Bacteriocin-like substance producing bacteria in cattle manure composting process

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論 文 内 容 要 旨

Introduction:

Composting is one of the most natural processes capable of stabilizing organic wastes. Because animal waste possibly contains viral, bacterial, and protozoan zoonotic pathogens, the application of untreated livestock waste could be a hygienic risk for human [3].

Application of cow manure or composted one in agricultural practice could potentially cause contamination of foodstuffs with pathogenic bacteria such as *E. coli* O157:H7, *Listeria monocytogenes*, *Clostridia* sp., *Mycobacterium* sp., and *Yersinia enterocolitica*. When the composting processes are carried out in an inefficient manner, the organic mater remains poorly stabilized and sanitized, recontamination can occur and the compost may become a source of pathogen. In addition the physical and chemical conditions involved during composting are variable and these may alter the results of the composting process unpredictably. There fore, new ways are experimented and searched to reach higher effectiveness of stabilization process of the animal manure. A new form of treatment could consist of the use of bacteriocins for waste treatment [7].

Bacteriocins are peptides produced by bacteria that kill or inhibit the growth of closely related bacteria or share the same ecological niche [5]. Recent studies revealed that bacteriocins play a critical role in mediating microbial interactions and may even play a more fundamental role in maintaining microbial diversity. Both gram-positive and gram-negative bacteria could produce these antimicrobial agents.

There has been no literature about the detection of bacteriocin-like substance producing bacteria in the compost pile or during the composting process. And one of the most important requirements from animal-manure composting is the great reduction of the pathogenic content of the manure before its application to decrease the possibility of disease transmission to human and animal. Furthermore, during the composting process the temperature of the compost pile changes dramatically between mesophilic and thermophilic stages. So it was interesting to search the presence of BLS producing bacteria during these various stages of compost-pile temperature.

In this study we report the production and partial characterization of the bacteriocin-like substance produced by bacterial strains from cow-manure compost during the various stages of the composting process. The activity of some of these

inhibitors against food-borne pathogenic and spoilage bacteria was studied. Further more, a bacteriocin-like substance from the compost-origin thermophilic *Bacillus licheniformis* H1 was characterized and partially purified.

Chapter 1: Isolation and identification of bacteriocin-like substance (BLS) producing bacteria from cattle-manure compost:

Introduction:

During the composting process the temperature of the compost pile changes dramatically, so it was interesting to search the presence of BLS producing bacteria during the various stages of compost- pile temperature.

Material & Methods:

The experiments were conducted on a field-scale cattle manure compost system in Kawatabi Field Science Center, Tohoku University, Japan. The compost pile was composed of cattle manure (beef and dairy) and saw dust. The pile temperature was measured throughout the composting process. Ten compost samples were collected at different stages of the composting cycle.

The pile temperature at the start was 9.1°C and the highest temperature was 76.3°C at day 9. The water content at the start was 76.1% and that of the finished compost was 54.8%. At the start of the process pH value was 7.3, the highest value (8.9) was recorded at the second day of the process.

Detection of BLS producing bacteria in the compost samples:

The compost samples were examined for BLS production against the pathogenic indicator strains: *Salmonella typhimurium* EF 85-9, *E. coli* O157:H7 ATCC 43888, *Enterococcus faecalis* ATCC 19433, *Staphylococcus aureus* ATCC 6538P and *Yersinia enterocolitica* ATCC 9610. Cell-free culture supernatants (CFCS) of the producer strains were prepared (pH 7.0). To analyze the Antimicrobial activity quantitatively, microtiter plate growth assay was applied as described by Dufour et al. [2]. The producer strains were identified based on their morphological and biochemical characteristics according to methods described in "Bergey's Manual of Systematic Bacteriology" [6, 8]. In order to confirm the taxonomical identity, the 16S rDNA of the producer strains was amplified using primer set "341 F & 907 R" [1].

