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The Effect of Egg-White Lysozyme on the Growth of Cheese Starter Organisms. I

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The effect of egg-white lysozyme was examined for two ***Streptococcus cremoris*** strains which are used in cheese processing ***as*** a starter. In a low concentration of lysozyme such as in 0.000025 % solution, these two strains were found to be activated to grow. The differences of 0. D. at 560 mμ between control and sample were increasing from 90 minutes after incubation at 30°C, and the difference at 480 minutes after incubation was 0.07 in AM_1 and was 0.09 in HP. These results are significant because the increased acid by the activated ***Streptococcus cremoris*** would inhibit the contaminating organisms to some extent.

The lytic activity of egg-white lysozyme to gram-positive organisms has been reported by Thompson (1952) and other several workers. However, the effect of egg-white lysozyme used for the infant diet has been reported recently by Ferlazzo (1961), Rossi (1961), Buccellato (1961) and Sukegawa (1967). By them a growth factor of lysozyme to ***Lactobacillus*** *bifidus* has been reported.

Weiss and Rettger (1934) reported that ***Lactobacillus*** *bifidus* was similar in a bacteriological nature to ***L. acidophilus.*** However, ***Lactobacilfus bijidus*** has been recognized as a different species by Hayward ***et al.*** (1955), Ochi and Mitsuoka (1968) because this strain is different from other ***Lactobacillus*** strains in the point of the cell-shape, the requirement of nutrition and the metabolism. Orla-Jensen (1924) has suggested to rank this strain as *Bifidobacterium.*

Recently, ***Lactobaciffus bijidus*** has been utilized in a fermentative milk product or lactic acid drink in which lactic ***Streptococcus*** such as S. *lactis* and S. ***cremoris*** are used as a starter. To utilize lysozyme in milk products, its effect to lactic ***Streptococcus*** must be examined. In this paper, the effect of egg-white lysozyme on two starter organisms is reported.

MATERIALS AND METHODS

Organisms : *Streptococcus cremoris.* AM, (slow fermentative strain) and HP (fast fermentative strain) which are stock strains of Dairy Research Institute, Palmerston North, New Zealand.

Egg-white lysozyme (Sigma Company) **:** This enzyme dissolved in 0.1 M phosphate buffer (pH 6.8) to 0.1 % concentration as stock solution was sterilized

by the millipore filter (G. S., 0.22 μ, Bedford, Massachusetts U.S.A.)

Medium : M_6 (lactose, 20 g ; ascorbic acid, 0.5 g ; sodium acetate . 3 H_2O, 2.8 g polypepton, 10 g ; beef extract, 5 g; yeast extract, 2.5 g ; phyton, 5 g ; agar, 1 g ;) was used.

Assay method: Assay method which was carried out by the method reporter by Akashi (1965), is shown in Fig. 1.

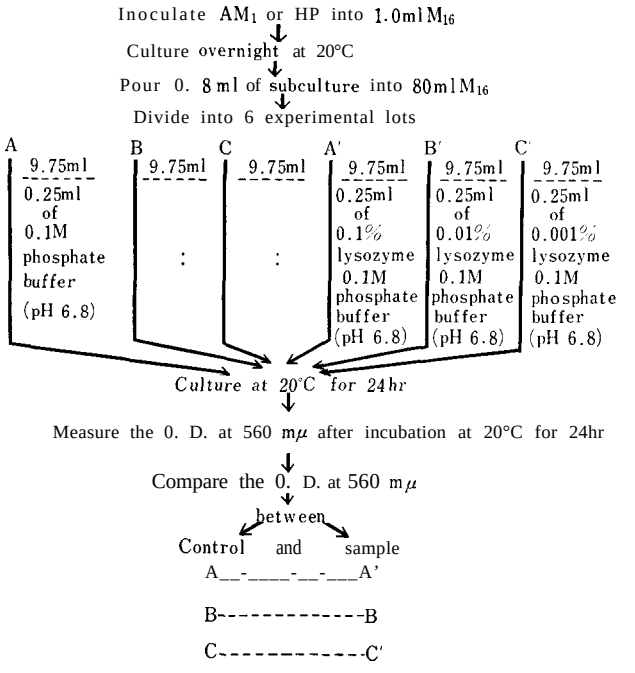

Fig. 1. Assay method carried out at *20°C.*

As shown in Fig. 1 the experiment was carried out in both strains. Subculture of each strain was poured into M,, in the rate of one percent. 0. D. at 560 mμ of A, B and C (without lysozyme) and A', B′ and C′ (with lysozyme) were measured respectively. Control and sample (A and A'), (B and B′), (C and C′) were compared each other.

In control (A, B and C), 0.25 ml of 0.1 M phosphate buffer (pH 6.8) was added to 9.75 ml of M_{16} containing subculture of *Streptococcus cremoris* (AM, and HP). In sample A', *0.25* ml of *0.1%* lysozyme solution was added to 9.75 ml of M_{16} containing subculture of AM, or HP strain, the final concentration of which turned out into 0.0025 %. In sample B′, 0.25 ml of 0.01% lysozyme was added to 9.75 ml of M_6 containing subculture of AM, or HP strain, the final concentration of which turned out into to 0.00025 %.

