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Activity of Some Intracellular Enzymes of Three Virulent *Erwinia* sp. in Presence of Some Heavy Metal Salts

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Abstract□Based on equal number of cells, supplementation of 10^{-6} M cadmium highly stimulated the intracellular amylase, GGT, LDH as well as the glucose and urea content of *E. carotovora* var. *carotovora* cells. This was coupled with initiation of highly active GOT, CPK as well as accumulation of cholesterol in the cells. Lanthanum was less active and unable to initiate GOT or CPK. Nickel was almost without effect though reduced LDH activity without initiating either enzyme or cholesterol production. Similar stimulations and/or initiations were observed, though to variable extents, when the same concentration of the three elements were supplied to *E. carotovora* var. *citullis* or *E. toxica*. In the meantime, lanthanum arrested GPT whereas nickel arrested GOT activity of *E. toxica*. The highest yield of amylase, GPT, GGT or glucose was obtained when *E. carotovora* var. *carotovora* was supplemented with Cd + Ni. The highest urea level was recorded in *Erwinia carotovara* var. *citrullis*, amended with Cd + La.

Keywords□*Erwinia* sp., heavy metal, intracellular enzymes.

In previous investigations, Saleh and Khalil[1], Khalil and Saleh[2], and Saleh[3] dealt with the effect of lanthanum, nickel and cadmium on the pathogenicity of *Erwinia carotovora* var. *carotovora* to melon seedlings growing under laboratory (water culture) and natural (graden soil in pots) conditions. Furthermore, Saleh[4] noticed that the enzymatic pattern in *E. carotovora* var. *carotovora*, *E. carotovora* var. *citrullis* and *E. toxica* showed differential affinities for the production of glutamic-oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), gamma glutamyl transpeptidase (GGT) as well as the glucose, urea and cholesterol content of their biomass, which may be used as criteria to differentiate these pathogens.

Kozareva *et al.*[5] found that the pathogenic properties of the microorganisms corresponded to the changes in the GOT and GPT, protease and catalase activity in *E. coli* and *Proteus* which were isolated from food-borne disease.

Suzuki *et al.*[6] reported that GGT of *E. coli* K-12 is localized in the periplasmic space. Nasir *et al.*[7] working on *Salmonella typhosa*, and Kidway and Murti[8] experimenting on *E. coli* showed that the activity of lactic dehydrogenase was located only on the plasma membrane fraction.

It was thought to perform the present study on the activities of some intracellular enzymes of three virulent plant pathogenic Erwiniae, in presence of some heavy metal salts and to correlate the results with the virulence of the heavy metal-treated pathogens.

MATERIAL AND METHODS

Three virulent pathogenic Erwiniae were used in this study: *Erwinia carotovora* var. *carotovora*[9] causing soft rot of melon plants in Egypt, *Erwinia carotovora* var. *citrullis*[10] causing wilt and rotting of melon plants in Egypt, and *Erwinia toxica*[11] toxigenic bacteria infecting the vascular system of melon and water melon in USSR.

One m*l* of bacterial suspension (2.5×10^8 cells/m*l*) was inoculated into 50 m*l* beef peptone broth amended with 10^{-6} M lanthanum chloride, nickel chloride or cadmium chloride, singly or in their possible combinations. The media were incubated at 28°C for 24 hours, then centrifuged at 5000 ppm for 15 minutes. The biomass was harvested, washed several times with distilled water, and then suspended in 2 m*l* of sterile acetone.

The biomass was analyzed for the activity of amylase, transaminase (GOT and GPT), alkaline phospha-

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Table I. Effect of heavy metals on intracellular enzymes of the produced biomass as well as compared with equal number of cells of respective controls

(International Units)