Results:

A total of 14 bacterial producer strains were isolated from compost samples. The inhibitory effects of the producer strains (H1-H14) are illustrated in Table1. None of these strains resulted in loss of viability of any of the tested indicator strains. The inhibitory effects were different depend on the BLS, although all BLS from the isolated strains had no an inhibitory effect against *Y. enterocolitica.* The inhibitory actions of BLS can be divided into 2 groups. One group BLSs can inhibit the lag growth phase and exponential phase of indicator strain. Second group BLSs can inhibit only the exponential growth phase of indicator strains. Cell- free culture supernatant (CFCS) of strains H1 and H6 caused a delay in the start of the exponential growth phase of *Ent. faecalis* ATCC 19433 when compared with controls without CFCS. Also they caused a reduction in the optical density at 24 h. Similar effects were exhibited by strains H4 and H7 against *Sal.* Typhimurium EF85-9. Strains H3, H8, H10 and H12 inhibit only exponential growth phase of *E. coli* O157:H7. In the case of H8, it can inhibit only exponential growth phase of *E. coli* 157:H7, but H8 can inhibit both lag and exponential growth phase of *Staph. aureus.*

These isolates were examined with gram stain; one isolate (H1) was gram-positive rods and the other producers (H2-H14) were gram–negative rods. Table 2 shows the most related species. Strain H1 was identified as *B. licheniformis.* It was motile, gram-positive rod; spore forming and can grow at 55°C. It reacts positively in the catalase and oxidase tests. Strain H6 identified as *Bacteroidetes bacterium* with 99% 16S rDNA sequences similarity. Strains H2, H7, H13 and H14 belonged to the genus *Salmonella.* Strains H3-H5 and H8-H12 belonged to the genus *Shigella.*

In summary, BLS producers (14 strains) were isolated from compost samples at different stages, (strain H1 at the start), (strains H2-H7 from the 5-day compost sample), (strain H8 from the 23-day sample), (strains H9-H14 from the finished pile). BLS either inhibits both lag and exponential phase or only exponential phase. The BLS producers isolated in this study included both gram-positive (strain H1) and gram-negative bacteria (strains H2-H14).

Chapter 2: Partial characterization of the inhibitory substances:

Enzymatic treatment and heat stability of the inhibitory substances:

Introduction:

Bacteriocins are proteins; some have been reported to consist of combinations of different proteins together with lipid or carbohydrate functional groups [4]. The kept CFCS of the producers were analyzed for their sensitivity to proteolytic enzymes as well as for lipolytic enzyme (Lipase enzyme). Additionally, heat stability of these inhibitory substances was examined.

Material and Methods:

Cell-free culture supernatant from these producers was treated with α– chymotrypsin, trypsin, pepsin, papain and lipase enzymes at 37°C for 3 hrs at a final conc. 1 mg enzyme /ml. Then the residual activity was tested using microtiter plate growth assay. CFCS was heated at 55°C and 75°C for 30 and 60 min as well as autoclaving at 121°C for 15 min. After cooling to room temperature, the residual activity was assayed with microtiter plate growth assay.

Results:

Table 3 presented the effect of α– chymotrypsin and heat on the antimicrobial activity of the all producers. CFCS of strains H1, H4, H5, H7, H9 and H11 lost its antimicrobial activity completely after treatment with α– chymotrypsin. The inhibitory effect of the other strains was greatly reduced by α– chymotrypsin. The inhibitory effect of H5, H8, H9 and H11 was greatly inactivated by lipase. Concerning the heat stability, the supernatants of all strains retained the inhibitory effect for 60 min at 55 and 75°C. CFCS of strains H2, H6, H11, H12 and H14 showed marked heat stability with 100% residual activity after autoclaving at 121°C for 15 min. There was a highly significant decrease in the activity of BLS from H1 and H10 with trypsin, ∝-chymotrypsin, and papain ($p<0.001$) (Table 4).

In summary, Activity of BLS from all producers (strains H1-H14) decreased after treatment with proteolytic enzymes (protein in nature). Activity of 4 producer strains (H5, H8, H9 &H11) decreased after treatment with proteolytic enzymes & lipase. BLSs were heat stable, which indicate that they will not loose their inhibitory effect during thermophilic stage of the composting process.