The same procedure mentioned above was carried out by using 0.001% lysozyme solution in sample C′, the final concentration of which became to 0.000025

%. After incubation at 20°C, O.D. at *560* mμ was measured in each solution respectively. The procedure shown in Fig. 1 was repeated in four experiments.

RESULTS

The mean values of 0. D. at 560 mμ in every experimental part after repeating the procedure shown in Fig. 1 are shown in Tables 1 and 2.

Table 1. The mean values of 0. D. at 560mp in four experiments by using AM,.

Exp. part	Lysozyme solution and reaction time					
	0.0025 %		0.00025 %		0.000025 %	
	0	24 (hr)	0	24 (hr)	0	24 (hr)
Control	0.184	0.810	0.183	0.800	0.184	0.800
Sample	0.185	0.820	0.184	0.860	0.185	0.990
Differences at 24hr after incubation		0.010		0.060		0.190

Table 2. The mean values of 0. D. at 560 mμ in four experiments by using HP.

Exp. part	Lysozyme solution and reaction time					
	0.0025 %		0.00025 %		0.000025 %	
	0	24 (hr)	0	24 (hr)	0	24 (hr)
Control	0.185	0.820	0.185	0.820	0.185	0.810
Sample	0.187	0.830	0.187	0.885	0.187	0.940
Differences at 24 hr after incubation		0.010		0.065		0.130

As shown in Tables 1 and 2, by using 0.0025 %, *0.00025* % and 0.000025 % lysozyme solution, the highest difference of 0. D. at 560 mμ between control and sample after 24 hr was observed in 0.000025 % solution. These values decreased in the order of 0.000025 %, 0.00025 % and 0.0025 % solution. Based upon these results, it might be presumed that lysozyme would activate the growth of *Streptococcus cremoris* in a low concentration such as 0. 000025 % solution.

The procedure carried out at 30°C is shown in Fig. 2. The adding lysozyme concentration used in this experiment was 0.000025 %, whereas in this concentration the largest difference of 0. D. at 560 mμ between control and sample was observed in the experiment carried out at 20°C for 24 hr as shown in Tables 1 and 2.

The procedure shown in Fig. 2 was repeated in four experiments. 0. D. at 560 mμ on 0', 30', 60', 90', 120', 240', 300', 360', 420', and 480' after incubation at 30°C were measured respectively. The mean values of 0. D. at 560 mμ after four experiments are shown in Tables 3 and 4. The growth curves of AM, and HP

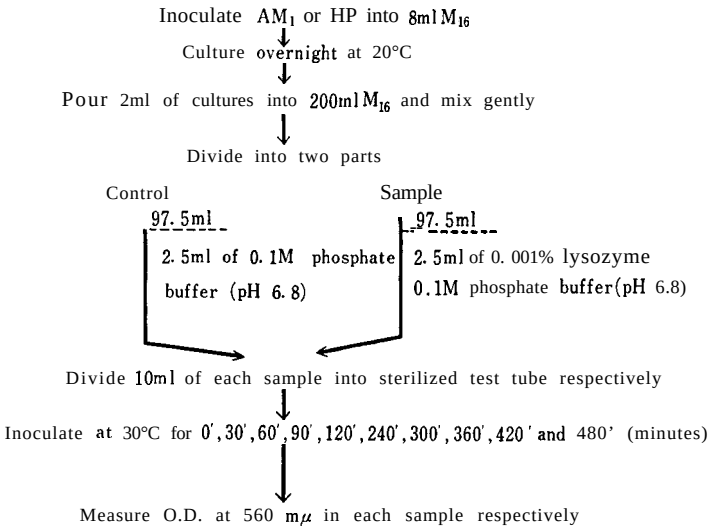

Fig. 2. Assay method carried out at 30°C.

Table 3. The growth rate of control (without lysozyme) and sample (with lysozyme) in AM_1.

(minutes)

Exp. part	Reaction time and the mean value of 0. D. at 560mp.									
	0	30	60	90	120	240	300	360	420	480
Control	0.210	0.213	0.2201	0.230	0.290'	0.3701	0.5001	0.503	0.504	0.510
Sample	0.210	0.213	0.2301	0.2401	0.310	0.410	0.560	0.5661	0.580	0.600
Difference at 480 minutes after incubation										0.09

Table 4. The growth rate of control (without lysozyme) and sample (with lysozyme) in HP.

