *toxica* species whereas the absence of glucose confirmed the *citrullis* variety.

Keywords□Enzymatic activity, *Erwinia carotovora, E. toxica.*

Several studies had been performed on the enzymatic activities of the plant pathogenic bacteria, genus *Erwinia,* but very little has been published on the effect of pollutants on such activities. Enzymes *viz.* amylase and transaminases (GOT and GPT) were found in comparatively large quantities, while others *e.g.* lactate dehydrogenase (LDH) and alkaline phosphatase (Al-P) were detected in smaller amounts.

Kozareva *et al.*[1], working on GOT and GPT, acid and alkaline phosphatase, protease and catalase of *Escherichia coli* and *Proteus,* concluded that the pathogenic properties of these microorganisms corresponded to changes in the enzyme activity of the bacterial cells.

Amylases are widely distributed in all members of the plant and animal kingdoms. Kuranova *et al.*[2] were able to isolate and purify α-amylase produced by the thermophilic *Bacillus subtilis* strain 110. Khan and Khan[3], proved that *Bacillus* sp., *Pseudomonas* and *E. coli,* isolated from the mid gut of adults of *Musca domestica,* exhibited amylase and invertase activity. Nakakuki *et al.*[4], demonstrated the presence of an exo-amylase in *P. stutzeri,* capable of degrading soluble starch. Nasir *et al.*[5] working on *Salmonella typhosa,* and Kidwey and Murti[6], experimenting on *E. coli,* showed that the activity of lactate dehydrogenase (LDH) was located in the plasma membrane fraction.

Williams and Withers[7, 8], noticed that the specific activity of the alkaline phosphatase, widely occurring in rumen bacteria, was affected by the carbohydrate source supplied in the growth medium of these hemicellulose decomposing bacteria; glucose being highly suppressive.

The aim of this investigation is to find out whether or not the activity of some intracellular enzymes may be a helpful tool to distinguish between the three species of the pathogenic *Erwinia.*

MATERIAL AND METHODS

In this investigation there virulant plant pathogenic bacteria were used: *Erwinia carotovora* var. *carotovora*[9] causing soft rot of melon plants in Egypt. *E. carotovora* var. *citrullis*[10] causing wilt and rotting of melon plants in Egypt. *E. toxica*[11], toxigenic bacteria infecting the vascular system of melon and water melon in USSR.

One m*l* of bacterial suspension containing 2.5×10^8 cells was inoculated in 50 m*l* beef-peptone broth, and incubated, at 28°C, for 24 hours. The bacterial growth was centrifuged, at 5000 rpm, for 15 minutes. The biomass was harvested, washed several times, and suspended in 2 m*l* acetone.

0.2 m*l* of this suspension was tested for amylase, transaminases (GOT and GPT), alkaline phosphatase, creatine phosphokinase, gamma glutamic transpeptidase (GGt) and lactic dehydrogenase. The reducing sugars were also determined in addition to urea and cholesterol. All these activites were measured by the Automatic Clinical Analyser 60-channel ACA-II (Du Pont Instruments, Wilmington, USA), at the specified wave length for each determination.

RESULTS AND DISCUSSION

Table I shows that amylase and lactic dehy-

drogenase were the most active enzymes that were detected in the three *Erwinia* species. Amylase was more active than LDH presumably due to the greater need for starch hydrolysis than the anaerobic reduction of pyruvate to lactate, since the organisms were cultured under strictly aerobic conditions.

The transaminase enzymes (GOT and GPT) were detected only in *E. carotovora* var. *citrullis* and *E. toxica* . It was not traced in *E. carotovora* var. *carotovora*. On the other hand, GGt was only detected in the latter variety. These criteria may be taken as a differential evidence for separating the *carotovora* from the *citrullis* variety as well as from the *toxica* species.

The three test bacteria were unable to produce alkaline phosphatase or creatine phosphokinase. Naguib *et al.*[12], found that several members of enterobacteriaceae were able to produce CPK when cultured on beef-peptone or single amino acid media. *E. toxica* produced this enzyme only in presence of isoleucine whereas *E. carotovora* var. *citrullis* was able to do so in presence of dihydroxyphenyl alanine, leucine, serine, tryptophane or tyrosine.

On biomass basis, *E. carotovora* var. *carotovora* showed the highest amylase and least LDH activities whereas per equal number of cells, LDH activity was highst in the *carotovora* variety and least in the *toxica* species. Amylase was almost equally high in both varieties of *E. carotovora*. Again, per biomass, GOT activity was higher in *toxica* species than the *citrullis* variety but the reverse was observed with GPT. Within equal number of cells, GOT was equally active in both organisms but GPT activity was higher in the *citrullis* variety than the *toxica* species.

The differentiation between the three *Erwinia* species may be supported by detecting some endometabolites. Thus, based on equal number of cells, the *citrullis* variety produced the highest urea content followed the *toxica* species and the *carotovora* variety. Cholesterol was only detected in the *toxica* species whereas glucose was not detected in the *citrullis* cells. The *toxica* species contained larger amounts of glucose than the *carotovora* variety.

Accordingly, detection of cholesterol in the cells confirms the *toxica* species whereas the absence of glucose confirms the *citrullis* variety.

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Table I. Activity of some endoenzymes and content of some endometabolites in three pathogenic bacteria of the genus *Erwinia* [I.U. (enzymes) or mg (metabolites) per biomass or defined number of cells (10^{17})]

Organism		Am.	GOT	GPT	Al-P	GGt	CPK	LDH	Glu.	Urea	Choles.
E. carotovora	Biomass	0.88	–	–	–	0.06	–	0.26	0.06	0.08	–
var. *carotovora*	10^{17}	1.83	–	–	–	0.12	–	0.54	0.08	0.17	–
E. carotovora	Biomass	0.84	0.21	0.16	–	–	–	0.16	–	0.16	–
var. *citrullis*	10^{17}	1.68	0.42	0.32	–	–	–	0.32	–	0.32	–
E. toxica	Biomass	1.01	0.36	0.12	–	–	–	0.12	0.12	0.16	0.45
	10^{17}	1.26	0.45	0.15	–	–	–	0.15	0.15	0.20	0.56

Number of cells produced in the biomass: *E. carotovora* var. *carotovora*, 4.6×10^{16}; *E. carotovora* var. *citrullis*, 5.0×10^{16}; *E. toxica*, 8.0×10^{16}.

GOT, glutamic-oxalacetic transaminase; GPT, glutamic-pyruvic transaminase; Alk-P, Alkaline phosphatatse; GGt, Gamma glutamic transpeptidase; CPK, Creatine phosphokinase; LDH, Lactate dehydrogenase; Am., Amylase; Glu., Glucose; Choles., Cholesterol.

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