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Lytic action of the egg-white lysozyme on *Micrococcus radiodurans* isolated from irradiated meats and sea foods,

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There are a lot of papers concerning the lytic action of lysozyme on several strains. Most of these works have been carried out in the biochemical field to clarify the mechanism of lysis by lysozyme, and in these studies the test strains were generally used for the experiment after being killed by exposing to ultraviolet ray.

Since the microorganism contaminating food generally grow well on food, it seems reasonable that in a study of the lytic action of lysozyme on the microorganism contaminating food, living test strains should be used.

Micrococcus radiodurans, a non-sporing spoilage organism highly resistant to ionizing radiation and to ultraviolet light has been reported by Duggan[2] ***et al.*** and Anderson.[1] This organism has been detected often from irradiated meats and sea foods. The present paper records the lytic action of egg-white lysozyme on ***Micrococcus radiodurans.***

Methods

Organism : Strain of ***Micrococcus radiodurans*** was cultured in 100 ml of TGY broth (trypton 5 g, glucose 1 g, yeast extract 1 g, D. L. methionine 10 mg, distilled water to one 1, adjusted to pH 7.2) on a shaker at 37°C for 24 hr.

Lysis test: Organism was grown on pepton medium (100 ml, pH 7.2) for 24 hr. Crystalline lysozyme is dissolved in 0.1 % potassium phosphate buffer (pH 7.2 or 5.5) in the rate of 1.0 % concentration.

Procedure of examination :

The procedure of examination was shown in Table 1.

A, B.....F in Table 1 are not inoculated solutions of each concentra-

Table 1. Culture method.

Kinds of solutions		Concentration of lysozyme in the test solutions (pH 7.2)											
		Control		0.0125 %		0.025 %		0.05 %		0.1 %		0.2 %	
		A	A'	B	B'	C	C'	D	D'	E	E'	F	F'
Amounts mixed (ml)	ph.	5.0	5.0	4.875	4.875	4.75	4.75	4.5	4.5	4.0	4.0	3.0	3.0
	T.	5.0		5.0		5.0		5.0		5.0		5.0	
	T-B.		5.0		5.0		5.0		5.0		5.0		5.0
	LY.			0.125	0.125	0.25	0.25	0.5	0.5	1.0	1.0	2.0	2.0

ph. ..... Phosphate buffer at pH 7.2.
T. ..... TGY medium.
T-B. ..... Organisms grown in TGY at 40°C for 24 hr.
Ly. ..... Lysozyme solution in 0.5 M phosphate buffer at pH 7.2.

tion of lysozyme. A', B'..... F' are inoculated solutions of each concentration of lysozyme.

Measuring of lytic action: Lytic action of lysozyme was determined at 560 mμ by using a spectrophotometer. The transmittance value effected by 0.0125 % lysozyme was obtained by decreasing the transmittance value of B from the transmittance value of A, whereas in increasing the concentration of lysozyme, transmittance value decreased.

Thus the transmittance values effected by lysozyme in each concentration were determined. The experimental procedure for the lytic action of lysozyme on organisms was as follows. The transmittance values (A', B'..... F') were measured after incubation for 0, 1, 24, 48 and 72 hr at 37°C. To these values, the transmittance values effected by lysozyme in each concentration were added respectively. These were the true transmittance values of organisms grown in phosphate buffer and TGY broth for 0, 1, 24, 48 and 72 hr in each lysozyme solution.

Results

Experiment I. Effect of lysozyme treatment of ***Micrococcus radiodurans*** on lysis.

(Experiment I-a) Lytic action of lysozyme incubated at 37°C in the test solution of pH 7.2.

The results were shown in Table 2 and Fig. 1.

As shown in Table 2 and Fig. 1, there were differences of transmittance value between control and each test solution, especially in 0.0125

Table 2. Lytic action of egg-white lysozyme on ***Micrococcus*** *radiodurans* (expressed per cent transmittance at 560 mμ).

