

H. SUMI¹, M. IMAI¹, T. MITSUO² and C. YATAGAI¹

¹ Kurashiki University of Science and the Arts, Kurashiki, Japan, ² Mitsuo Clinic, Tokyo, Japan

PURPOSE / OBJECTIVES

Commercially available natto has strong nattokinase, which works for thrombolysis when eaten, while it has been reported to have antibacterial activity against *Escherichia coli* or O-157 bacterial.

In this work, we focused on the cell-lysis (lysozyme) activity in natto, which is usually consumed by Japanese people.

MATERIALS & METHODS

Bacillus natto (12 species) were isolated in our laboratory. It was cultured on 3% dry broth medium (Nippon Seiyaku) as the basic medium, and incubated for 48hr in a static culture or 24-168hr with shaking (100rpm).

Thrombolytic activity was measured by using the fibrin plate method. Amounts of 10ml of 0.5% fibrinogen solution (0.17M of borate-saline buffer:pH7.8) and 500μl of 50U/ml thrombin were used to prepare fibrin plates, where 30μl of each sample was placed. The solution was incubated at 37°C and then the lysis area formed was measured (mm²).

The cell-lysis activity (CL₅₀, μg/ml) was calculated from the enzyme concentration required to lyse 50% of *Micrococcus luteus* by the method of Shugar *et al.* Egg lysozyme was purchased from Sigma Co. Ltd..

RESULTS

The cell-lysis activity after static incubation showed that no lysis was observed in general bacteria, but No.1-3 bacteria showed particularly strong activity among the 12 species of *Bacillus natto* (Table 1).

From the shaking culture of No.1, the yield of cell-lysis activity was equivalent to 1.5g of lysozyme per liter. It was found that the bacteria (*Bacillus*) could utilize ammonium sulfate as a nitrogen source, and that the activity was transient and very strong at 37°C for 24 to 48hr of incubation, after which it gradually decreased. The optimal pH of this enzyme was about 6.0, and pI was about 9.5 by isoelectric point electrophoresis (LKB column method) (Fig.1).

There was a fairly large difference in cell-lysis activity among commercial natto (10 varieties), but three products (No.1-3) had cell-lysis activities of 1.70, 0.83, and 0.53μg/ml, in order from strongest to weakest (Fig.2).

Table1 The cell lysis activity in species of *Bacillus natto*

Species	No.	Lysozyme activity (μg/ml)
Natto bacteria	No.1	307.7
Natto bacteria* (for starter)	No.2	240.0
"	No.3	10.9
"	No.4	7.2
"	No.5	—
"	No.6	—
"	No.7	—
"	No.8	—
"	No.9	—
"	No.10	—
"	No.11	—
"	No.12	—

Tempeh
E.coli

It was cultured on 3% dry broth medium incubated for 48hr. The cell lysis (lysozyme) activity (CL₅₀) was 1.10mg/ml. *Micrococcus luteus* 1.15mg/3ml phosphate buffer, pH6.2 and incubated for 24hr.

Table 2 The cell lysis activity in *Bacillus natto* (No.1)

Shaking culture	pH	Incubation time (hr)			
		0	24	48	168
3% dry broth, 1% ammonium sulfate	6.3	—	603	1,523	—
3% dry broth	6.4	—	243	479	74

Each value represent lysozyme activity (CL₅₀ μg/ml).