Chapter 3:Bacteriocin-like substance from producer strains H1 & H10:

Introduction:

BLS from two producer strains (H1 and H10) was used for more characterization. The inhibitory effect of BLS from H1 and H10 against some food-borne spoilage and pathogenic bacteria was studied. The inhibitory activity can be reduced by long-term storage also pH can affect the activity. Incubation temperature for obtaining high yield of BLS varies from strain to strain. It was essential to study the effect of various treatments on the antibacterial activity of the obtained BLS to get more details about possibility of the use of BLS under different field conditions.

3.1. Efficiency of BLS from H1 and H10 against some food-borne pathogenic and spoilage bacteria:

Material and Methods:

Antimicrobial activity of CFCS was examined against: *Listeria monocytogenes* ATCC 19111, *Pseudomonas fluorescens* ATCC 11251, *Bacillus cereus* ATCC 14579, *Bacillus subtilis* ATCC 6633, *Lactobacillus species* ATCC 33198, *Lactobacillus fermentum* ATCC 11739, *Lactobacillus plantarum* sub. plantarum. ATCC 14917, *Enterobacter aerogenes* ATCC 13048, and *Serratia marcescens* ATCC 6911.

Results:

The BLS produced by H1 did not inhibit the growth of *Serratia marcescens, Lactobacillus plantarum* sub. plantarum and *Enterobacter aerogenes*. Strains of gram-positive bacteria like *L. monocytogenes*, *Bacillus cereus, Bacillus subtilis*, *Lactobacillus species,* and *Lactobacillus fermentum* and also a strain of gram-negative bacterium *Pseudomonas fluorescens* were inhibited (Fig. 1). The BLS from H10 showed an inhibitory action against the all tested food-spoilage and food-borne bacteria except *Enterobacter aerogenes* ATCC 13048 and *Lactobacillus plantarum* subsp. plantarum ATCC 14917. Analyzing the growth curve of the sensitive bacteria after incubation with BLS of either H1 or H10, there was a highly significant reduction in the growth rate of the bacterial cells treated with cell-free culture supernatant compared with the control ($p < 0.001$). *L. moncytogenes* was the highly

sensitive (49% inhibition after 24 hr) for the inhibitory effect of BLS from H1, but in case BLS from H10, *B. subtilis* was the highly sensitive (55% inhibition after 24 hr).

3.2. Efficiency of BLS from H1 and H10 against some plant pathogenic bacteria:

The inhibitory activity of BLS from H1 and H10 against 11 bacterial plant pathogens was studied. These plant pathogens included; *Pantoea ananatis* (2 isolates), *Erwinia carotovora* subsp. carotovora (6 isolates), *Burkholderia glumae, Clavibacter michiganensis* subsp. michiganensis and *Ralstonia solanacearum.*

Results:

BLS from H1 exhibited slightly (9-14%) inhibitory effect against *Erwinia carotovora* subsp. carotovora and *Pantoea ananatis.*

3.3. Effect of different incubation temperatures:

Material and Methods:

H1 and H10 were used to study the effect of different incubation temperatures on both cell density and BLS production. The technique was done according to von Mollendorff et al. [9] with some modification. In case of strain H10 incubation was done at 25, 37 and 45°C. An overnight culture of *B. licheniformis* H1 was inoculated (1% v/v) into 50 ml tryptic soy broth (TSB). Incubation was done at 55°C for 5 days. The antimicrobial activity was analyzed and the bacterial cell count was determined. Simultaneously, *B. licheniformis* H1 was incubated at 37°C.

Results:

At 37 and 25°C the stationary phase of strain H10 started after 4 and 6 h, respectively. The bacterial cell density after 24 h was about 1.3 times higher at 25°C than at 37°C. The lowest cell density was observed at 45°C (Fig. 2A). Figure 2B illustrates the production of bacteriocin-like substance by H10 during the stationary phase when the incubation was done at 25°C. At 37°C the antimicrobial effect could be detected during the exponential growth phase and reached the highest activity at the early stationary phase. Moreover, there was a slow decrease in the antagonistic activity in the later stationary phase. When incubation was done at 45°C the antimicrobial activity was detected during the stationary phase till 15 h after that the antimicrobial activity could not be detected.