Conditions of samples		Concentration of Iysozyme in the test solutions (pH 7.2)					
		Control	0.0125 %	0.025 %	0.05 %	0.1 %	0.2 %
Hours of incubation at 37°C	0	51.0	50.0	51.0	51.0	51.3	54.0
	1/2	47.0	51.4	52.0	54.2	55.0	57.5
	1	47.5	51.4	50.5	54.2	53.5	56.0
	19	46.0	64.0	58.2	52.5	49.3	40.0
	24	45.0	58.0	56.0	53.5	47.0	36.0

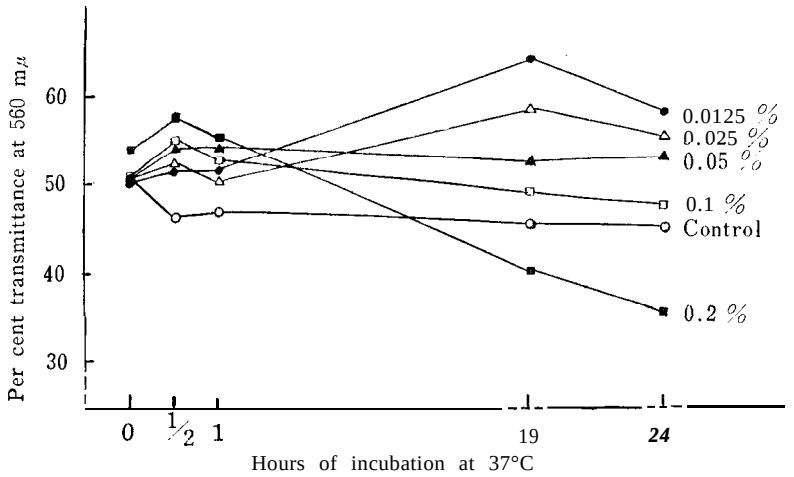

Fig. 1. Lysis of ***Micrococcus radiodurans*** by lysozyme.

% and ***0.025*** % lysozyme solutions. An increase in lysozyme concentration at levels was accompanied by an increased rate of lysis, but at levels above 0.05 % there was a rapid decrease in activity.

(Experiment I-b) Lytic action of lysozyme incubated at 40°C for O-24 hr in the test solution of pH 7.2.

The results were shown in Table 3 and Fig. 2.

As shown in Table 3 and Fig. 2, the test solutions of lysozyme showed a high lysis than the control, and a maximum lysis was observed in 0.0125 % concentration. In this experiment the lysis in a low concentration of lysozyme was greater than that of in a high concentration of lysozyme.

Table 3. Lytic action of egg-white lysozyme on ***Micrococcus radiodurans*** (expressed per cent transmittance at 560 mμ).

Conditions of samples		Concentration of lysozyme in the test solutions (pH 7.2)				
		Control	0.0125 %	0.025 %	0.05 %	0.1 %
Hours of incubation at 40°C	0	51.0	52.0	51.0	52.0	54.0
	1/2	50.0	55.0	54.0	51.0	51.0
	1	46.0	49.0	47.5	46.9	49.0
	19	42.0	65.0	61.0	61.0	45.0
	21	41.0	64.0	61.0	63.5	44.8
	24	40.0	63.0	59.8	57.5	41.2

