Biodegradation **4**: 115–123, 1993.

Synergistic interaction between two bacterial isolates in the degradation of carbofuran

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Received 15 June 1992; accepted in revised form 12 January 1993

Key words: insecticides, methylcarbamates, carbofuran, carbaryl, bendiocarb, carbosulfan, biodegradation, bacterial degradation, synergism

Abstract

The dominant bacteria *Pseudomonas* sp. and *Arthrobacter* sp. were isolated from the standing water of carbofuran-retreated *Azolla* plot. *Arthrobacter* sp. hydrolysed carbofuran added to the mineral salts medium as a sole source of carbon and nitrogen while no degradation occurred with *Pseudomonas* sp. Interestingly, when the medium containing carbofuran was inoculated with both *Arthrobacter* sp. and *Pseudomonas* sp., a synergistic increase in its hydrolysis and subsequent release of CO_2 from the side chain was noticed. This synergistic interaction was better expressed at 25° C than at 35° C. Likewise, related carbamates, carbaryl, bendiocarb and carbosulfan were more rapidly degraded in the combined presence of both bacterial isolates.

Introduction

Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl-*N*-methylcarbamate) has been a widely used broadspectrum insecticide for controlling insect pests of economically important crops. This insecticide is also effective in controlling common pests of *Azolla,* a fern that harbors a nitrogen-fixing blue-green alga, *Anabaena azollae* and is used as a biofertilizer in rice culture (Singh 1989). Carbofuran can undergo degradation by both chemical and biological means. In alkaline soil and water environments, degradation is largely by chemical hydrolysis (Rajagopal et al. 1984a). But, in near neutral soils and in acclimatized soils, degradation of carbofuran is mediated essentially by microorganisms. In the last one decade, considerable literature has accumulated on the accelerated biodegradation of carbofuran in acclimatized soils (Felsot et al. 1981; Read 1983, 1986; Kaufman & Edward 1983; Suett 1986; Racke & Coats 1988). More recently, we found that the phenomenon of accelerated biodegradation of carbofuran developed in *Azolla* plots after repeated applications of carbofuran (Singh et al. 1990). There are reports of degradation of carbofuran in enrichment cultures and in individual cultures of microorganisms (Karns et al. 1986; Ramanand et al. 1988a, b; Chaudhry & Ali 1988). The present study is concerned with the synergistic interaction between two bacterial isolates in the degradation of carbofuran.

Materials and methods

Insecticides and their metabolites

Analytical grade carbofuran (99.4% purity), uniformly ring-labelled-^{14}C-carbofuran (specific activity 39.4 mCi/mmol; 50% purity), carbonyl-^{14}C-car-

bofuran (specific activity, 13.3 mCi/mmol; 98% purity), carbofuran phenol, 3-hydroxycarbofuran and 3-ketocarbofuran were gifts from FMC Corporation, Middleport, New York. Technical carbaryl (99.4% purity) was obtained from Union Carbide (India) Ltd., Bombay, India. Technical grade bendiocarb (100% purity) and carbosulfan (89.5% purity) were gifts from M/s Rallis India Ltd., Bangalore, India.

Isolation of carbofuran degrading bacteria

An enrichment culture with an exceptionally high ability to degrade carbofuran was obtained by repeated additions of carbofuran to the standing water from carbofuran-retreated *Azolla* plot (Singh et al. 1990). For the isolation of carbofuran-degrading microorganisms from this enrichment culture, 1 ml of this enrichment culture after 5th addition was serially diluted. The sterile mineral salts medium ($MgSO_4 \cdot 7\ H_2O$, 0.200 g; K_2HPO_4, 0.1 g; $FeSO_4 \cdot 7\ H_2O$, 0.001 g; $CaSO_4$, 0.40 g; water, 1 l, pH 6.2] containing 20 µg/ml carbofuran was inoculated with 1 ml of each 10^{-1} to 10^{-10} dilution. After 5 days of incubation at room temperature (28± 2° C), sample from each flask was analysed for carbofuran by gas-liquid chromatography (GLC). The maximum dilution (10^{-7}) from which carbofuran disappeared completely in 5 days was plated on mineral salts agar medium supplemented with 3% sucrose and 10 µg of carbofuran per ml of medium. Of the four bacterial isolates that appeared on agar plates after 5 days of incubation at room temperature (28± 2° C), two dominant bacterial isolates (1 and 2) were selected and purified by successive transfers on sucrose-agar medium containing carbofuran (10 µg/ml).

