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Degradation of Carbofuran by *Azospirillum lipoferum* and *Streptomyces* spp. Isolated from Flooded Alluvial Soil

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In rice culture, carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl N-methylcarbamate) is being extensively used in view of the recent outbreak of the brown planthopper (Nilaparvata lugens Stal.). Most of the studies indicate an indirect involvement of soil microorganisms in the degradation of carbofuran (Getzin 1973, Williams et al. 1976, Siddaramappa et al. 1978). Microorganisms have been implicated in the metabolism of carbofuran in flooded soils after an initial lag of about 20 days (Venkateswarlu et al. 1977, Venkateswarlu and Sethunathan 1979). We now report the metabolic pathway in the degradation of ring-14C-carbofuran by a bacterium (Azospirillum lipoferum) and two actinomycetes (Streptomyces spp.), isolated from flooded alluvial soil.

MATERIALS AND METHODS

A thin, undulating film characteristic of the genus Azospirillum was observed in the standing water of a flooded alluvial soil taken in a test tube and amended with technical carbofuran (40 ppm). One loopful of this was transferred to a semisolid malate medium, used for the isolation of Azospirillum lipoferum (Dobereiner et al. 1976). A typical white, dense, fine pellicle developed, a few mm below the surface of the semisolid malate medium within 24 h at 30°C. The organism was cultured following the technique of Neyra and Dobereiner (1977) and identified as Azospirillum lipoferum (Beijerinck), based on the description of Tarrand et al. (1978).

In another attempt, 10 g portions of the alluvial soil were spread as a thin layer in 250 mL Erlenmeyer flasks and then flooded with 12.5 mL of distilled

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water to simulate the oxidized surface of flooded soil. The soil was then supplemented with Furadan 3G (3% carbofuran) to provide 5000 ppm of carbofuran. The two distinct white, visible colonies appeared on the surface of the flood water of the soil were tentatively identified as Streptomyces spp.

Uniformly ring-labeled ^{14}C-carbofuran (specific activity, 2.20 mCi/mmol; 98% purity) was first dissolved in 100 mL acetone. The acetone in a 5 mL aliquot of this stock solution was evaporated to dryness at room temperature and the residue was equilibrated with distilled water for 8 h before Millipore filtration. To 25 mL portions of the sterile salts medium (pH 7.0) without any carbon source, taken in 100 mL Erlenmeyer flasks, the filtered labeled-carbofuran together with technical carbofuran was added to provide a final concentration of 40 ppm. The flasks were inoculated either with one milliliter suspension of A. lipoferum culture maintained in mineral salts medium containing carbofuran or with seven-day old cultures of Streptomyces spp. from solid agar medium. Uninoculated medium served as control. After 20 and 30 days of incubation at 30°C in a BOD incubator, two replicates were removed for residue analysis.

The residues of carbofuran from two replicate samples were extracted thrice using chloroform-diethyl ether, (1:1) as described earlier (Venkateswarlu et al. 1977). After evaporating the solvent from the pooled fractions, the residues were redissolved in 2 mL methanol. The radioactivity partitioned into the solvent and the medium left after solvent extraction was determined in a liquid scintillation counter, LSS 20, Electronics Corporation of India Ltd., Hyderabad (Venkateswarlu and Sethunathan 1979). After thin-layer chromatographic separation, the silica gel areas on TLC plates opposite to the authentic compounds (carbofuran and its predicted metabolites, carbofuran phenol and 3-hydroxycarbofuran) were taken into 5 mL scintillator for radioactivity determination.

RESULTS AND DISCUSSION

At the end of 30 days of incubation, nearly 73% of carbofuran was degraded in the mineral salts medium inoculated with A. lipoferum (Table 1) as compared to only 37% loss from the uninoculated medium. The slow decomposition of carbofuran which occurred in uninoculated medium might be due to its chemical

Table 1. Degradation of ^{14}C-carbofuran by *Azospirillum lipoferum* and *Streptomyces* spp isolated from flooded alluvial soil

Incubation (days)	Treatment	%Radioactivity recovered/25 mL medium[a]				
		Solvent phase[b]	Carbofuran[c]	3-Hydroxy-carbofuran[c]	Carbofuran phenol[c]	Water soluble[d]
20	Uninoculated	83.65	65.45	1.73	4.14	2.41
	A. lipoferum	76.55	46.41	1.73	14.32	8.00
	Streptomyces sp 1	61.77	41.55	0.82	12.18	44.55
	Streptomyces sp 2	73.55	48.95	1.36	9.68	18.32
30	Uninoculated	75.14	62.59	0.77	0.05	2.64
	A. lipoferum	51.05	27.23	0.73	4.64	8.59
	Streptomyces sp 1	37.14	24.37	0.86	5.73	35.50
	Streptomyces sp 2	62.91	32.59	0.73	13.50	17.73

