Chapter 24

Accelerated Degradation of Carbaryl by Acclimated Bacterial Isolates from *Apis cerana* Bees

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Pesticide metabolism in microorganisms results in formation of toxic and/or non-toxic products (Kearney *et al.*, 1963). Stenerson (1965) first speculated upon the role of bacteria-influenced metabolism of DDT in resistant mosquitoes. Sharma and Nath (1996) showed adaptation to carbaryl of bacterial isolates from *Apis cerana* bees. Treatment of bees with these modified isolates resulted in decontamination of bees and enhanced tolerance to carbaryl. This paper reports carbaryl degradation by acclimated bacterial isolates in different media and discusses the effects of supplemented carbon sources on degradation rates.

Materials and Methods

Bacterial strains (*Citrobacter* sp., *Enterobacter aerogenes* and an unidentified bacterium) active in degrading carbaryl were the isolates of *Apis cerana* bees obtained by the enrichment and adaptation technique used Sharma and Nath (1996). The stock solution of carbaryl was prepared in dimethyl sulphoxide followed by filter sterilisation. These bacteria were made adaptive to carbaryl by transferring them every week from a medium containing a lower concentration of carbaryl to a similarly prepared medium containing a higher concentration of carbaryl. Every transfer to the higher concentration was made when more than 2000 colonies per ml of suspension were observed. The process was continued until 600 ppm were obtained.

Estimation of carbaryl and l-naphthol

Estimation of carbaryl was as used by Benson and Finocchiaro (1965); l-naphthol, an initial degradation product of carbaryl, was estimated by the same technique but without alkali hydrolysis of the samples.

Reagents and growth media

Growth and carbaryl degradation experiments were carried out in nutrient broth and basal salts medium. Experiments were also conducted with and without exogenous-added carbon sources in basal salts medium to see their effect on carbaryl degradation. The constituents of nutrient broth were peptone 5 g l^{-1}, NaCl 5 g l^{-1}, beef extract 3 g l^{-1} and distilled water 11 g l^{-1}. The basal salts medium contained L K_2HPO_4 $3H_20$ 5.8 g l^{-1}, KH_2PO_4 (anhydrous) 4.5 g l^{-1}, $(NK_4)_2So4$ (anhydrous) 2.0 g l^{-1}, $MgCl_2$ $6H_20$ 0.16 g l^{-1}, $CaCl_2$

$2H_20$ 0.02 g l^{-1}, Na_2MoO_4 $2H_20$ 0.002 g l^{-1}, $FeSO_4$ H_2O 0.001 g l^{-1}, $MnCl_2$ $4H_20$ 0.001 g l^{-1} and distilled water 11 g l^{-1} (Niemeela and Vaatanem, 1982). To study the effect of exogenous-added carbon sources on growth and carbaryl degradation, the basal salts medium was supplemented with glucose (0.2 g l^{-1}) and yeast extract (0.2 g l^{-1}) alone and in combination.

Inoculum

The isolates (24–28-h-old bacterial culture) were inoculated into nutrient broth and basal salts medium independently. Bacterial-cell suspension (0.5 ml) of 0.5 O.D. at 600 nm was routinely added to each flask containing 50 ml of medium to give a final concentration of about 1.5×10^5 cells ml^{-1}.

Carbaryl degradation and growth of bacteria

Carbaryl was added to the flasks containing 50 ml of media-inoculated bacterial isolates to reach a final concentration of 1 mM for determining growth and carbaryl degradation capacities of isolates. Each treatment was replicated three times. In every experiment there were controls for carbaryl degradation (i.e., medium containing only carbaryl but no bacterial cells) and for growth (i.e., medium containing only bacteria but no carbaryl). There was no degradation of carbaryl nor production of material from cells that interfered with the assay. Carbaryl enrichment cultures were incubated at 30°C under stationary conditions. Samples were drawn at 0–1, 24, 48, 72, 96 and 120 h of incubation for estimating loss of carbaryl and naphthol formation. Samples from these aliquots were also used for determining bacterial-cell population by measurement of cell survival by serial dilution and plating. The two media, nutrient broth and basal salts medium, were compared for degradation of carbaryl per unit biomass by various bacterial isolates by using t tests at 1 and 5% levels of significance. The effect of exogenous-added carbon sources on carbaryl degradation and growth of isolates was determined by comparing the basal salts medium with and without supplemented glucose and yeast extract. The data (mg μg^{-1} dry wt) were analysed statistically using CRD at 5% level of significance. Biomass was computed on the basis of the formula: 10^9 bacterial population = 380 µg biomass dry wt.

Results and Discussion

Degradation of carbaryl in culture media

All the bacterial species (*Citrobacter* sp., *Enterobacter aerogenes* and unidentified sp.) have the ability to grow and degrade carbaryl in nutrient broth and basal salts medium. Maximum growth and degradation of carbaryl were observed in nutrient broth compared to basal salts medium. All organisms exhibited about 25% carbaryl degradation at log phase (24–36 h). Maximum degradation (more than 80%) was during stationary phase of growth in nutrient broth and about 32–48% carbaryl degradation was during 72–96 h in basal salts medium. The carbaryl recovery in the absence of organisms at 1 mM concentration was about 95% in 120 h.