Bacterial metabolism of carbofuran

The bacterial isolates (1 and 2) isolated from carbofuran degrading enrichment culture by serial dilution technique were tested for their ability to degrade carbofuran as a sole source of carbon and nitrogen individually or in combination. One millilitre aliquots of 400 µg/ml carbofuran (analytical grade) in acetone were dispensed in sterilized 100 ml Erlenmeyer flasks. After 24 h for evaporation of acetone at room temperature, 20 ml portions of sterilized mineral salts medium were added aseptically to each flask and equilibrated for 4 h. The sterile mineral salts medium (20 ml) containing 20 µg/ml carbofuran was inoculated with 0.2 ml of sterile water suspension of isolate 1 (approximately 22×10^3 cells) and isolate 2 (approximately 50×10^3 cells), individually and in combination. Uninoculated medium served as control. Uninoculated and inoculated flasks were incubated in a B.O.D. incubator at 25± 1° C and 35± 1° C. At periodic intervals, samples were removed aseptically and analysed for carbofuran by GLC after extraction by ethyl acetate.

Isotope studies

In a follow-up study, U-phenyl-^{14}C-carbofuran and carbonyl-^{14}C-carbofuran were used. ^{14}C-carbofuran (ring labelled or carbonyl labelled) in 0.5 ml acetone was added aseptically to sterilized 100 ml Erlenmeyer flasks. After evaporation of acetone at room temperature, 20 ml portions of sterilized mineral salts medium containing 10 µg/ml carbofuran were dispensed aseptically to each flask and equilibrated for 24 h. The mineral salts medium containing labelled carbofuran was inoculated with 0.2 ml of sterile water suspension of isolate 1 (approximately 20×10^3 cells) and isolate 2 (approximately 45×10^3 cells), individually and in combination. Uninoculated medium served as control. Flasks were closed with a rubber bung fitted with an inlet and an outlet, which were closed with a pinch cock. The assembly was then incubated at room temperature (28± 2° C). At periodic intervals, the inlet was connected to an air generator through a trap containing 25 ml of 2N KOH solution to remove $^{14}CO_2$ if any, from the air. The $^{14}CO_2$ that evolved from ^{14}C-carbofuran from each of the duplicate flasks containing inoculated and uninoculated medium was purged, after the pinchcock was released into 5 ml of $^{14}CO_2$ scintillation cocktail (repurged with nitrogen) containing pseudocumene (R.J. Harvey In-

strument Corporation, 123 Patterson Street, New Jersey, U.S.A.). The contents in each reaction flask were shaken with chloroform-diethyl ether (1:1 v/v) for extraction of residues and subsequent assay of the radioactivity by liquid scintillation after separation by thin-layer chromatography (TLC).

Assay of radioactivity

In isotope studies, residues in the medium from each flask were extracted 3 times with 30ml portions of chloroform-diethyl ether (1:1 v/v) (Venkateswarlu & Sethunathan 1978). The chloroform-diethyl ether fractions from three extractions were pooled and after evaporation to about 0.5ml at room temperature, the residues were redissolved in 1ml of methanol. The radioactivity remaining in water phase after organic solvent extractions were determined by liquid scintillation. Residues finally eluted in methanol were separated by TLC (Ramanand et al. 1988a). The standards were located by spraying successively with 2N NaOH in absolute methanol and a solution of *p*-nitrodiazonium fluoroborate (5mg dissolved in 25ml of methanol and 25ml of diethyl ether) (Archer 1976). The silica gel areas corresponding to authentic compounds (carbofuran, carbofuran phenol, 3-hydroxycarbofuran and 3-ketocarbouran) were scraped into 5ml of scintillation cocktail, Optiphase Hisafe II liquid scintillation cocktail (FSA Laboratory Suppliers, Loughborough, Leies, England) and radioactivity assayed in Rackbeta Liquid Scintillation Spectrometer Model 1209 (LKB, Wallac, Finland) programmed for colour and chemical quenching correction. DPM conversions with background quenching correction was done by Facit B 1100 printer interfaced with liquid scintillation spectrometer. $^{14}CO_2$ sorbed in scintillation cocktail was assayed directly by liquid scintillation.