[a]Per cent recovery of the initially added radioactivity, mean of two replicates.
[b]Partitioned into chloroform-diethyl ether.
[c]After tlc separation of the residues.
[d]Partitioned into the water phase after solvent extraction.

hydrolysis at pH 7.0 as evidenced by the formation of carbofuran phenol even in uninoculated medium. Carbofuran phenol was the major product of bacterial metabolism of carbofuran with 3-hydroxycarbofuran as minor product. Carbofuran phenol decreased between 20 and 30 days indicating its further transformation. The decrease in the recovery of total radioactivity in organic solvent extract of the inoculated medium was accompanied by an increase in the radioactivity in the water phase remaining after organic solvent extraction indicating the conversion of carbofuran past carbofuran phenol and 3-hydroxycarbofuran. It is very interesting to note that A. lipoferum isolated in our laboratory from rice roots and flooded alluvial soil also exhibited greater 15N incorporation and acetylene reduction (Nayak et al. 1981).

Of particular interest is also the higher capacity of both Streptomyces spp. to degrade carbofuran (Table 1). Within 10 to 20 days of incubation with Streptomyces spp, the color of the medium turned from colorless to reddish brown indicating the conversion of carbofuran to colored metabolite(s). The extent of carbofuran degradation was 76 and 67% after 30 days of incubation with Streptomyces isolates 1 and 2, respectively, as compared to the 37% in uninoculated medium. Carbofuran phenol was recovered as a major product of carbofuran metabolism by Streptomyces spp. Carbofuran phenol was metabolized further, as indicated by its decrease, probably to the colored metabolite. Interestingly, the colored metabolite was not extracted by the chloroform-diethyl ether unlike carbofuran and carbofuran phenol but remained in the water phase. Thus, at the end of 30 days, 36 and 18% of added carbofuran was recovered from the water phase (remaining after organic solvent extraction) of the medium inoculated with isolates 1 and 2, respectively, as against the corresponding value of only 3% from the water phase of the uninoculated medium. This showed that the degradation of carbofuran was fairly rapid in the presence of the isolates of Streptomyces resulting in the formation of water soluble products. According to an earlier report (Williams et al. 1976), actinomycetes have been particularly implicated in the degradation of carbofuran in aerobic soils.

The present data would indicate that A. lipoferum, shown to occur in most of the rice soils (Charyulu and Rao 1980), besides enriching the soil with biologically fixed nitrogen appears to play a major role in the degradation of carbofuran under flooded conditions.

Streptomyces spp which predominated in the aerobic zones of the flooded soil may also facilitate the carbofuran loss from the rice fields considerably leading to the formation of colored water soluble products.

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REFERENCES

Charyulu PBBN, Rao VR (1980) Influence of various soil factors on nitrogen fixation by Azospirillum spp. Soil Biol Biochem 12:343-346

Dobereiner J, Marriel IE, Nery M (1976) Ecological distribution of Spirillum lipoferum Beijerinck. Can J Microbiol 22:1464-1473

Getzin LW (1973) Persistence and degradation of carbofuran in soil. J Environ Entomol 2:461-467

Nayak DN, Charyulu PBBN, Rao VR (1981) $15N_2$ incorporation and acetylene reduction by Azospirillum sp isolated from rice roots and soils. Plant Soil 61: 429-436

Neyra CA, Dobereiner J (1977) Nitrogen fixation in grasses. Adv Agron 29:1-38

Siddaramappa R, Tirol AC, Seiber JN, Heinrichs EA, Watanabe I (1978) The degradation of carbofuran in paddy water and flooded soil of untreated and re-treated rice fields. J Environ Sci Health 13B:369-380

Tarrand JF, Krieg NR, Dobereiner J (1978) A taxonomic study of the Spirillum lipoferum group, with description of a new genus, Azospirillum gen.nov. and two species, Azospirillum lipoferum (Beijerinck) comb. nov. and Azospirillum brasilense sp.nov. Can J Microbiol 24:967-980

Venkateswarlu K, Gowda TKS, Sethunathan N (1977) Persistence and biodegradation of carbofuran in flooded rice soils. J Agric Food Chem 25:533-536

Venkateswarlu K, Sethunathan N (1979) Metabolism of carbofuran in rice straw-amended and unamended rice soils. J Environ Qual 8:365-368

Williams IH, Pepin HS, Brown MJ (1976) Degradation of carbofuran by soil microorganisms. Bull Environ Contam Toxicol 15:244-249

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