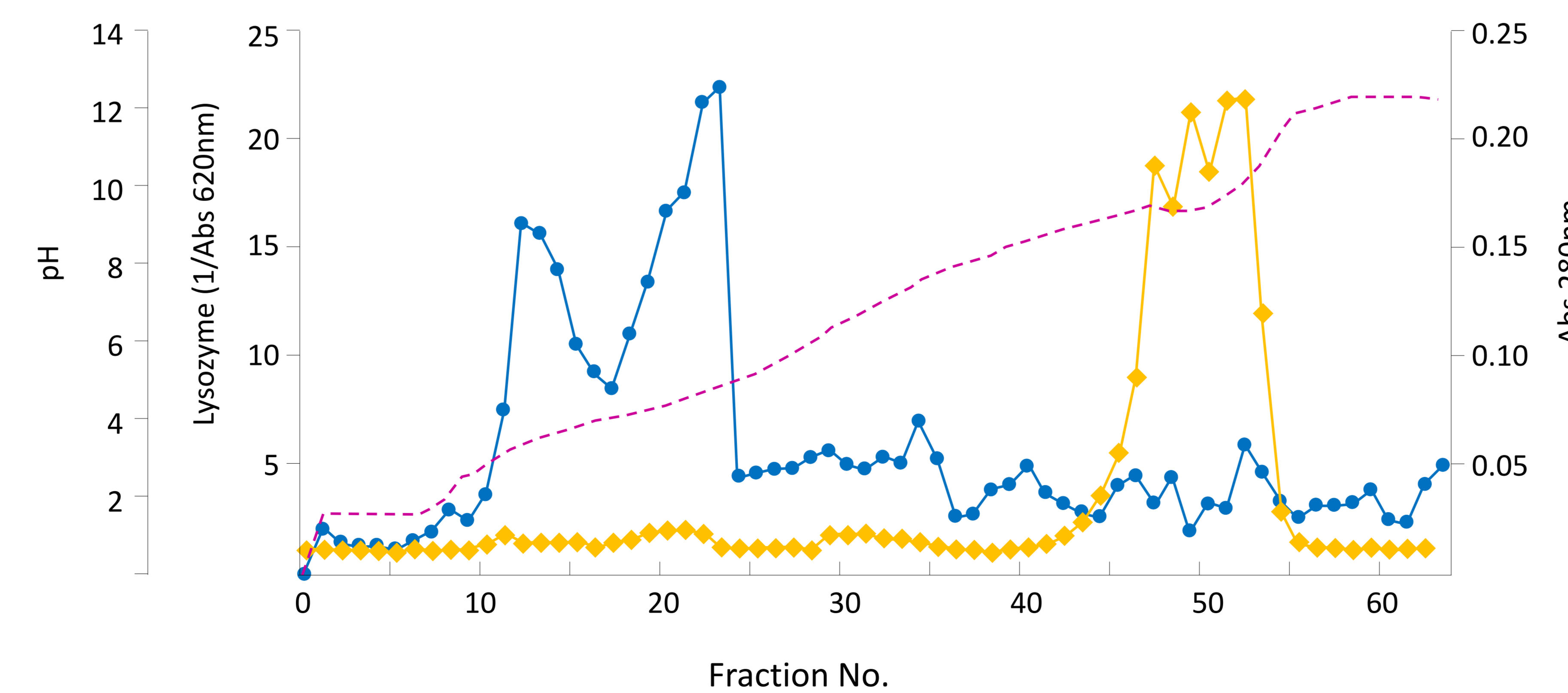


Fig.1 Isoelectric focusing pattern (LKB) of *Bacillus natto* (No.1)
Column method (110ml) at 900V for 19hr, 5°C.
Lysozyme (◆—◆), protein (●—●), pH (---).