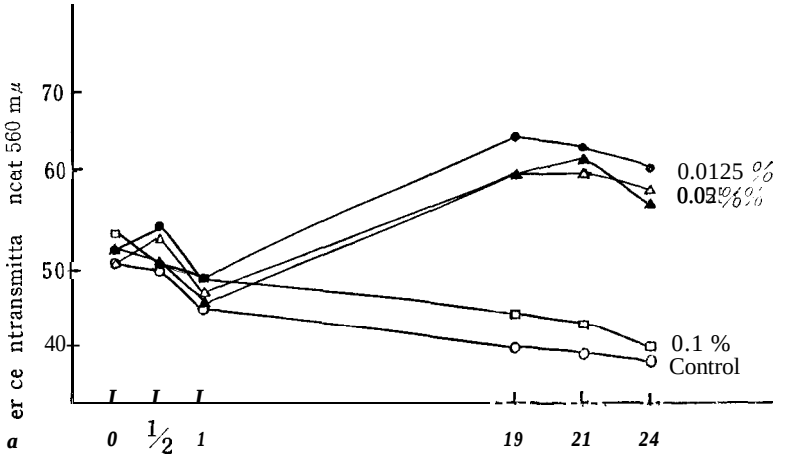

Fig. 2. Lysis of ***Micrococcus radiodurans*** by lysozyme.

(Experiment I-c) Lytic action of egg-white lysozyme incubated at 40°C for O-48 hr in the test solution of pH 5.5.

The results were shown in Table 4 and Fig. 3.

Similar tendency was obtained as shown in Tables 2 and 3. However the lytic activity of enzyme in this experiment I-c was higher in comparing with two other experiments (experiment I-a and I-b).

Experiment II. Effect of lysozyme treatment on the growth of ***Micrococcus radiodurans.***

The results were shown in Figs. 4, 5, 6 and 7.

Table 4. Lytic action of egg-white lysozyme on ***Micrococcus radiodurans*** (expressed per cent transmittance at 560 mμ).

Conditions of samples		Concentration of lysozyme in the test solutions (pH 5.5)					
		Control	0.0125 %	0.025 %	0.05 %	0.1 %	0.2 %
Hours of incubation at 40C°	0	63.0	***62.0***	***62.0***	***63.0***	***63.5***	***64.0***
	1/2	64.0	61.0	***59.0***	***63.2***	***61.5***	***63.0***
	1	***63.0***	***62.0***	***59.0***	***60.5***	***61.0***	***62.0***
	24	***55.0***	***66.0***	***64.0***	***62.0***	***59.0***	***55.0***
	48	***55.0***	***70.0***	***68.5***	***61.5***	***57.5***	***56.5***

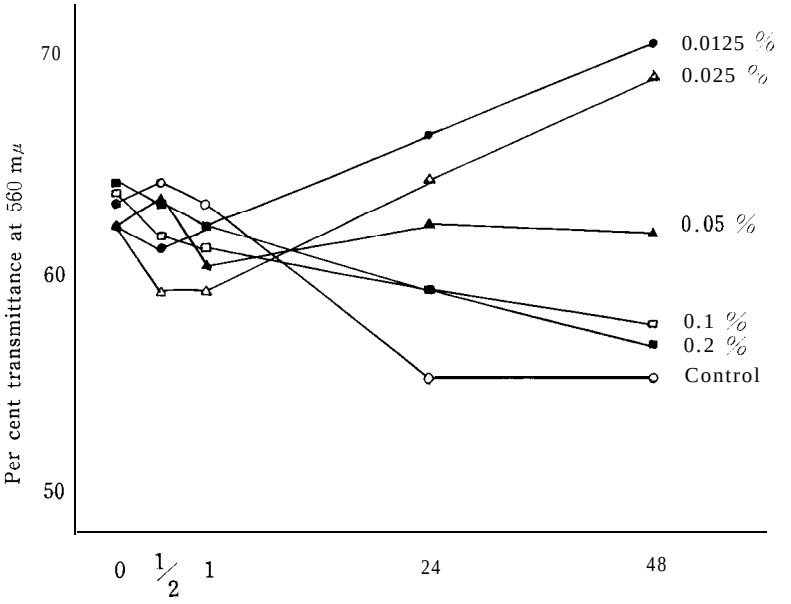

Fig. 3. Lysis of ***Micrococcus radiodurans*** by lysozyme.