The thin-layer chromatograms of the solvent extracts of the mineral salts medium containing ^{14}C-carbofuran and its degradation products were exposed to Kodak X-ray screen film for 30 days in a Siemen's metal casette and the film was developed with X-ray developer.

Degradation of carbaryl, bendiocarb and carbosulfan

Carbaryl (1-naphthyl-*N*-methylcarbamate), bendiocarb (2,2-dimethyl-1, 3-benzioxiol-4-yl-N-methylcarbamate) and carbosulfan (2,3-dihydro-2, 2-dimethyl-7-benzofuranyl (di-n-butyl) aminosulfenyl methylcarbamate) were added in 1ml acetone (400µg) to presterilized 100ml Erlenmeyer flasks. After acetone evaporation 20ml portions of mineral salts medium were added to the flasks and equilibrated for 4h. The mineral salts medium thus containing 20µg/ml of respective insecticide was inoculated with 0.2ml of sterile water suspension of 5 day old culture of isolate 1 (approximately 25×10^3 cells) and isolate 2 (approximately 60×10^3 cells) individually and in combination. Uninoculated medium for each insecticide served as control. Flasks were incubated in an incubator at 25±1° C. At periodic intervals, samples were withdrawn from duplicate flasks and analysed for respective insecticide by GLC after separation by TLC.

Gas liquid chromatography (GLC)

The carbofuran, carbaryl, bendiocarb and carbosulfan in the media (uninoculated and inoculated) of duplicate flasks were extracted by shaking 1ml of the sample in each flask with 2 to 4ml of ethyl acetate and organic fraction was dried over anhydrous sodium sulphate. The residues in ethyl acetate fraction were analysed by injecting 1 to 2µl portions into gas chromatograph (Varian Model 3400) equipped with a thermionic specific detector (TSD) by using a 5% OV 101 stainless steel column. The operating parameters for carbofuran, carbaryl and bendiocarb were: argon (carrier gas flow 30ml/min; hydrogen, 3ml/min; air, 150ml/min; column temperature, 190° C; injector temperature, 240° C; detector temperature, 250° C. Under these conditions, the retention time (R_t) for carbofuran, carbaryl and bendiocarb were 1.0, 1.5 and 0.6 min, respectively. For estimation of carbosulfan, injector, column and detector temperature were 250, 250 and 275° C, respectively with identical gas flow rates. Under these conditions, the retention time (R_t) for carbo-

sulfan was 1.8 min. All the four carbamate insecticides were quantified using a calibration graph of external standards which was linear over a range of 2 to 10 ng.

Using this extraction and analysis procedure, the recovery of carbofuran, carbaryl and bendiocarb ranged between 95 and 100% while for carbosulfan it ranged between 75 and 85%.

Results and discussion

Isolation and identification of bacteria

Two dominant bacteria (isolate 1 and 2) were selected out of four colony types that appeared on the agar plate after serial dilution technique. Isolate 1 was a strict aerobe, Gram negative, non-acid fast, nonspore-forming rods, while isolate 2 was a strict aerobe, Gram positive, non-acid fast, mostly consisted of jointed non-spore forming rods, irregular which were replaced by coccoid cells in aged culture. Based on various morphological, physiological and biochemical tests carried out in accordance with Bergey's Manual of Determinative Bacteriology (1974), isolates 1 and 2 were tentatively identified as *Pseudomonas* sp. and *Arthrobacter* sp., respectively.

