IN VITRO DEFLUORINATION OF MONOFLUOROACETATE BY SOME BACTERIA ISOLATED FROM SOILS

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INTRODUCTION

A few literatures are available on the defluorination of organic fluorine compounds (HORIUCHI, 1961, 1962; KAUFMAN, 1961; MOUNTER et al., 1955; TONOMURA et al., 1965). HORIUCHI (1961) isolated a bacterium which is capable of splitting the C-F bond of monofluoroacetate (FA) and identified it to be *Pseudomonas indoloxidans*. TONOMURA et al. (1965) also made a search for dehalogenation bacteria. In the preceding paper the authors investigated the dynamic change of microbial population in soils sprayed with FA or monofluoroacetamide (FAA) and assumed that defluorination of these compounds in soils might not be as extensive as had been expected (Ouchi et al., 1971).

It is yet evident, however, that some bacteria contain an enzyme which catalyzes defluorination in an *in vitro* system. Question then arose as to whether the pesticide ingredient-resistant bacteria are indeed able to defluorinate these organic fluorine compounds. In this paper the authors will describe the growth and defluorination patterns of some bacteria isolated from soils and will discuss the possible participation of these organisms in the *in vivo* degradation of the pesticides.

MATERIAL AND METHOD

Bacteria: The bacterial cultures used in this experiment were selected from the organic fluorine-resistant bacteria discussed in the preceding paper (OUCHI et al., 1971).

Growth test and defluorination test: A basal medium containing K_2HPO_4 0.5 g, KCl 0.25 g, $NaNo_3$ 1.0 g, $MgSo_4 \cdot 7H_2O$ 0.25 g, $FeSo_4 \cdot 7H_2O$ 0.02 g, meat extract 0.2 g per liter (Cme medium) was used for the primary culture. Five ml of this medium was inoculated with a loopful test bacterium and incubated at 30°C for 48 hrs. For the secondary culture, the Cme medium deprived of meat extract was added with 5.0 g of sodium monofluoroacetate (CFA medium) or monofluoroacetamide (CFAA medium). The whole content of the primary culture was added to 100 ml of CFA or CFAA medium and the inoculated medium was incubated at 30°C for 28 days. An aliquot of the culture was taken with an appropriate time interval

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and subjected to turbidity measurement for estimating growth and to quantitative determination of fluorine for knowing the degree of defluorination. The turbidity was measured with a spectrophotometer at 640 nm. Fluorine was determined photometrically by the zirconium-alizalin method of MEGREGIAN (1954). Applicability of this method to the present experimental system was checked as follows: The medium not inoculated with bacteria was diluted 25 times and added with a known amount of NaF; To 2 ml of this solution was added 2.5 ml of zirconium-alizalin reagent and the developed color intensity was compared at 528 nm with water control containing the same amount of fluoride. No difference was detected between the medium and water samples suggesting that obstruction of color development by ions in the medium is negligible if ever existed.

RESULT

1. Bacterial Growth in Defined Media Containing The Pesticide Ingredients.

All the bacterial isolates were tested for their growth in media containing FA or FAA as a sole source of carbon. One of the representative results is shown in Table 1. It clearly shows that some of the ingredient-resistant bacteria are unable to utilize these pesticide ingredients while some are indeed capable of utilizing them. It also shows that some isolates grew well in the presence of FA but not in the presence of FAA and *vice versa*. This fact suggests that enzyme systems are different from each other and substantiate the notion that the enzyme induced by FA has a high specificity to FA (TONOMURA et al., 1965).

Table 1. Growth of some bacterial isolates in a defined medium containing organic fluorine compound

Isolate No.	Growth (Optical Density at 640 nm)*	
	CFA medium	CFAA medium
2D-186	0.012	0.035
2D-188	0.013	0.026
2D-196	0.002	0.022
2D-207	0.010	0.013
2D-209	0.022	0.005
2D-212	0.022	0.020
2D-222	0.035	0.008
2D-229	0.023	0.036
2D-237	0.023	0.001
2D-241	0.018	0.008
2D-258	0.052	0.008
2D-264	0.002	0.061
2D-265	0.063	0.004
2D-270	0.039	0.008
2D-278	0.075	0.086

* Turbidity of each tube was measured immediately after inoculation and substracted from the final turbidity (10 days after inoculation)

2. Defluorination of Monofluoroacetate

Most of the isolates were tested for their capability to grow in the defined medium containing the pesticide ingredient as was illustrated in the preceding section. The isolates which gave high turbidity in the growth test were selected for the defluorination test. The result is represented in Table 2.

It is apparent that some bacteria defluorinate FA extensively while others do

Table 2. Defluorination of monofluoroacetate in medium by some bacterial isolates*

Isolate No.	Growth (O. D_{640})	Defluorination** (%)
15	0.027	3.9
30	0.215	85.5
53	0.102	88.8
59	0.222	96.7
69	0.032	2.6
70	0.009	4.6
88	0.060	82.9
0-12	0.032	2.6
0-15	0.023	2.0
0-18	0.012	5.9
0-26	0.200	67.8
0-32	0.167	54.6
0-35	0.207	71.7
0-37	0.033	5.3
0-38	0.142	67.8
0-41	0.102	65.8
0-43	0.318	62.5
0-50	0.133	55.9
0-71	0.275	75.7
0-73	0.119	75.7
CFD***	0.062	67.8

* Refer to the text for the detailed experimental conditions. The data in this table was obtained of cultures incubated for 28 days.