B. licheniformis H1 produced bacteriocin-like activity when incubated at 55°C with significant decrease in the growth rate of *B. subtilis* ATCC 6633 (Fig. 3b). The incubation of *B. subtilis* ATCC 6633 for 24 h in the presence of CFCS collected after incubation at 55°C resulted in a significant decrease in the optical density at 595 nm compared with the control ($p<0.05$). There was no significant change in the cell count of *B. licheniformis* H1 producer strain when incubated at 55°C for 5 days (Fig 3a). Cell count of the producer strain at 37°C incubation (2 $x10^7$ CFU/ml) was significantly higher compared with the cell count at 55°C incubation (1.3 $x10^6$ CFU/ml).

3.4. Effect of pH and storage at different temperature on the activity of BLS from strains H1and H10:

Material and Methods:

CFCS pH was adjusted to 3, 5, 7, 9, 11, and kept at 4°C overnight. Then each sample was adjusted to pH 7.0 and assayed for residual activity. To analyze the stability at different storage temperatures, samples of the CFCS were stored at 4, 28, -20, and -80°C for 3- 4 weeks. During the period of storage the antimicrobial activity was analyzed weekly using microtiter plate growth assay.

Results:

BLSs from H1 and H10 were stable at pH values ranging from 3 to 9 (Fig. 4). More over, there was no loss of activity of CFCS from H1 when stored for up to 4 weeks at 4 and -20°C, and for 3 weeks when stored at 28°C (Table 4). There was no loss of activity of CFCS from H10 when stored for 2 weeks at 28°C and -80, and for 3 weeks at 4 and -20°C (Table 4).

In summary, BLS from strains H1 and H10 were stable to various pH values and storage at different conditions. BLS from strains H1 & H10 will be able to retain its activity at the alkaline pH during the composting process. H1 producer is thermophilic with optimum BLS production at 55°C. CFCS of strains H1 and H10 exhibited an inhibitory effect against *Listeria monocytogenes* ATCC19111. *L. moncytogenes* was the highly sensitive for the inhibitory effect of BLS from H1, but in case BLS from H10, *B. subtilis* was the highly sensitive.

Chapter 4: Partial purification of BLS produced by thermophilic Bacillus licheniformis H1

Material and Methods:

BLS from strain H1 was partially purified from 500 ml broth culture. Cells were grown in TSB at 55°C and then collected by centrifugation. The obtained CFCS was precipitated with solid ammonium sulfate (55, 60, and 80% saturation) with overnight agitation at 4°C. The solid fraction was obtained by centrifugation. The pellet was dissolved in 10 ml sodium phosphate buffer. That concentrated supernatant was dialyzed using dialysis membrane (Spectra/Por®, MWCO 1 KD). The protein content was measured along the course of BLS preparation using Coomassie Plus (Bradford) Assay Kit with Nanodrop ND-1000 Spectrophotometer. SDS-PAGE was performed to detect the molecular mass of the active protein fraction.

Results:

The best recovery (90.7%) of the protein was obtained with 60% ammonium sulfate saturation. SDS-PAGE analysis of the protein in the concentrated partially purified supernatant of *B. licheniformis* H1 revealed a protein with an approximate molecular mass of 3.5 kDa (Fig. 5), that exhibited antibacterial activity against *L. monocytogenes* ATCC 19111.

In summary, SDS-PAGE analysis of the concentrated supernatant from H1 revealed a bacteriocin-like activity against *L. moncytogenes* ATCC 19111 with a molecular mass of approximately 3.5 kDa.

Conclusion:

Chapter 1: BLS producers against pathogenic bacteria were isolated from compost samples at different stages of the process. The inhibitory activity of BLS varies according to the sensitivity of the target bacteria and BLS itself. BLS can be divided into 2 groups: the first group inhibits both lag phase and exponential phase. The second group BLS inhibits exponential phase. The producer strains included both gram-positive and negative bacteria.

Chapter 2: Decreasing activity of the BLSs by α-chymotrypsin suggested the characteristics of protein of BLSs. The heat stability of BLSs indicated that they

would not loose their inhibitory effects during thermophilic stage of the composting process.