Carbofuran degradation by the bacteria in pure cultures

The ability of the bacteria to degrade carbofuran individually and in combination as a sole source of carbon and nitrogen was studied in mineral salts medium incubated at 25 and 35° C. Carbofuran disappeared completely within 2 days from the mineral salts medium inoculated with *Arthrobacter* sp. alone or *Arthrobacter* sp. + *Pseudomonas* sp. at 35° C (Table 1). The loss of carbofuran from the medium inoculated with *Pseudomonas* sp. alone and from the uninoculated medium was negligible during the corresponding period. Evidently, *Pseudomonas* sp. alone was not capable of degrading carbofuran while *Arthrobacter* sp. could degrade carbofuran as a sole source of carbon and nitrogen. Data indicate a synergistic interaction between *Pseudomonas* sp. and *Arthrobacter* sp. in enhancing the degradation of carbofuran in the combined presence of both isolates. This synergistic action was expressed more distinctly at 25° C than at 35° C. Carbofuran disappeared completely in 3 days from the mineral salts medium inoculated with a combination of *Pseudomonas* sp. and *Arthrobacter* sp. at 25° C as compared to 60% loss from the medium inoculated with *Arthrobacter* sp. alone and negligible loss from the medium inoculated with *Pseudomonas* sp. Thus, *Pseudomonas* sp., albeit its inability to degrade carbofuran even after 5 days, synergistically increased the degradation of carbofuran by *Arthrobacter* sp.

In a follow-up study, ring-labelled ^{14}C-carbofuran was used to get further insight into the pathway of degradation of carbofuran by the bacterial isolates. The concentration of the insecticide decreased to about 16% of initial concentration in the medium inoculated with a combination of *Pseudomonas* sp. and *Arthrobacter* sp. after 2 days incubation (Table 2). While during the corresponding period only 48% of carbofuran was degraded in medium inoculated with *Arthrobacter* sp. alone. Assay of radioactivity after thin-layer chromatographic separation of the residues in organic solvent showed that carbofuran phenol accounted for 41 and 56% of the ^{14}C in ring-^{14}C-carbofuran in the medium inoculated with a combination of *Pseudomonas* sp. and *Arthrobacter* sp. at 1 and 2 days, respectively, as compared to 6 and 23% in the presence of *Arthrobacter* sp. alone. Radioautograph of this organic solvent extract showed the presence of only one prominent metabolite which had the same Rf of 0.78 as that of authentic carbofuran phenol. $^{14}CO_2$ evolution from the ring ^{14}C-carbofuran during bacterial metabolism was negligible. In uninoculated medium and medium inoculated with *Pseudomonas* sp. there was no appreciable decrease in the total radioactivity partitioned into organic solvent and radioautograph of this extract showed the presence of carbofuran only. These observations suggested that degradation of carbofuran by *Arthrobacter* sp. (individually or in combination with *Pseudomonas* sp.) led to the formation of carbofuran phenol as the major

metabolite and these bacterial cultures were not capable of mineralizing the ring moiety of carbofuran.

Bacteria capable of degrading carbofuran as sole source of carbon and nitrogen have been isolated earlier (Rajagopal et al. 1984 a, b; Venkateswarlu & Sethunathan 1984); but, these isolates took 40 days to degrade 60 to 80% of added carbofuran and most of the radioactivity accumulated as carbofuran phenol. An *Achromobacter* sp. isolated from retreated soil (Karns et al. 1988) effected most rapid degradation of carbofuran. Growing cells of this bacterium completely hydrolyzed carbofuran (20 µg/ml) within 42 h as a sole source of nitrogen in the mineral salts medium supplemented with glucose as additional carbon source. Recently, an *Arthrobacter* sp. isolated from carbofuran-retreated flooded alluvial soil incubated at 35° C, mineralized ^{14}C-carbofuran to $^{14}CO_2$ with ease (Ramanand et al. 1988 b).