** $\frac{\text{fluorine liberated}}{\text{fluorine added}} \times 100$

*** A stock culture known to defluorinate FA

slightly. It will be noticed that the defluorination activity roughly parallels with the growth rate in the defined medium. The growth curves of some representative isolates ere shown in Figure 1. Most of the isolates which are shown to efficiently defluorinate FA had a relatively long lag phase and grew slowly comparing to the reference Pseudomonad (a culture stock from Daikin Industrial Company). Further analyses of defluorination pattern indicated that when the primary inoculum was cultured in a medium containing 1.0 g of meat extract, the defluorination was not as extensive as seen in Table 2. This would probably be attributed to the disturbance of preadaptation by the meat extract added excessively to the medium, in the light of finding that the defluorinating

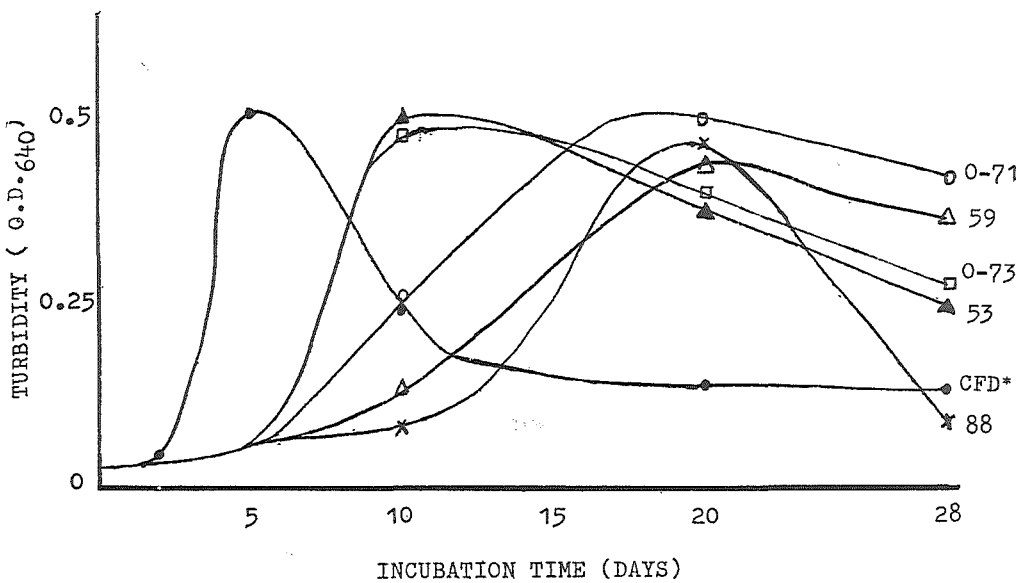

Fig. 1. Growth of several bacterial isolates in a defined medium containing monofluoroacetate.
Number attached to each growth curve represents isolate number.
CFD* is a stock culture known to defluorinate FA.

bacteria are easily deadapted in the presence of excess nutriennts (HORIUCHI, 1962). This fact would then suggest that the bacterial adaptation to and defluorination of these pesticide ingredients could hardly be expected as far as other organic nutrients are available in soil for the bacterial growth.

DISCUSSION

It has been clearly demonstrated that some of the ingredient-resistant bacteria isolated from soils are indeed capable of rupturing C-F link of FA or FAA in a liquid media. The defluorination activity of these isolates, however, seems to vary depending on the conditions of the primary culture. As was shown by HORIUCHI (1962), the adaptive defluorination system is apparently disturbed by the presence of nutrients in the medium. Taking this fact into consideration, it seems to be reasonable to conclude that the microbial defluorination in nature might not be as extensive as had been expected unless a massive addition of the pesticide was executed in orchards. A preliminary experiment suggests that defluorination would be secured when the preadapted bacteria were added to soils sprayed with the pesticide ingredients.

SUMMARY

1. Some of the bacterial isolates are capable of growing in a defined medium containing FA or FAA as a sole source of carbon and are indeed able to defluorinate these pesticide ingredients.

2. The defluorination activity roughly parallels to the growth rate in the medium.

3. The apparent disturbance of the adaptation process by the nutrients carried over from the inoculum suggests that the bacterial adaptation to defluorination could hardly be expected in the ordinary field soil.

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土壌より分離した細菌によるモノフルオロ酢酸の分解

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前報において分離された細菌について，培地中における脱ふっ素活性を測定した結果，かなりの分離株において脱ふっ素活性の高いことが明らかになった．モノフルオロ酢酸（FA）を用いて測定した脱ふっ素活性は FA 含有培地中における生育度とほぼ比例する．しかしながら，接種源培養を多量の肉エキス存在下で行なった場合には，脱ふつ素活性は低くなるから，微生物栄養源の多い通常の果樹園，森林等における有機ふつ素化合物の分解にはかなりの困難がともなうと考えた．