Chapter 3: BLS from strain H1 inhibited *L. moncytogenes, B. cereus, B. subtilis, Pseud. fluorescens, lact. fermentum* & *Lact. species*. BLS from strain H10 inhibited *L. moncytogenes, B. cereus, B. subtilis, Pseud. fluorescens, Lact. fermentum, Lact. species & Serr. Marcescens.* BLS from H1 and H10 are stable to pH (3-9) and storage at different conditions. Strain H1 producer is thermophilic with 55°C optimum production temperature.

Chapter 4: SDS-PAGE of concentrated supernatant from *B. Licheniformis* H1 revealed a bacteriocin-like activity against *L. moncytogenes* with an approximate molecular mass of 3.5 kDa.

Finally, This study represents the first report about BLS producing bacteria against pathogenic bacteria from compost samples at different stages of the composting process. We could detect various BLSs in the composting process. *B. licheniformis* H1 isolated from compost sample is thermophilic and produced thermostable BLS. Also its inhibitory activity differs from reported and the molecular weight of the active fraction differs from the previously reported. Therefore, our strain is original one.

The obtained results about isolation of BLSs producing bacteria from the compost samples are very important from the hygienic point of view. During the animal manure treatment, it is essential to produce compost that is free of pathogens or at least contains a greatly reduced pathogenic content. For this, the obtained BLS or BLS producer bacteria may be used to control some pathogens during animal manure treatment. By this way we can produce safe compost that can be applied to agricultural field and produce safe vegetables and decrease the risk of disease transmission to human. Further more, these BLS or BLS producer bacteria may be applied to land or soil that is contaminated with pathogens (as *L. monocytogenes*) to control these pathogens.

References:

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Table 1. The inhibitory effect of the isolated strains:

Isolate	*S. typhimurium* EF 85-9		*E. coli* O157:H7		*Ent. faecalis* ATCC 19433		*Staph. aureus* ATCC 6538P	
	Inhib. %	Lag phase [a]	Inhib. %	Lag phase	Inhib. %	Lag phase	Inhib. %	Lag phase
H1					14%	8%		
H2					13%	ND		
H3			14%	ND [b]	21%	ND	27%	ND
H4	11%	8%					13%	4%
H5							15%	ND
H6					7%	8%		
H7	9%	8%						
H8			14%	ND			13%	4%
H9							18%	ND
H10			15%	ND	10%	ND	17%	ND
H11							14%	ND
H12			12%	ND	12%	ND	16%	ND
H13					20%	ND		
H14					20%	ND		

(a): This criteria express the increase in the lag growth phase of the indicator strain by the effect of the producer-strain supernatant.

(b): ND means not detected.

Table 2: The most related species of the producer strains:

Isolate	Identified Species, accession no.	Homology (%)	Growth temp. (°C)	Count (CFU/g compost)
H1	*Bacillus licheniformis*, EU869262	551/551(100%)	55	$1x10^4$
H2	*Salmonella* Typhimurium LT2, AE008895	504/507 (99%)	37	$1X10^4$~10^6
H3	*Shigella* sp. 4109, FJ405330	507/508 (99%)	37	
H4	*Shigella* sp. 4109, FJ405330	504/507 (99%)	37	
H5	*Shigella sonnei* AU65, EF032687	499/503 (99%)	37	
H6	*Bacteroidetes bacterium* 37LGX-1, AB375750	497/498 (99%)	37	
H7	*Salmonella* Typhimurium LT2, AE008895	450/450 (100%)	37	
H8	*Shigella sonnei* AU65, EF032687	500/507 (98%)	37	$1x10^4$
H9	*Shigella* sp. 4109, FJ405330	505/507 (99%)	37	$1X10^3$
H10	*Shigella* sp. 4109, FJ405330	506/508(99%)	37	
H11	*Shigella* sp. 4109, FJ405330	506/507 (99%)	37	
H12	*Shigella* sp. 4109, FJ405330	506/508(99%)	37	
H13	*Salmonella* Typhimurium LT2, AE008895	502/503 (99%)	37	
H14	*Salmonella* Typhimurium LT2, AE008895	505/507 (99%)	37	

Table 3: Effect of enzymes and heat on inhibitory effect of CFCS of strains H1-H14