Treatment	Organism	Cell count	Amylase	GOT	GPT	Al-P	GGT	CPK	LDH
Control	*E. carotovora* var. *carotovora*	Biomass	0.88	–	–	–	0.06	–	0.26
		4.8×10^{16}	0.88	–	–	–	0.06	–	0.26
	E. carotovora var. *citrullis*	Biomass	0.84	0.21	0.16	–	–	–	0.16
		5.0×10^{16}	0.84	0.21	0.16	–	–	–	0.16
	E. toxica	Biomass	1.01	0.36	0.12	–	–	–	0.12
		8.0×10^{16}	1.01	0.36	0.12	–	–	–	0.12
10^{-6} M La	*E. carotovora* var. *carotovora*	Biomass	0.76	0.30	–	–	0.06	–	0.10
		4.8×10^{16}	2.28	0.90	–	–	0.18	–	0.30
	E. carotovora var. *citrullis*	Biomass	0.86	0.54	0.12	–	–	–	0.14
		5.0×10^{16}	1.12	0.70	0.16	–	–	–	0.18
	E. toxica	Biomass	1.05	0.45	–	–	–	–	0.12
		8.0×10^{16}	2.10	0.90	–	–	–	–	0.24
10^{-6} M Ni	*E. carotovora* var. *carotovora*	Biomass	0.80	–	–	–	0.06	–	0.10
		4.8×10^{16}	1.20	–	–	–	0.09	–	0.15
	E. carotovora var. *citrullis*	Biomass	0.84	0.27	0.14	–	–	–	0.08
		5.0×10^{16}	0.76	0.24	0.13	–	–	–	0.07
	E. toxica	Biomass	1.10	–	0.10	–	–	–	0.10
		8.0×10^{16}	1.43	–	0.13	–	–	–	0.13
10^{-6} M Cd	*E. carotovora* var. *carotovora*	Biomass	0.76	0.33	–	0.04	0.04	0.02	0.22
		4.8×10^{16}	9.12	3.96	–	0.48	0.48	0.24	2.64
	E. carotovora var. *citrullis*	Biomass	1.08	0.27	0.14	–	–	–	0.08
		5.0×10^{16}	2.70	0.68	0.35	–	–	–	0.20
	E. toxica	Biomass	1.15	0.36	0.12	–	–	–	0.08
		8.0×10^{16}	2.88	0.90	0.30	–	–	–	0.20
10^{-6} M La + Ni	*E. carotovora* var. *carotovora*	Biomass	0.82	–	–	–	0.10	0.14	0.28
		4.8×10^{16}	3.28	–	–	–	0.40	0.56	1.12
	E. carotovora var. *citrullis*	Biomass	0.90	0.36	0.14	–	–	–	0.08
		5.0×10^{16}	1.60	0.65	0.35	–	–	–	0.20
	E. toxica	Biomass	1.09	0.36	0.10	–	–	–	0.10
		8.0×10^{16}	3.16	1.04	0.29	–	–	–	0.29
10^{-6} M La + Cd	*E. carotovora* var. *carotovora*	Biomass	0.80	–	0.18	–	0.02	0.04	0.16
		4.8×10^{16}	6.40	–	1.44	–	0.16	0.32	1.28
	E. carotovora var. *citrullis*	Biomass	0.89	–	–	–	–	–	0.10
		5.0×10^{16}	8.90	–	–	–	–	–	1.0
	E. toxica	Biomass	1.10	–	0.16	–	–	–	0.12
		8.0×10^{16}	2.75	–	0.40	–	–	–	0.30

Table I. Continued

Treatment	Organism	Cell count	Amylase	GOT	GPT	Al-P	GGT	CPK	LDH
10^{-6} M Ni + Cd	*E. carotovora* var. *carotovora*	Biomass	0.94	–	0.18	–	0.10	–	–
		4.8×10^{16}	15.88	–	2.88	–	1.60	–	–
	E. carotovora var. *citrullis*	Biomass	0.95	–	0.12	–	–	–	0.10
		5.0×10^{16}	4.75	–	0.60	–	–	–	0.50
	E. toxica	Biomass	1.21	–	0.18	–	–	0.04	0.06
		8.0×10^{16}	2.42	–	0.36	–	–	0.08	0.12
10^{-6} M La + Ni + Cd	*E. carotovora* var. *carotovora*	Biomass	0.62	–	–	–	0.16	–	0.34
		4.8×10^{16}	0.74	–	–	–	0.19	–	0.41
	E. carotovora var. *citrullis*	Biomass	1.16	–	0.12	–	–	–	0.06
		5.0×10^{16}	1.16	–	0.12	–	–	–	0.06
	E. toxica	Biomass	1.24	–	0.10	–	–	–	0.08
		8.0×10^{16}	1.61	–	0.13	–	–	–	0.10

tase (Al-P), gamma glutamyl transpeptidase (GGT), lactic dehydrogenase (LDH), creatine phosphokinase (CPK) by the Automatic Clinical Analyzer 60-Channel ACA II (Du Pont Instruments, Wilmington, USA) using the specific wave length for each enzymes. In addition, glucose, urea and cholesterol were assayed by the same apparatus.

