Kinetics of DDT Degradation as Influenced by Selected Strains of Micrococci

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INTRODUCTION

Studying the possibility of organochlorine pesticide degradation influenced by microorganisms isolated from meat products, we found that several strains of micrococci were able to degrade these compounds. The obtained results were presented in a previous report (7) in which we described the incubation of isolated strains in physiological saline in the presence of defined concentrations of pesticides. As pesticides were then the only source of energy, we directed our further work to an examination of the influence of selected strains of micrococci on pesticide degradation in the presence of nutritive matter, as well as to an examination of the kinetics of this process.

MATERIALS AND METHODS

The kinetics of DDT degradation was examined in bouillon under the influence of three strains of micrococci marked as III, IX and X. Namely, 5 $(m^3)^{-6}$ amounts of 48-hour old bouillon cultures of each of the examined strains, with an initial bacterial count of $10^6/(m^3)^{-6}$, were poured into tubes. An aliquot of $10(m^3)^{-9}$ of a solution of pp'DDT at a concentration of 1 $nkg/(m^3)^{-9}$ was added to each tube under sterile conditions. Bouillon cultures treated in this way were incubated at 37°C. A few tubes of each of the examined strains were not incubated. Their content was used for the analysis of pesticides in order to determine the recovery of the analytical procedure.

The rate of DDT degradation was followed by analysis after 2, 4, 6, 8, 10, 12, 16, 20, 24, 28, 32, 36, 43, 48, 54 and 60 hours of incubation.

The kinetics of pesticide degradation takes place according to the law given in the Michaelis-Menten equation which is characteristic for enzymatic reactions. Proceeding from this, we determined the maximum reaction velocity (Vmax) and the Michaelis constant (Km) in the way described by Counotte and Prins (4):

$$\frac{d[S]}{dt} = v = \frac{V_{max} \cdot [S]}{K_m + [S]}$$, where

$[S]$ - substrate concentration (DDT in this case) at the time t
v - reaction velocity
V_{max} - maximum reaction velocity
K_m - Michaelis constant

If the above equation is integrated, we obtain:

$$\int_0^{\infty} \frac{K_m \cdot d[S]}{V_{max} \cdot [S]} + \int_0^{\infty} \frac{d[S]}{V_{max}} = \int_0^{\infty} dt$$

$[S_o]$ - initial substrate concentration

at $t = 0 \dashrightarrow [S] = [S_o]$, and therefore:

$$K_m \cdot \ln \frac{[S]}{[S_o]} + [S] - [S_o] = V_{max} \cdot t$$

The last equation can be written as $y = ax + b$, where

$$y = K_m \cdot \ln \frac{[S]}{[S_o]} + [S] - [S_o]$$

$x = t$

$a = V_{max}$, while b is a constant

Since the concentration of DDT in these experiments was low, the expression $[S] - [S_o]$ was of irrelevantly small value and we omitted it in our calculations.

When K_m is known and the "y" value is plotted egainst "x", a straight line is obtained with a slope equal to "a", while "b" = 0.

As K_m for DDT degradation as influenced by micrococci strains was not known, some arbitrary values for K_m were taken (K_m^1, K_m^2, K_m^3 and K_m^4) and we obtained straight lines with segments "b" $\neq$ 0 and slopes giving corresponding values for V_{max} (V_{max}^1, V_{max}^2, V_{max}^3 and V_{max}^4), (Figure 1.b). In our diagrams, substitution of only two K_m values (K_m^1 and K_m^2) is presented. Deviations of the "b" values from zero can be used for the calculation of real K_m and V_{max} values (Figure 1.c). When the values for "b" were plotted against the corresponding values for maximum velocities, the real V_{max} value was obtained at "b" = 0. The real K_m value can be calculated when the arbitrary values for K_m^1, K_m^2, K_m^3 and K_m^4 are plotted against V_{max}^1, V_{max}^2, V_{max}^3 and V_{max}^4. In that case, the real K_m value (Figure 1.d) will correspond to the real V_{max} value obtained according to the relationship presented in diagram 1.c. Using this procedure, K_m and V_{max} were obtained by measuring the DDT concentrations, during the reaction, as a function of time (Table 1, Figure 1.a).

The results were evaluated with the least squares method, using a programmable pocket calculator - Texas Instruments TI-59.

Degradation of DDT under the influence of strains III, IX and X at different time intervals

Table 1.