Treatment	Relative activity (%)[a]													
Enzymes	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14
∝-chymotrypsin	0	42.5	54	0	0	45.2	0	23	0	12.5	0	8.3	39	54
Lipase	83	100	100	92	0	100	100	38	0	94	43	86	82	100
Heat														
55°C/ 30 min	100	100	100	100	78.3	100	100	100	100	100	77	100	97	100
55°C/ 60 min	100	100	100	100	72.7	100	100	100	100	100	60	100	88	88
75°C/30 min	100	100	100	100	81.8	100	100	100	79	100	62	81	100	100
75°C/60 min	84.6	100	94.7	73	78.3	100	69	83.3	79	100	59	75	72	82
Autoclaving (121°C/15min)	16.7	100	70	63	61.5	100	59	55	0	50	100	100	67	100

[a] The activity of the control preparation without any treatment was defined as 100%.

Table 4: Effects of various enzymes and storage on the antimicrobial activity of BLS from H1 & H10:

Treatment	Relative activity (%)[a]	
Enzymes	H1	H10
∝- chymotrypsin	0	12.5
Trypsin	0	32.5
Papain	0	17.5
Pepsin	92	100
Lipase	82.8	93.8
Storage condition		
4°C/1, 2, 3 weeks	100	100
4°C/4 weeks	100	80
28°C/1,2 weeks	100	100
28°C/ 3 weeks	100	74
-20°C/ 1, 2, 3 weeks	100	100
-20°C/ 4 weeks	100	91
-80°C/1, 2 weeks	100	100
-80°C/3 weeks	76	57

[a] The activity of the control preparation without any treatment was defined as 100%.

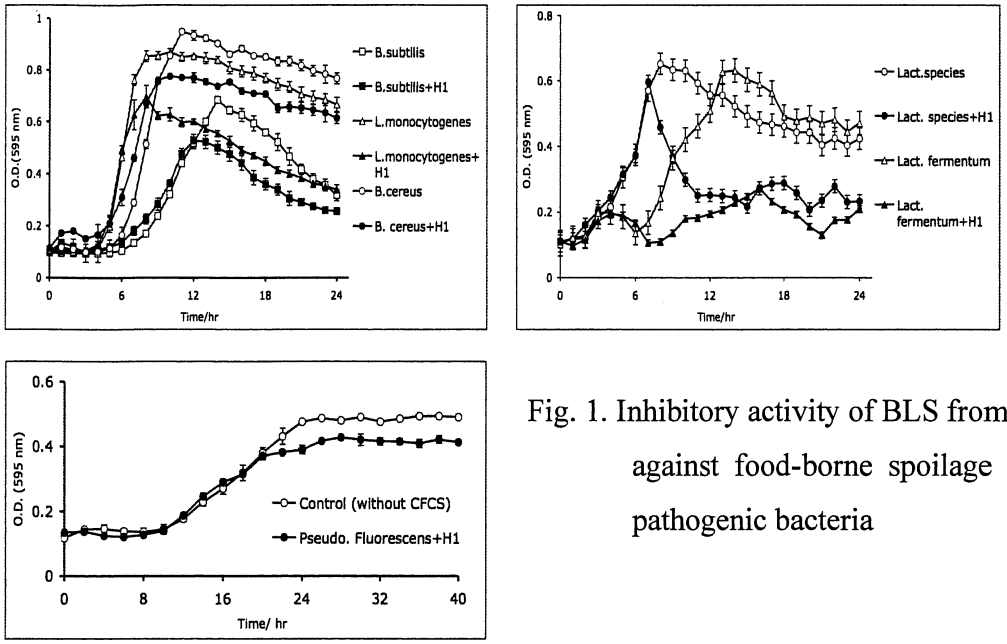

Fig. 1. Inhibitory activity of BLS from H1 against food-borne spoilage and pathogenic bacteria