RESULTS AND DISCUSSION

Table I shows that, per biomass yield, the amylase activity was high in *E. toxica* than both *carotovora* varieties that were equally active. Lanthanum or cadmium equally lowered amylase activity of *E. carotovora* var. *carotovora* more effectively when mixed together. Only cadmium and to a lesser extent its mixture with lanthanum or nickel seemed to stimulate amylase activity in *E. carotovora* var. *citrullis* but the three elements together were highly stimulatory. *E. toxica* similarly responded to the heavy metal application.

GOT and GPT were not traced in *E. carotovora* var. *carotovora* but lanthanum or cadmium (singly) could induce GOT formation whereas La + Cd or Ni + Cd were able to initiate GPT activity in this organism. GOT of *E. carotovora* var. *citrullis* was stimulated by lanthanum and to a lesser extent by La + Ni. Nickel or cadmium alone were without effect whereas the remaining mixtures totally abolished the activity of the enzyme. GOT of *E. toxica* was also activated by lanthanum but was not affected by cadmium or La + Ni whereas the remaining treatments arrested the enzyme activity within the organism.

GPT of *E. carotovora* var. *citrullis* or *E. toxica* was almost unaffected by either of the heavy metal treatments except La + Cd (*E. carotovora* var. *citrullis*) or lanthanum alone (*E. toxica*) that promoted the formation of the enzyme in both organisms respectively.

Al-P was not traced in either of the Erwiniae except *E. carotovora* var. *carotovora* supplemented with cadmium, whereas GGT was only traced in small amounts in *E. carotovora* var. *citrullis* that highly increased in presence of either mixtures except La + Cd which suppressed its activity. CPK was also traced in *E. toxica* following Ni + Cd treatment. LDH was highest in *E. carotovora* var. *carotovora* and least in *E. toxica*. Lanthanum or nickel highly suppressed LDH of *E. carotovora* var. *carotovora* whereas cadmium or La + Ni exerted no effect. La + Cd also suppressed LDH but further addition of nickel stimulated its activity whereas Ni + Cd totally arrested its activity. All treatments but lanthanum suppressed LDH of *E. carotovora* var. *citrullis*. The latter seemed without effect. On the other hand, cadmium and all its combinations except with lanthanum suppressed LDH of *E. toxica* while the other treatments were hardly effective.

Compared with equal number of respective control cells (Table I), it seems that most treatments tended to stimulate the enzyme activities of the test organisms. The mixture of the three elements seemed

Table II. Effect of heavy metals on intracellular enzymes of equal number of cells of three virulent *Erwinia* species
(International Units)