Time (h)	Strain III $[S].10^3$(nkg)	Strain IX $[S].10^3$(nkg)	Strain X $[S].10^3$(nkg)
0	4.00	4.00	4.00
2	-	3.69	-
4	-	3.74	-
6	-	3.70	3.68
8	-	3.78	3.42
10	-	3.66	3.72
12	3.67	3.58	-
16	3.57	-	3.18
20	3.25	2.79	3.04
24	3.02	-	2.88
28	2.82	2.50	3.89
32	2.69	-	-
36	2.46	3.06	2.70
43	1.88	2.36	-
48	1.96	2.27	2.32
54	1.97	1.68	-
60	-	1.47	1.90

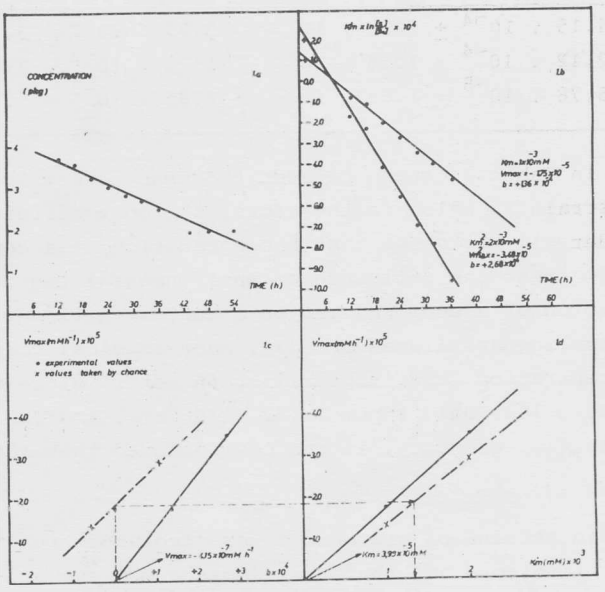

Fig. 1. Calculation of K_m and V_{max} for strain III from substrate concentration versus time plots.

RESULTS AND DISCUSSION

Enzymatic reactions are investigated in order to evaluate the affinity of enzymes for certain substrates and to determine the reaction velocity. On the other hand data on the activity of a particular microorganism are obtained from the degradation of certain substrates (4). The results obtained in this study have been used to compare the efficiency of the examined strains during degradation of pesticides.

Besides theoretical value, determination of the Michaelis constant (K_m) also has significant practical value. Thus, a definition of K_m is that it is the substrate concentration at half maximum velocity. This may be shown algebraically by

$$v = \frac{V_{max}[S]}{K_m + [S]} \quad \text{if} \quad v = \frac{V_{max}}{2}$$

$$K_m = [S]$$

According to the opinion of Neilands and Stumpf (6), this constant is by far the most fundamental constant used in enzyme chemistry. On the basis of the K_m value, many practical conclusions can be made. For changes in a substrate brought about by enzymes, the most suitable enzyme is the one having the lowest K_m value. Using values for K_m, the substrate concentration giving the maximum velocity for a reaction can be calculated. In addition, on the basis of K_m values data on the identity of enzymes izolated from different sources can also be obtained.

The maximum velocity of pp'DDT degradation under the influence of enzymes from micrococci strains III, IX and X, and the corresponding Michaelis constants are given in Table 2.

Kinetic parameters of pp'-DDT degradation in bouillon as influenced by different strains of micrococci

Table 2.

Micrococci strains	V_{max} (μmol h^{-1})	K_m (μmol)
III	$-4.15 \cdot 10^{-4} \pm 5.58 \cdot 10^{-4}$	$3.99 \cdot 10^{-2} \pm 3.19 \cdot 10^{-2}$
IX	$-2.12 \cdot 10^{-4} \pm 1.38 \cdot 10^{-4}$	$1.72 \cdot 10^{-2} \pm 1.00 \cdot 10^{-2}$
X	$-5.78 \cdot 10^{-5} \pm 1.09 \cdot 10^{-4}$	$4.85 \cdot 10^{-3} \pm 9.46 \cdot 10^{-3}$

From the results presented in Table 2 it can be seen that the lowest value for the Michaelis constant was shown by strain X, which is,therefore, the most efficient one in the case of DDT degradation. Alexander (1) has shown that biodegradation of a compound is dependent on its chemical structure, whereas the influence of environmental factors has only partially been examined. Concentration of a compound can be a very significant factor for its degradation. According to Boethling and Alexander (3), many chemical compounds are persistent owing to their low concentration and insolubility in water. Since the concentrations of DDT in our experiments were low, only 1 ppm ($2.82 \cdot 10^{-5}$mM), and the examined strains degraded about 50% of the initial quantity, it can be concluded that their efficiency is satisfactory.

If we have in mind that these strains of micrococci were isolated from fermented sausages, that they represent useful microflora and that they degrade DDT even in the presence of the nutritive constituents of bouillon, then it is quite justifiable to presume that all the examined strains could be used to degrade residues of this pesticide in other substrates as well and, consequently, in meat products.

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