In another study, the mineral salts medium containing carbonyl-^{14}C-carbofuran was inoculated with *Pseudomonas* sp. and *Arthrobacter* sp. individually and in combination. Total radioactivity in the organic solvent extract of the mineral salts medium inoculated with combination of *Pseudomonas* sp. and *Arthrobacter* sp. decreased to 6.8% after 2 days (Table 3) while from medium inoculated with *Arthrobacter* sp. alone it was 25.2%. On the other hand the recovery of radioactivity from medium inoculated with *Pseudomonas* sp. and uninoculated medium ranged between 84 and 85%. Analysis of the extract by thin-layer chromatography indicate that concentration of carbofuran reached undetectable levels in medium inoculated with combination of both isolates in 2 days while it took 4 days for complete loss in medium inoculated with *Arthrobacter* sp. alone. Data in Table 3 indicate that the combination of *Pseudomonas* sp. and *Arthrobacter* sp. was distinctly more effective in degrading carbofuran than the *Arthrobacter* sp. alone while virtually no degradation occurred with *Pseudomonas* sp. alone. Most of the activity lost from inoculated medium was accounted for as $^{14}CO_2$. Thus, about 45% of carbonyl-^{14}C was accounted for as $^{14}CO_2$ in medium inoculated with the combination of both bacterial isolates at day 1, as compared to 3% with *Arthrobacter* sp. alone. According to earlier studies (Rajagopal et al. 1984 a, b; Ramanand et al. 1988 a, b) substantial portion of radioactivity from carbonyl-^{14}C-carbofuran during its degradation by soil enrichment cultures and bacterial cultures was not accounted for and $^{14}CO_2$ evolved was negligible. Kuhr & Dorough (1976) reported that degradation of carbofuran pro-

Table 1. Degradation of carbofuran in mineral salts medium by *Pseudomonas* sp. and *Arthrobacter* sp. alone, and in combination at 25 and 35° C.

Incubation (days)	Carbofuran recovered[a] (µg·ml^{-1})							
	25° C				35° C			
	UI[b]	I[c]	I[d]	I[e]	UI[b]	I[c]	I[d]	I[e]
0	19.7± 0.1[f]	19.8± 0.0	20.0± 0.1	20.0± 0.0	19.9± 0.0	20.0± 0.5	20.0± 0.0	19.9± 0.0
1	19.8± 0.0	19.8± 0.1	17.1± 0.1	15.5± 0.0	19.6± 0.0	19.6± 0.2	3.6± 0.2	2.0± 0.0
2	19.7± 0.1	19.7± 0.1	15.7± 0.3	6.4± 0.4	19.5± 0.3	19.5± 0.1	0	0
3	19.7± 0.1	19.6± 0.1	8.0± 0.0	0	19.1± 0.1	19.1± 0.1	NE[g]	NE
4	19.5± 0.2	19.4± 0.1	3.7± 0.1	NE	18.8± 0.2	19.0± 0.0	NE	NE
5	19.1± 0.1	19.1± 0.1	0	NE	18.6± 0.0	18.6± 0.1	NE	NE

[a] The mineral salts medium was supplemented with carbofuran (20 µg·ml^{-1}).
[b] Uninoculated.
[c] The mineral salts medium was inoculated with *Pseudomonas* sp. alone.
[d] The mineral salts medium was inoculated with *Arthrobacter* sp. alone.
[e] The mineral salts medium was inoculated with *Pseudomonas* sp. + *Arthrobacter* sp. simultaneously.
[f] Mean of duplicate estimations ± mean deviation.
[g] Not estimated.

Table 2. Distribution of radioactivity during degradation of U-phenyl-^{14}C-carbofuran in mineral salts medium by *Pseudomonas* sp. and *Arthrobacter* sp. alone and in combination.

