

Increase of proteolytic activity in *Lactobacillus casei* by X-ray irradiation

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Summary. A study was conducted on the effect of X-ray irradiation on proteolytic activity of *Lactobacillus casei* (RTS). Substantial increase (82%) in the proteolytic activity was noted among the isolates of X-ray irradiation survivors after 18,000 R.

As proteolytic activity of microorganisms contributes significantly to the texture and flavour by the degradation of casein during cheese ripening, it was considered worthwhile to explore the possibility of obtaining mutants of *L. casei* (RTS) with increased proteolytic activity. Such genetic variants may have practical significance in cheese ripening.

Material and methods. A strain of *Lactobacillus casei* (RTS) maintained in the culture collection of National Dairy Research Institute was selected for the present

study. 25 ml of an enriched medium¹ were inoculated with 10⁷ colony forming units of 16-h-old culture. After incubation for 9 h at 37°C, cells were harvested, washed twice with phosphate buffer (0.02 M; pH 7.0) and resuspended in the same buffer. 20 ml of the washed cell suspension having an OD of 0.55, adjusted in a Hilger Absorptiometer at 430 µm, were subjected to various doses of X-rays from a Philips Contact Therapy Installation (Type No. 11606/58, 50 KV, 220 V tube). After plating control and experimental samples on an enriched tomato juice agar supplemented with 10% sterilized skim milk¹, a survival curve was prepared by plotting the log number of survivors against X-dose ranging from 0 to 27,000 R. 5 irradiation trials were conducted and survivors were isolated at random from petri dishes showing 1.0–0.1% survival. The isolates were compared with non-irradiated control for variations in proteolytic activity. For proteolytic activity, isolates from control as well as experimental samples were grown in sterilized skim milk for 24 h at 37°C and tyrosine released was determined according to the procedure of Hull².

Results and discussion. Approximately 10–30 colonies per plate were examined both in control and irradiated samples. Before screening the mutants for proteolytic activity on the basis of clearance zones, colonies of the non-irradiated culture were examined for the same. A relationship between the zones of clearance and proteolytic

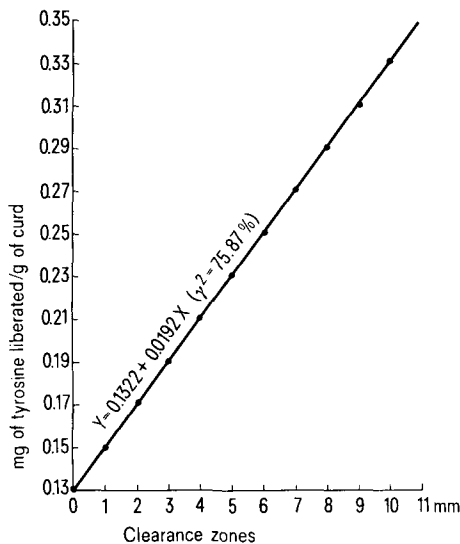


Fig. 1. Linear regression of tyrosine liberated with clearance zone.

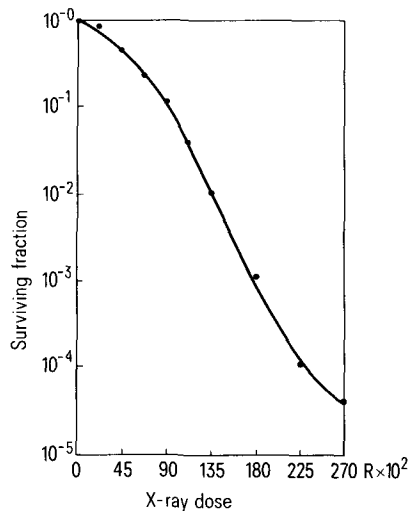


Fig. 2. Survival curve of *Lactobacillus casei* after exposure to X-ray irradiation.

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Table 1a. Relationship between clearance zones and proteolytic activity in the untreated *Lactobacillus casei*

Type	Clearance zones (mm)	No. of colonies showing proteolytic activity (mg of tyrosine liberated/g of curd)*		
		0.00–0.23	0.24–0.31	0.32–0.40
1	8–10	8	27	15
2	5–7	32	14	4
3	0–4	45	5	0

*No. of colonies examined in each type = 50.