(minutes)

Exp. part	Reaction time and the mean value of 0. D. at 560 mp.									
	0	30	60	90	120	240	300	360	420	480
Control	0.2901	0.290	0.2991	0.300)	0.320	0.4001	0.5001	0.6001	0.590	0.620
Sample	0.290	0.293~	0.3001	0.3101	0.3301	0.4401	0.580	0.6401	0.6851	0.690
Difference at 480 minutes after incubation										0.070

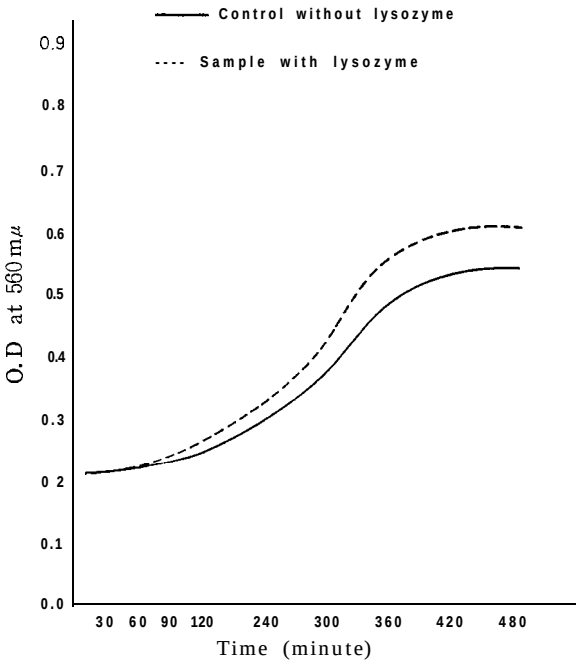

Fig. 3 The growth curve of AM_1 strain cultured at 30°C for O-480 minutes.

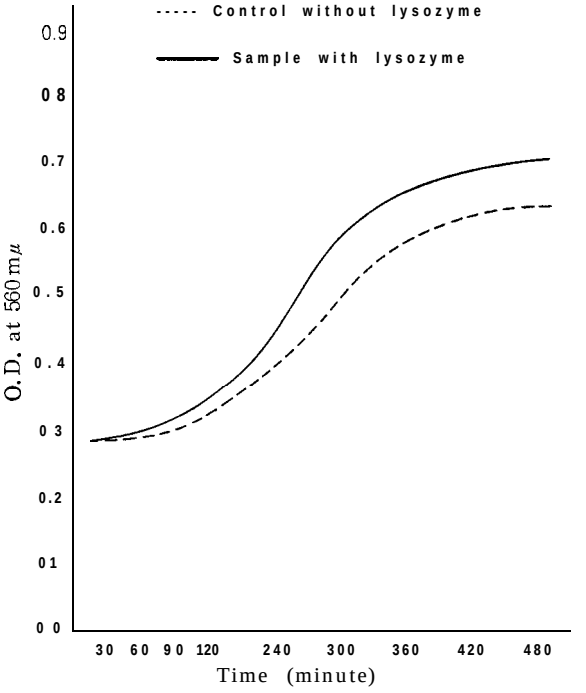

Fig. 4 The growth curve of HP strain cultured at 30°C for O-480 minutes.

strain are shown in Figs. 3 and 4.

As shown in Tables 3 and 4, the difference of 0. D. at 560 mμ between control (without lysozyme) and sample (with lysozyme) after incubation at 30°C for 480 minutes was 0.07 in HP strain and 0.09 in AM, strain. In each strain, 0. D. at 560 mμ of sample at every reaction time was higher than that of control. Especially, these differences increased after 90 minutes. As shown in Figs. 3 and 4, HP strain reached to the stational phase faster than AM, strain.

DISCUSSION

It is well known that egg-white lysozyme lyses the gram-positive organisms, however, in this experiment, two strains of *Streptococcus cremoris* were activated to grow especially in 0.000025 % lysozyme solution. By Ferlazzo, Rossi, Buceellato and Sukegawa, the growth factor of lysozyme to *Lactobacillus bifidus* was reported.

These results in *Lactobacillus* and the data of *Streptococcus* in this experiment might be caused by the different chemical constitutions of their cell walls comparing with those of other organisms which are lysed by lysozyme.

By Ochoa (1958) and Brien (1960), the cell wall of *Lactobacillus* was reported as a mutant cell wall which lacks acetyl-glucosamine containing oligosaccharide. By Brockaman (1954) a-lipoic acid was reported as a growth factor for lactic *Streptococcus.* Whether α-lipoic acid would be increased in a lactate medium by adding lysozyme would be examined in another experiment, and another mechanism in activating the growth of *Streptococcus cremoris* would be also confirmed afterwards. To confirm egg-white lpsozyme as a growth factor to *Streptococcus cremoris,* further experiment on the viable bacterial count in the growth of these strians would be needed.

However, though the differences of 0. D. at 560 mμ between control (without lysozyme) and sample (with lysozyme) were 0.07-o. 09 in two strains after incubation at 30°C for 480 minutes, these results might be significant because the contaminating organisms would be inhibited by the increased acid produced by the activated *Streptococcus cremoris* to some extent.

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