Incubation (days)	Treatment	Radioactivity recovered[a] from 20ml of medium (%)							
		Aqueous phase	Organic fraction	Carbofuran[b]	Carbofuran phenol[b]	CO_2	3-Keto-carbofuran[b]	3-Hydroxy-carbofuran[b]	Total recovery
0	Uninoculated[c]								
	Pseudomonas sp.	1.2± 0.1[d]	97.6± 3.4	92.9± 2.1	0.4± 0.0	–	–	–	98.8
	Arthrobacter sp.								
	Pseudomonas sp.+ *Arthrobacter* sp.								
1	Uninoculated	1.2± 0.2	97.1± 2.9	92.4± 3.1	2.8± 0.1	0.05± 0.01	1.1± 0.1	1.3± 0.2	98.35
	Pseudomonas sp.	1.2± 0.1	97.0± 3.0	93.2± 6.0	2.5± 0.0	0.3± 0.00	0.8± 0.2	1.4± 0.1	98.23
	Arthrobacter sp.	1.3± 0.3	96.5± 2.0	87.3± 2.0	6.1± 0.1	0.08± 0.00	0.9± 0.3	2.0± 0.3	97.88
	Pseudomonas sp+ *Arthrobacter* sp.	1.3± 0.1	97.4± 2.8	53.1± 1.0	41.8± 0.2	0.12± 0.02	0.4± 0.1	2.1± 0.2	98.82
2	Uninoculated	0.9± 0.3	96.6± 3.0	87.5± 2.4	3.6± 0.1	0.06± 0.01	1.5± 0.1	0.9± 0.1	97.56
	Pseudomonas sp.	1.3± 0.2	96.1± 1.4	87.8± 2.0	3.7± 0.2	0.07± 0.02	1.5± 0.2	0.7± 0.0	97.47
	Arthrobacter sp.	1.7± 0.1	96.4± 0.3	48.1± 1.8	23.0± 0.9	0.08± 0.01	2.3± 0.2	2.9± 0.3	98.18
	Pseudomonas sp.+ *Arthrobacter* sp.	1.8± 0.4	94.5± 1.3	14.8± 0.5	55.8± 0.4	0.10± 0.02	4.0± 0.1	2.0± 0.1	96.40
4	Uninoculated	2.1± 0.2	95.0± 3.2	85.9± 5.0	6.5± 0.6	0.07± 0.01	1.8± 0.2	0.6± 0.2	97.17
	Pseudomonas sp.	2.2± 0.0	95.4± 2.1	84.2± 3.0	5.7± 0.3	0.07± 0.02	1.9± 0.1	0.9± 0.2	97.67
	Arthrobacter sp.	2.7± 0.2	94.1± 3.4	1.1± 0.2	73.2± 2.1	0.08± 0.01	0.4± 0.0	1.7± 0.3	96.88
	Pseudomonas sp.+ *Arthrobacter* sp.	2.5± 0.1	94.0± 6.0	0.9± 0.0	75.2± 2.0	0.08± 0.01	0.5± 0.0	1.6± 0.2	96.58

[a] The mineral salts medium was supplemented with 1.7×10^5 dpm of labelled carbofuran·20ml^{-1} of medium.

[b] After separation of the residues by thin-layer chromatography.

[c] The mineral salts medium was not inoculated.

[d] Mean of duplicate estimations ± mean deviation.

Table 3. Distribution of radioactivity during degradation of carbonyl-^{14}C-carbofuran in mineral salts medium by *Pseudomonas* sp. and *Arthrobacter* sp. alone, and in combination.

Incubation (days)	Treatment	Radioactivity recovered[a] from 20 ml of medium (%)						
		Aqueous phase	Methanol fraction	Carbofuran[b]	3-Hydroxy-carbofuran[b]	3-Keto-carbofuran[b]	CO_2	Total recovery
0	Uninoculated[c]							
	Pseudomonas sp.	1.3± 0.3[d]	92.5± 5.0	89.3± 3.1	–	–	–	93.8
	Arthrobacter sp.							
	Pseudomonas sp.+ *Arthrobacter* sp.							
1	Uninoculated	1.3± 0.1	92.2± 4.3	82.1± 3.6	2.7± 0.2	1.7± 0.4	1.6± 0.2	95.1
	Pseudomonas sp.	NE[e]	NE	NE	NE	NE	NE	NE
	Arthrobacter sp.	1.1± 0.0	91.9± 4.1	80.2± 4.2	2.5± 0.4	1.8± 0.3	2.7± 0.5	91.3
	Pseudomonas sp.+ *Arthrobacter* sp.	1.1± 0.4	47.8± 3.3	40.0± 2.1	1.8± 0.3	2.5± 1.0	45.4± 2.2	94.3
2	Uninoculated	1.5± 0.2	86.2± 2.9	74.9± 4.2	5.0± 0.1	2.1± 0.2	2.0± 0.1	89.7
	Pseudomonas sp.	NE	NE	NE	NE	NE	NE	NE
	Arthrobacter sp.	1.5± 0.3	25.2± 1.2	19.9± 0.8	3.2± 0.0	2.8± 0.3	64.8± 3.8	91.5
	Pseudomonas sp.+ *Arthrobacter* sp.	1.6± 0.1	6.8± 0.8	3.5± 0.2	2.6± 0.1	0.8± 0.2	76.2± 5.2	84.6
4	Uninoculated	1.5± 0.2	85.0± 3.9	78.8± 2.2	4.9± 0.4	2.3± 0.0	2.2± 0.2	88.8
	Pseudomonas sp	1.4± 0.2	84.0± 4.1	79.0± 3.1	4.8± 0.2	2.3± 0.2	2.1± 0.3	87.5
	Arthrobacter sp.	1.8± 0.1	6.6± 0.3	1.3± 0.1	1.4± 0.1	0.9± 0.1	76.3± 4.4	84.7
	Pseudomonas sp.+ *Arthrobacter* sp.	NE	NE	NE	NE	NE	NE	NE