Treatment	Organism	Cell Count	Amylase	GOT	GPT	Al-P	GGT	CPK	LDH
Control	*E. carotovora* var. *carotovora*	10^{17}	1.83	–	–	–	0.12	–	0.54
	E. carotovora var. *citrullis*	10^{17}	1.68	0.42	0.32	–	–	–	0.32
	E. toxica	10^{17}	1.26	0.45	0.15	–	–	–	0.15
10^{-6} M La	*E. carotovora* var. *carotovora*	10^{17}	4.74	1.87	–	–	0.37	–	0.62
	E. carotovora var. *citrullis*	10^{17}	2.24	1.40	0.32	–	–	–	0.36
	E. toxica	10^{17}	2.63	1.13	–	–	–	–	0.30
10^{-6} M Ni	*E. carotovora* var. *carotovora*	10^{17}	2.50	–	–	–	0.19	–	0.31
	E. carotovora var. *citrullis*	10^{17}	1.52	0.48	0.26	–	–	–	0.14
	E. toxica	10^{17}	1.79	–	0.16	–	–	–	0.16
10^{-6} M Cd	*E. carotovora* var. *carotovora*	10^{17}	18.97	8.24	–	1.00	1.00	0.50	5.49
	E. carotovora var. *citrullis*	10^{17}	5.40	1.36	0.70	–	–	–	0.40
	E. toxica	10^{17}	3.60	1.13	0.38	–	–	–	0.25
10^{-6} M La + Ni	*E. carotovora* var. *carotovora*	10^{17}	6.82	–	–	–	0.83	1.16	2.33
	E. carotovora var. *citrullis*	10^{17}	3.20	1.30	0.25	–	–	–	0.44
	E. toxica	10^{17}	3.95	1.30	0.36	–	–	–	0.36
10^{-6} M La + Cd	*E. carotovora* var. *carotovora*	10^{17}	13.31	–	3.00	–	0.33	0.67	2.66
	E. carotovora var. *citrullis*	10^{17}	17.80	–	–	–	–	–	2.00
	E. toxica	10^{17}	3.44		0.50	–	–	–	0.38
10^{-6} Ni + Cd	*E. carotovora* var. *carotovora*	10^{7}	31.28		6.0	–	3.33	–	–
	E. carotovora var. *citrullis*	10^{17}	9.50	–	1.20	–	–	–	1.0
	E. toxica	10^{7}	3.03	–	0.45	–	–	0.10	0.15
10^{-6} M La + Ni + Cd	*E. carotovora* var. *carotovora*	10^{17}	1.54	–	–	–	0.40	–	0.85
	E. carotovora var. *citrullis*	10^{17}	2.32	–	0.24	–	–	–	0.12
	E. toxica	10^{17}	2.01	–	0.16	–	–	–	0.13

to be least effective if not slightly suppressive to amylase of *E. carotovora* var. *carotovora* as well as GPT and LDH of *E. carotovora* var. *citrullis* and *E. toxica*. Similarly nickel suppressed amylase, GPT and LDH of *E. carotovora* var. *citrullis*. Cadmium induced the highest GOT, Al-P, LDH and second best amylase, GGT activity in *E. carotovora* var. *carotovora*. Ni + Cd induced the highest amylase, GPT and GGT in *E. carotovora* var. *carotovora*. La + Ni procedure the second best LDH activity in *E. carotovora* var. *carotovora*. Accordingly it seems probable to add this variety to the list of organisms of possible role in large scale enzyme production particularly digestive (amylase) or transminases (GOT and GPT) enzymes.

Comparison between the three Erwinae based on fixed number of cells (Table II) reveals that the activity of the test intracellular enzymes was highest in *E. carotovora* var. *carotovora* and least in *E. toxica*. This trend was almost consistent (except for few cases) after heavy metal supplementation.

Nickel or lanthanum, singly or combined, though comparatively were less initiative to amylase activity than cadmium yet the amylase of *E. toxica* was higher in activity than that of *E. carotovora* var. *citrullis*. Cadmium alone was most effective for amylase activity of the three test organisms compared with nickel or lanthanum; *Erwinia carotovora* var. *carotovora* being most sensitive. Its combination with lanthanum slightly suppressed *E. carotovora* var. *carotovora* activity but stimulated that of *E. carotovora* var. *citrullis* without affecting the amylase activity of *E. toxica*. Its combination with nickel induced the highest amylase activity among the three test organisms (in *E. carotovora* var. *carotovora*) with less effectiveness on *E. carotovora* var. *citrullis* compared with Cd + La, but still almost without effect on the amylase activity of *E. toxica*. The three elements together reduced the amylase activity of the three test organisms to almost the control level or less.

Table III shows that glucose is one of the intracellular metabolites when *E. carotovora* var. *carotovora* or *E. toxica* were cultured on beef-peptone broth whereas urea was a common nitrogen and metabolite in the three Erwiniae. Cholesterol was only produced by *E. toxica*. Lanthanum and/or cadmium did not initiate glucose formation in *E. carotovora* var. *citrullis* whereas nickel seemed highly stimulatory to a lesser extent when coupled with lanthanum in absence or presence of cadmium. The presence of the latter counteracted the stimulatory effects of nickel on glucose accumulation in *E. carotovora* var. *citrullis*.