Table 1b. Analysis of variance

Source of variation	Degree of freedom	Mean sum of squares	Significance of F-ratio
Between types	2	0.2098	45.6086
Within types	147	0.0046 (EMS)	

EMS = Error mean square.

Table 2. Proteolytic activity of *L. casei* recovered after exposure of X-ray irradiation (mg of tyrosine/g of curd)

Irradiation trial	<i>L. casei</i> *	
	Parent strain	Mutants
1	0.320	0.655
2	0.375	0.610
3	0.300	0.625
4	0.380	0.670
5	0.350	0.600
Mean	0.345	0.632

*No. of colonies of parent strain or mutants tested in each trial = 50.

activity of the colonies was established (table 1a). Out of 50 colonies of the parent culture, 45 with 0–4 mm clearance zones showed poor proteolytic activity. A similar trend was observed in case of 5–7 mm. However, clearance zones of 8–10 mm exhibited higher proteolytic activity. The analysis of variance data (table 1b) in regard to the proteolytic activity of 3 types of colonies selected on the basis of diameter of clearance zones showed the significance of F-ratio between the types. The relationship of proteolytic activity with the zonal diameter was tested in a 3×3 contingency table, and it was observed that the proteolytic activity is highly associated with the diameter of the zone in mm

$$(\chi^2 = 39.49; p < 0.05).$$

A linear regression of tyrosine liberated with clearance zone was fitted as

$$(Y = 0.1322 + 0.0192 X) (r^2 = 75.87\%),$$

where Y = mg of tyrosine liberated/g,
X = clearance zone (mm).

The above equation is presented in figure 1. The correlation between tyrosine liberated and clearance zone was 0.871 ($p < 0.01$).

The X-ray irradiation survival curve of *L. casei* (RTS) is illustrated in figure 2. An X-ray dose of 18,000 R gave less than 1.0% survival and mutants (only those with more than 10 mm clearance zone) were isolated from such petri dishes. 100 isolates from each of the 5 trials exhibited an appreciable increase in proteolytic activity in milk (table 2) as compared to the unirradiated parent culture. These mutants retained their altered characteristics even after several subcultures. These results on enhanced proteolysis are comparable to earlier observations of Dilanyan et al.^{3–5}, who demonstrated increased proteolytic activity of X-ray mutants of lactic acid bacteria.

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The peritoneal fluid cytology of adult female dogs

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Summary. The percent distribution of peritoneal fluid cellular content of adult female dogs was recorded and found to be dissimilar to all other species examined, especially in regard to mesothelial cell, polymorphonuclear leukocyte and lymphocyte proportions.

Peritoneal fluid cytologic specimens obtained from different mammalian species provide basic data to study the cellular response of serous abdominal fluid in health and disease. In normal women, pelvic peritoneal fluid may be considered a function of cell renewal and degeneration whose balance changes in pathological conditions so that cul-de-sac cytologic aspirations may be used to detect early ovarian cancer^{1,2}. Peritoneal fluid also provides a useful tool for studying inflammation^{3,4}, endocrine factors^{5,6}, estrous and menstrual cycles⁷ and pregnancy^{8,9}. The effect of human pregnancy on peritoneal fluid cytology was found to be quite similar to that observed in mice. In the present study, we analyzed the percent distribution of peritoneal fluid in adult female dogs to extend our species knowledge of peritoneal fluid as well as to determine how the cellular count compared with women and other species so that we might consider this animal as a future model for experiments involving peritoneal fluid cytology.

23 adult female mongrel dogs (3–4 years, 9.9 ± 0.2 kg) in good health and nutrition with normal heart rates, blood pressure and electrocardiogram readings were used.

The animals were tranquilized with 0.5 ml acepromazine maleate administered i.m. A cytologic specimen was then aspirated through the ventral side of the manually restrained, prone animal using a tuberculin (1.0 ml) syringe with a 23 gauge needle. The aspirated specimen was placed on an albumin coated slide and stained by

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