[a] The mineral salts medium was supplemented with 2.2×10^5 dpm of labelled carbofuran·20 ml^{-1} of medium.
[b] After separation of residues by thin-layer chromatography.
[c] The mineral salts medium was not inoculated.
[d] Mean of duplicate estimations ± mean deviation.
[e] Not estimated.

Table 4. Degradation of carbaryl, bendiocarb and carbosulfan in mineral salts medium by *Pseudomonas* sp. and *Arthrobacter* sp. alone and in combination at 35° C.

Incubation (days)	Insecticide recovered[a] (μg·ml^{-1})											
	Carbaryl				Bendiocarb				Carbosulfan			
	UI[b]	I[c]	I[d]	I[e]	UI[b]	I[c]	I[d]	I[e]	UI[b]	I[c]	I[d]	I[e]
0	20.0	19.7	19.8	19.6	19.6	19.7	20.0	20.0	15.8	15.9	15.7	15.8
1	19.7	19.7	10.0	4.6	10.2	10.1	2.4	0	NE[f]	NE	NE	NE
2	19.6	19.5	tr	0	3.6	3.4	0	NE	15.8	15.8	15.0	12.4
4	19.4	19.3	0	NE	NE	NE	NE	NE	15.0	14.8	14.1	8.2
6	NE	NE	NE	NE	NE	NE	NE	NE	14.5	14.5	10.5	4.1

[a] The mineral salts medium was supplemented with 20 μg of insecticides·ml^{-1} of medium.
[b] Uninoculated.
[c] The mineral salts medium was inoculated with *Pseudomonas* sp. alone.
[d] The mineral salts medium was inoculated with *Arthrobacter* sp. alone.
[e] The mineral salts medium was inoculated with *Pseudomonas* sp. + *Arthrobacter* sp. simultaneously.
[f] Not estimated.

ceeded by primary hydrolysis of the carbamate (O-C-NH-) linkage leading to the formation of carbofuran phenol and methylisocyanate. Methylisocyanate can undergo further decomposition to CO_2 and methylamine. Alternatively, methylisocyanate being highly volatile, may escape from the system through rapid volatilization. Interestingly, however, in the present study most of the carbonyl-^{14}C in carbofuran was accounted for as $^{14}CO_2$ during its degradation by the bacterial cultures.

As noticed with carbofuran in earlier experiments, *Arthrobacter* sp. effected rapid degradation of related carbamates, carbaryl and carbosulfan (Table 4), and their degradation by *Arthrobacter* sp. was further enhanced in the presence of *Pseudomonas* sp. which alone was not effective in degrading these carbamates. Bendiocarb was degraded in uninoculated controls almost as fast as in the presence of *Arthrobacter* sp. probably due to its chemical instability; but evidence suggested that its degradation was further enhanced in the combined presence of *Arthrobacter* sp. and *Pseudomonas* sp. Such cross adaptation of bacterial cultures to carbamate pesticides has been reported earlier (Harris et al. 1984; Felsot 1986; Racke & Coats 1988).

Most of the studies on the degradation of pesticides by microorganisms have been confined to individual cultures while pesticides, applied to natural ecosystems, are subjected to attack by diverse microorganisms. Our studies show that synergistic interaction between bacterial strains can help in the accelerated degradation of carbofuran especially at 25° C.

Acknowledgement

We thank Dr P.K. Singh (Director) for permission to publish this work. This research was supported by a grant from US-India Funds (FG-In-693).

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