Lanthanum or nickel arrested glucose formation in *E. toxica* cells whereas cadmium was suppressive. All test combinations also arrested glucose formation except La + Ni that furthered glucose accumulation (compared with respective equal number of control cells). The three test elements apparently reduced the glucose content of *E. carotovora* var. *carotovora* but the presence of lanthanum and nickel, in any combination, totally arrested glucose accumulation. Comparing equal number of respective control cells, cadmium or lanthanum as well as their combinations stimulated glucose accumulation (Ni + Cd was most effective).

Based on biomass yield, all treatments seemed without effect on the intracellular urea but compared

Table III. Effect of heavy metals on intracellular metabolites of the produced biomass as well as compared with equal number of cells of respective controls of three *Erwinia* species

(mg per Count)

Treatment	Organism	Cell count	Glucose	Urea	Cholesterol
Control	*E. carotovora* var. *carotovora*	Biomass	0.12	0.16	–
		4.8×10^{16}	0.12	0.16	–
	E. carotovora var. *citrullis*	Biomass	–	0.32	–
		5.0×10^{16}	–	0.32	–
	E. toxica	Biomass	0.24	0.32	0.90
		8.0×10^{16}	0.24	0.32	0.90
10^{-6} M La	*E. carotovora* var. *carotovora*	Biomass	0.08	0.20	0.56
		4.8×10^{16}	0.24	0.60	1.68
	E. carotovora var. *citrullis*	Biomass	–	0.32	–
		5.0×10^{16}	–	0.48	–

Table III. Countinued

Treatment	Organism	Cell count	Glucose	Urea	Cholesterol
	E. toxica	Biomass	–	0.32	0.84
		8.0×10^{16}	–	0.64	1.68
10^{-6} M Ni	*E. carotovora* var. *carotovora*	Biomass	0.08	0.20	–
		4.8×10^{16}	0.12	0.30	–
	E. carotovora var. *citrullis*	Biomass	0.24	0.32	0.90
		5×10^{16}	0.21	0.29	0.80
	E. toxica	Biomass	–	0.32	84
		8.0×10^{16}	–	0.43	1.12
10^{-6} M Cd	*E. carotovora* var. *carotovora*	Biomass	0.08	0.20	0.72
		4.8×10^{16}	0.96	2.40	8.64
	E. carotovora var. *citrullis*	Biomass	–	0.32	0.88
		5×10^{16}	–	0.80	2.20
	E. toxica	Biomass	0.04	0.32	0.84
		8.0×10^{16}	0.10	0.80	2.10
10^{-6} M La + Ni	*E. carotovora* var. *carotovora*	Biomass	–	0.16	–
		4.8×10^{16}	–	0.64	–
	E. carotovora var. *citrullis*	Biomass	0.08	0.32	0.92
		5.0×10^{16}	0.14	0.57	1 64
	E. toxica	Biomass	0.08	0.32	0.80
		8.0×10^{6}	0.23	0.91	2.19
10^{-6} M La + Cd	*E. carotovora* var. *carotovora*	Biomass	0.04	0.20	–
		4.8×10^{16}	0.32	1.6	–
	E. carotovora var. *citrullis*	Biomass	–	0.32	0.84
		5×10^{16}	–	3.20	8.40
	E. toxica	Biomass	–	0.32	–
		8.0×10^{16}	–	0.80	–
10^{-6} M Ni + Cd	*E. carotovora* var. *carotovora*	Biomass	0.12	0.16	–
		4.8×10^{16}	1.92	2.56	–
	E. carotovora var. *citrullis*	Biomass	–	0.32	–
		5.0×10^{16}	–	1.92	–
	E. toxica	Biomass	–	0.36	–
		8.0×10^{16}	–	0.72	–
10^{-6} M La + Ni + Cd	*E. carotovora* var. *carotovora*	Biomass	–	0.20	–
		4.8×10^{16}	–	0.24	–
	E. carotovora var. *citrullis*	Biomass	0.20	0.32	–
		5×10^{16}	0.20	0.32	–
	E. toxica	Biomass	–	0.32	–
		8.0×10^{16}	–	0.43	

Table IV. Effect of heavy metals on intracellular metabolites of equal number of cells of three virulent *Erwinia* species.
(mg per 10^{17} cells)