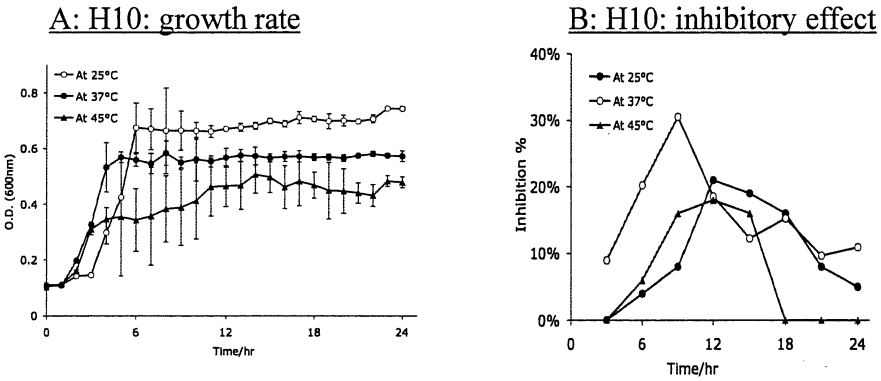

Fig. 2. Effect of different incubation temperature (25, 37 and 45°C) on growth and bacteriocin- like substance production by strain H10

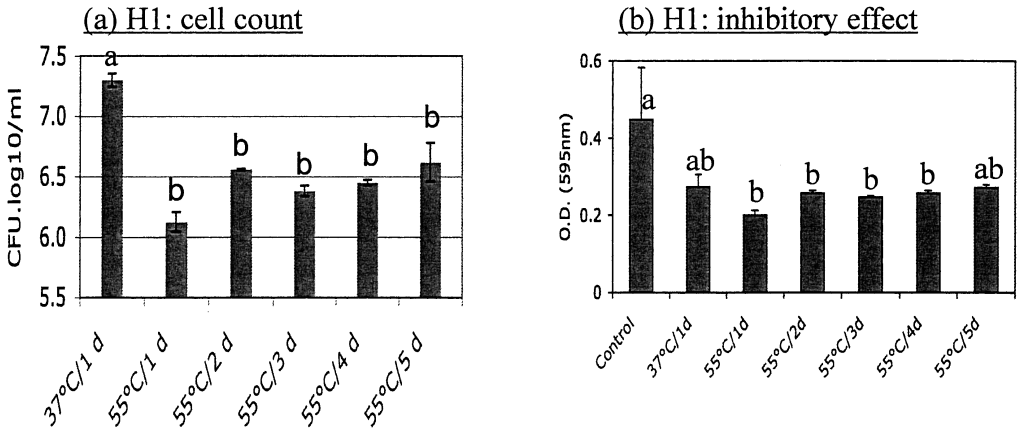

Fig. 3. Effect of different incubation temperature (37 and 55°C) on growth and bacteriocin- like substance production by strain H1

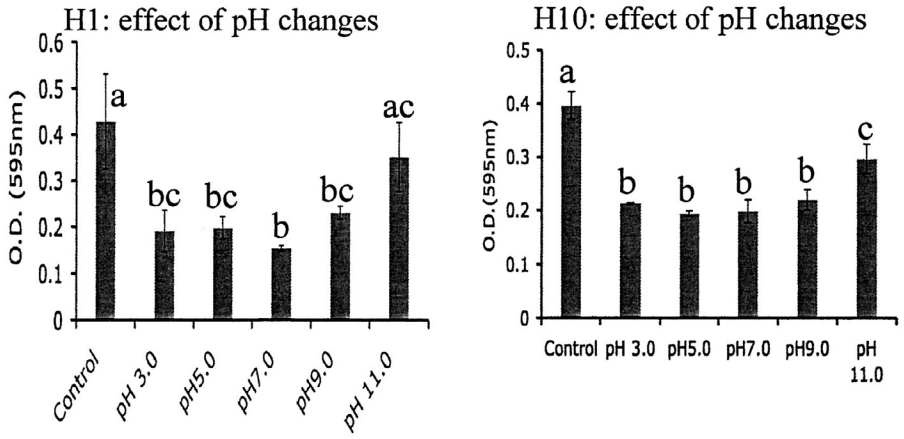

Fig. 4. Effect of pH changes on the antimicrobial activity of BLS produced by H1 and H10. The supernatant pH was adjusted to a range of 3-11, kept at 4°C overnight, then neutralized and the inhibitory effect against *B. subtilis* ATCC 6633 was analyzed. Control means *B. subtilis* bacterial cells without supernatant.

abc: means with different letters are highly significantly different ($p<0.001$). Bar show standard deviation (n=3)