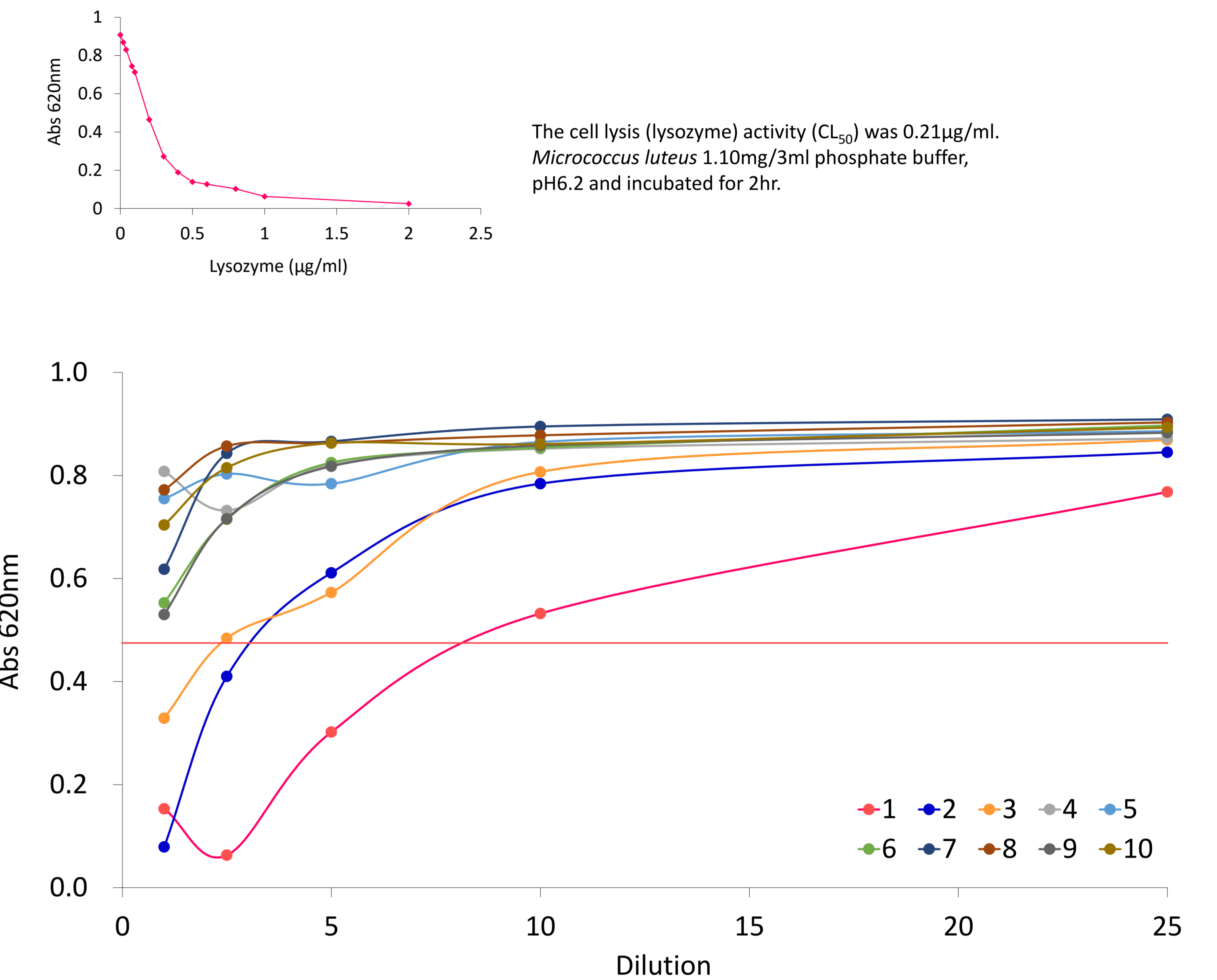


Fig.2 The cell lysis activity in 10 commercial natto

SUMMARY/CONCLUSION

Although the cell-lysis activity in natto probably extends to all microorganisms or even cancer, the relationship between this bacteriolysis activity and antibacterial activity and nattokinase activity is still unknown and remains a future challenge.

REFERENCES

- SUMI H. Advanced food ingredients and functions CMC, Tokyo1997; p.190-196
- SUMI H *et al.* Anti-bacterial component dipicolinic acid measured in Natto and Natto Bacilli. *Nippon Nogeikagaku Kaishi* 1999; 73: 1289-1291
- SUMI H *et al.* Anti-bacterial and cell activation ability of *Bacillus natto*. *New Food Industry* 2019; 61: 95-98
- SUMI H. Discovery of new ingredient "lysozyme" expected to revitalize the market. *The health industry news* 2001; 976: 26
- SHUGAR D. The measurement of lysozyme activity and the ultra-violet inactivation of lysozyme. *Biochim Biophys Acta* 1952; 8:302-309