The total bacterial number was counted to investigate the effect of lysozyme on the growth of ***Micrococcus radiodurans.*** From each sample of the tested solutions of lysozyme after incubation for 48 hr in the experiment II, one ml of the diluted culture was inoculated on TGY agar and incubated at the 37°C for 48 hr. Fig. 4 shows the colonies of ***Micrococcus radiodurans.*** The total bacterial number was 82×10^7. Fig. 5 shows the colonies of ***Micrococcus radiodurans*** in the solution of 0.025 % lysozyme. The total bacterial count was 5x 10. Fig. 6 shows

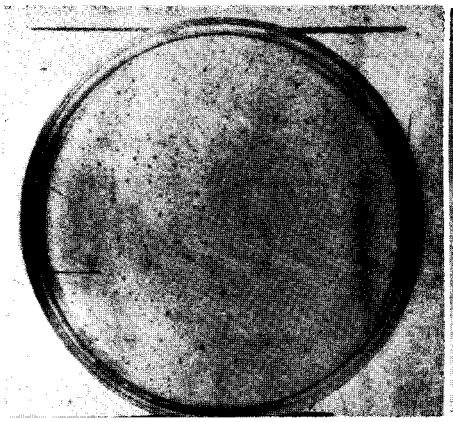

Fig. 4. Colonies of ***Micrococcus radiodurans*** grown on TGY agar after incubation at 37 C for 48 hr.

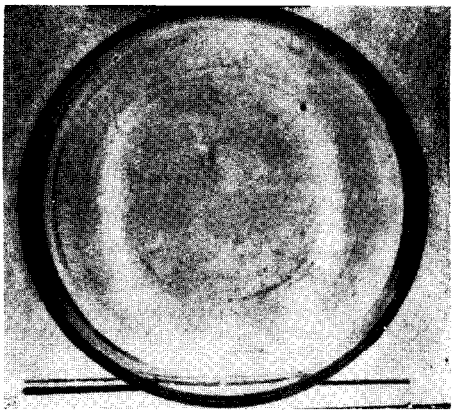

Fig. 5. Colonies of ***Micrococcus radiodurans*** grown on TGY agar in the test solution of 0.025% lysozyme solution after incubation at 37°C for ***48*** hr.

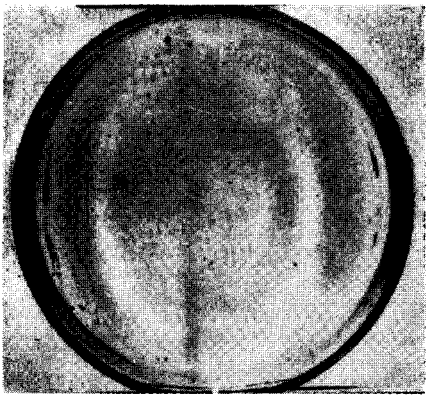

Fig. 6. Colonies of ***Micrococcus radiodurans*** grown on TGY agar in the test solution of 0.05 % lysozyme solution after incubation at 37°C for 48 hr.

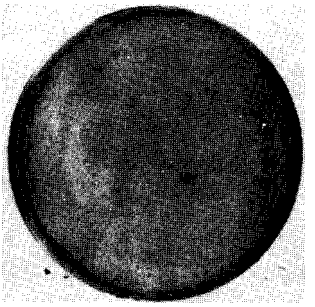

Fig. 7. Colonies of ***Micrococcus radiodurans*** grown on TGY agar in the test solution of 0.1 % lysozyme solution after incubation at 37°C for 48 hr.

the colonies in the solution of 0.05 % lysozyme. A total of 6x10 bacteria was counted. Fig. 7 shows the colonies in the solution of 0.1 % lysozyme. A total of 38x 10^5 bacteria was counted.

Experiment III. Microscopic observation after transmittance of lysozyme.

Smear and gram stain were made after incubation for 48 hr in the

experiment I-c. Fig. 8 shows ***Micrococcus radiodurans*** (lx 1200), and Fig. 9 shows ***Micrococcus radiodurans*** (1 x 1200) treated with 0.025 % lysozyme. ***Micrococcus radiodurans*** is a tetracoccus or diplococcus organism and stained gram positive, while [after treating with lysozyme it stained negative. Fig. 9 shows these tetracoccus organisms changed into pieces after treating with lysozyme.