Treatment	Organism	Cell count	Glucose	Urea	Cholesterol
Control	*E. carotovora* var. *carotovora*	10^{17}	0.25	0.33	–
	E. carotovora var. *citrullis*	10^{17}	–	0.64	–
	E. toxica	10^{17}	0.30	0.40	1.13
10^{-6} M La	*E. carotovora* var. *carotovora*	10^{17}	0.50	1.25	3.50
	E. carotovora var. *citrullis*	10^{17}	–	0.96	–
	E. toxica	10^{17}	–	0.80	2.10
10^{-6} M Ni	*E. carotovora* var. *carotovora*	10^{17}	0.25	0.63	–
	E. carotovora var. *citrullis*	10^{17}	0.42	0.58	1.60
	E. toxica	10^{17}	–	0.54	1.40
10^{-6} M Cd	*E. carotovora* var. *carotovora*	10^{17}	2.00	5.00	18.00
	E. carotovora var. *citrullis*	10^{17}	–	1.60	4.40
	E. toxica	10^{17}	0.13	1.00	2.63
10^{-6} M La + Ni	*E. carotovora* var. *carotovora*	10^{17}	–	1.33	–
	E. carotovora var. *citrullis*	10^{17}	0.28	1.14	3.28
	E. toxica	10^{17}	0.29	1.14	2.86
10^{-6} M La + Cd	*E. carotovora* var. *carotovora*	10^{17}	0.67	3.33	–
	E. carotovora var. *citrullis*	10^{17}	–	6.40	16.80
	E. toxica	10^{17}	–	1.00	–
10^{-6} M Ni + Cd	*E. carotovora* var. *carotovora*	10^{7}	4.00	5.33	–
	E. carotovora var. *citrullis*	10^{17}	–	3.20	–
	E. toxica	10^{7}	–	0.90	–
10^{-6} M La + Ni + Cd	*E. carotovora* var. *carotovora*	10^{17}	–	0.50	–
	E. carotovora var. *citrullis*	10^{17}	0.40	0.64	–
	E. toxica	10^{17}	–	0.54	–

with respective number of control cells, all treatments increased the urea level in the three test organisms. In most cases, the combinations of the test elements were most effective than single elements.

Table III further shows that nickel did not initiate cholesterol accumulation in *E. carotovora* var. *carotovora* whereas lanthanum or cadmium were highly effective particularly the latter. Coupling lanthanum with either nickel or cadmium abolished such effect. Lanthanum alone seemed ineffective for cholesterol formation in *E. carotovora* var. *citrullis* but nickel or cadimum and their combinations with lanthanum were very effective. On the other hand, the lesser stimulatory effect of either of the three elements (alone) on cholesterol production by *E. toxica* was still apparent when lanthanum was coupled with nickel.

It is interesting to note that all cadmium combinations arrested cholesterol production by either test organisms except *E. carotovora* var. *citrullis* when it initiated a high level of the compound.

Based on equal number of treatment cells (Table IV), cadmium alone was most effective in inducing the highest cholesterol yield by *E. carotovora* var. *carotovora* as well as very large amounts of glucose or urea. In presence of lanthanum, cadmium had similar effects on *E. carotovora* var. *citrullis*. In most cases, the treatments were less effective on *E. toxica*, if not suppressive.

In previous publications Khalil and Saleh[2], and Saleh and Khalil[12] showed that addition of 10^{-5} or 10^{-6} M cadmium to the culture media of *E. carotovora* var. *carotovora* highly increased its virulence. The same was observed when the element was amended to the soil (Saleh[3]). As already reported, in this investigation cadmium highly stimulated the intracellular amylase, GGT and LDH and initiated the formation of GOT, CPK and Al-P. Accordingly, it seems that increased pathogenicity (following cadmium treatment) was a function of increased enzyme activity.

In this connection, it may be mentioned that Kozareva *et al.*[5] stated that high values of acid phosphatase activity were observed in the intracellular protein of *E. coli* and *Proteus* with enhancement of their pathogenic properties. They further showed that the pathogenic properties of the microorganisms corresponded to changes in GOT and GPT, protease and catalase.

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