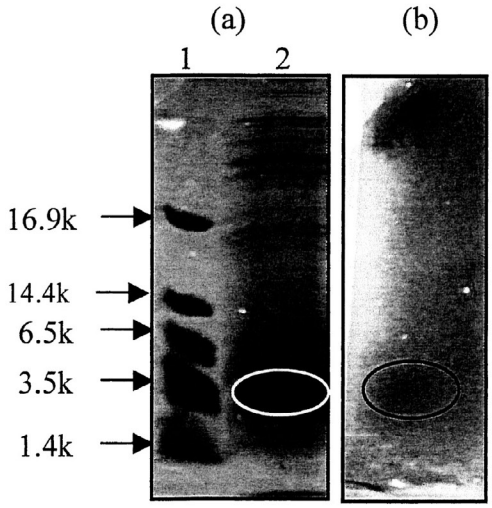

Fig. 5: (a) Tricine-SDS-PAGE. Lane 1. Molecular-weight marker. Lane 2. Protein profile of the concentrated partially purified culture supernatant of *B. licheniformis* H1. The circle denotes the active protein band. (b) Direct overlay of SDS-PAGE gel demonstrating clear inhibition zone against *L. moncytogenes* ATCC 19111. The circle denotes the inhibition zone.

論文審査結果要旨

コンポスト化は、世界各地で広く用いられている家畜排せつ物の処理・利用方法である。コンポスト化過程で微生物が重要な役割を担っていることは広く知られているが、その種および機能に関しては不明な点が多い。コンポスト化過程には多種多様な微生物が存在することから、微生物間に競争が存在すると考えられるがその因子の解明はほとんど行われていない。微生物が生産して他の微生物を制圧する因子であるバクテリオシンは広く研究され、すでに食品保存剤として実用化されているが、コンポストにおけるその存在は明らかではない。そこで、実規模施設の牛ふんコンポスト化過程を対象として、バクテリオシン産生細菌を検索することとした。

病原細菌 *Salmonella typhimurium*、*E. coli* O157:H7、*Enterococcus faecalis*、*Staphylococcus aureus*、*Yersinia enterocolitica* を指標細菌として、これらの増殖を抑制するバクテリオシン様物質（BLS）を産生する細菌の分離を試みた。その結果、コンポスト化開始時（0 日）から 1 株（H1）、高温期(開始後 5 日)から 6 株（H2-H7）、一次発酵終了時（開始後 23 日）から 1 株（H8）、終了時（開始後 28 日後）から 6 株（H9-H14）、指標細菌のいずれかを抑制する株が分離された。H1 のみが 55℃培養可能な高温細菌であり、他はすべて中温菌であった。産生された BLS は 55℃および 75℃60 分間の感作で失活しなかった。なかでも、H2、H6、H11、H12 および H14 が産生する BLS は 121℃15 分間感作でも安定であった。H1 および H10 が産生する BLS について食品腐敗細菌および食中毒菌に対する作用を観察した。H1 の BLS はグラム陽性細菌である *Listeria monocytogenes*、*Bacillus cereus*、*Bacillus subtilis*、*Lactobacillus species* および *Lactobacillus fermentum* およびグラム陰性細菌である *Pseudomonas fluorescens* の増殖を抑制した。H10 の BLS はこれらに加えて、*Serratia marcescens* にも抑制効果を示した。H1 の BLS は植物病原細菌の *Erwinia carotovora* subsp. Carotovora および *Pantoea ananati* にも効果は弱いものの抑制を示した。両 BLS ともに pH3 から 9 および 28℃から-80℃で保存した場合も安定した活性を示した。H1 は遺伝子解析等の結果、*Bacillus licheniformis* と同定された。

本研究は、実規模施設のコンポスト化過程から、初めて BLS を産生する微生物を発見したもので、コンポストの微生物群集成立の一要因を明らかにした点で重要な知見を与えるものである。とくに、それらの中で *B. licheniformis.* は pH および温度条件に影響されにくく、比較的広い範囲の細菌の増殖を抑制する BLS を産生する株であり、実用化の可能性を有する株として評価される。これらのことから、本論文は、博士論文に相応しいと結論された。