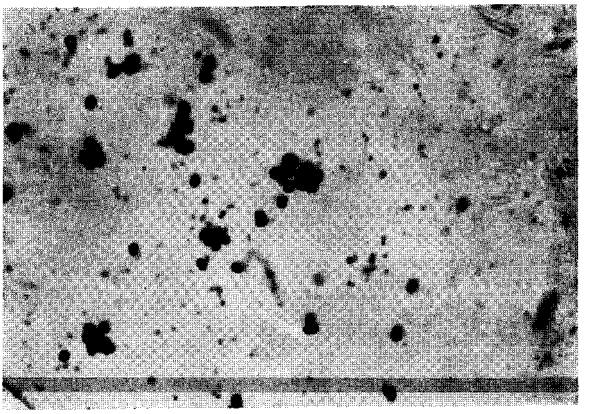

Fig. 8. ***Micrococcus radiodurans (1 X 1200).***

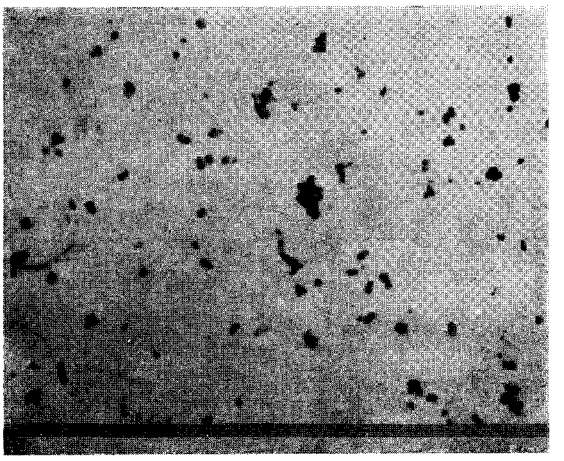

Fig. 9. ***Micrococcus radiodurans*** treated with lysozyme (1 × 1200).

Discussion

Transmittance values of 0.0125 % lysozyme solution in these experi-

ments were higher in comparing with the values of 0.025 % lysozyme solution, however these differences are not significant differences, and it can be explained that the lytic activity was observed especially in a low concentration such as 0.0125 % or 0.025 % lysozyme and this activity decreased in a high concentration such as 0.1% or 0.2 % lysozyme. The reason why the lytic action to this organism became higher in a low pH value must be determined by the study about the physiological change of this organism or by the chemical and analytic study of the cell wall.

Data presented in this paper show a similar result by Wilcox.[1] However, the conditions of experiments and organism of this study were different from those of Wilcox. Wilcox reported that the inhibition of lysozyme activity at a high concentration appears to be a function of the relative amount of enzyme and sodium ion. The reason why the activity of lysozyme decreased in a high concentration of lysozyme must be also studied afterwards. Tomcsik[4] reported that the crystal violet uptake ability of bacteria was changed by the action of lysozyme, and that lysozyme changed the absorptive properties for crystal violet. Salton[2] found the same effects of lysozyme upon gram-stained organisms observed in this experiment.

Summary

Micrococcus radiodurans, a non-sporing spoilage organism highly resistant to ionizing radiation and to ultraviolet light was inoculated into pepton broth adjusted to pH 7.2 with 0.1 M phosphate buffer. Crystallized lysozyme was added to this inoculated pepton broth in a rate of 0.0125, 0.025, 0.05, 0.1 and 0.2 % concentration.

Each of these test inoculated lysozyme solutions was incubated at 37°C or 40°C for 24 hr. After incubation, the transmittance values at 560 mμ were measured. The lytic activity in a low concentration of lysozyme was higher in comparing with that of in a high concentration of lysozyme.

Smear was made after incubation at 40°C for 48 hr. ***Micrococcus radiodurans*** which is a gram-positive and tetracoccus shape organism stained gram negative and this tetracoccus shape changed into pieces.

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