Quantitatively the breakdown of carbaryl per unit of biomass was more in basal salts medium compared to nutrient broth. When comparing these two media statistically, the value of 't' was

Table 1. Comparative degradation of carbaryl (mg/µg dry weight) by bacterial spp. in nutrient broth (N.B.) and basal salts medium (B.S.M.)

Time (h)	Carbaryl (mg/µg dry weight)					
	Citrobacter sp.		*Enterobacter aerogenes*		Unidentified sp.	
	N:B.	B.S.M.	N.B.	B.S.M.	N.B.	B.S.M.
24	0.034	0.438	0.063	3.509	0.082	7.895
48	0.019	0.132	0.025	1.716	0.022	3.289
72	0.025	0.111	0.039	1.786	0.026	1.316
96	0.026	0.150	0.039	1.933	0.028	1.404
120	0.027	0.204	0.041	1.907	0.029	1.624
't'	3.16*		6.41**		2.48*	

* Significance at 5% level
** Significance at 1% level

significant at 5% in all bacterial species, and in *Enterobacter aerogenes* it was significant at 1% (Tables 1 and 2). The degradation was greatest in the cultures of *Enterobacter aerogenes* and unidentified sp. compared to culture of *Citrobacter* sp. in basal salts medium. This difference in degradation of carbaryl could be because l-naphthol formed in basal salts medium with carbaryl as the sole source of both carbon and nitrogen concomitant with faster carbaryl degradation. In nutrient broth, because of preferential utilisation of available energy sources, conversion of 1-naphthol to other metabolites did not take place. Liu and Bollag (1971) demonstrated that the influence of changed growth conditions on microbes, heavy

Table 2. Efficacy of bacterial isolates to degrade carbaryl (mg μg^{-1} weight) in nutrient broth and basal salts medium

Time (h)	Carbaryl (mg μg^{-1} dry weight)					
	Nutrient broth			Basal salts medium		
	Citrobacter sp.	*Enterobacter*	Unidentified	*Citrobacter*	*Enterobacter*	Unidentified
024	0.034	0.063	0.082	0.438	3.509	7.895
048	0.019	0.025	0.022	0.132	1.716	3.289
072	0.025	0.039	0.026	0.111	1.786	1.316
096	0.026	0.039	0.028	0.150	1.933	1.404
120	0.027	0.041	0.029	0.204	1.907	1.624
F(CD)	2.76(0.055)				5.46(2.062)*	

* Significance at 5% level

Table 3: Effect of exogenously added carbon sources on degradation of carbaryl (mg/μg dry weight) by carbaryl enrichment cultures in basal salts medium (BSM).

Bacterial sp.	Time (h)	Carbaryl (mg μg^{-1} dry weight)			
		BSM	BSM	BSM	BSM
			+glucose (g)	+yeast extract(y)	+(g+y)
	24	3.51	7.39	2.81	0.50
	48	1.72	0.22	1.49	0.10
Enterobacter	72	1.79	0.11	0.70	0.06
aerogenes	96	1.94	0.09	0.59	0.06
	120	1.91	0.07	0.64	0.06
F(C.D)		1.91 (1.80)			
	24	0.44	0.50	2.01	0.16
	48	0.13	0.10	0.10	0.08
Citrobacter spp.	72	0.11	0.10	0.07	0.09
	96	0.15	0.10	0.08	0.11
	120	0.20	0.14	0.08	0.14
F		0.81			
	24	7.89	4.31	0.76	1.0
	48	3.29	1.63	0.27	0.66
Unidentified sp.	72	1.32	0.20	0.28	0.60
	96	1.40	0.15	0.31	0.64
	120	1.26	0.16	0.31	0.67
F (C.D.)		4.40 (1.78)*			

*Significance at 5% level

inoculum or a different nutrient media promoted the formation of specific metabolites.

Recoveries of carbaryl and naphthol were better in nutrient broth than in basal salts medium, i.e., 82–94% at the end of 120 h of incubation. Reduction in recovery per centage (6–18%) in basal salts medium may be a result of opening of the aromatic ring of naphthol. Soil isolates have been reported to open the aromatic ring (Rajagopal *et al.*, 1983). It is possible that in nutrient broth other sources of carbon are available and microorganisms require limited metabolism of carbaryl for their needs, i.e., simple hydrolysis of the ester bond.

Effect of exogenously added carbon sources on degradation of carbaryl. The rate of total carbaryl degradation was lower in minimal media inoculated with all enrichment cultures, except in the presence of media containing glucose and yeast extract alone or in combination. In this case, there was an increase in growth compared to minimal media. Breakdown of carbaryl was significant with the unidentified species in basal salts media with and without glucose and yeast extract, while in other bacterial enrichment cultures the degradation was almost the same in these media (Table 3). The lower rate of carbaryl degradation compared to growth in the presence of additional carbon sources probably resulted from preferential utilisation of easily available sources of carbon and energy.

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