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Study on Bacterial Strain UL188 Isolated from Fermented Meat of Phu Tho Province in Vietnam

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Abstract: Lactic acid bacteria (LAB) and bacteriocins are of great interest to scientists due to their valuable properties. Therefore, LAB and bacteriocins have been applied in many fields such as food industry, pharmacy and medicine. In this study, one LAB strain, isolated from the fermented meat of Phu Tho province, Vietnam was studied. Some primary characteristics of the LAB strain, including morphology and classification as well as the bacteriocins synthesized by this strain were investigated. In particular, the bacteriocins were primarily evaluated for their activity unit (AU) and the effects of temperature and pH on the activities were examined as well. In addition, the bacteriocins were primarily purified by using ion exchange chromatography.

Keywords: Lactic acid bacteria (LAB), bacteriocins, activity unit (AU), chromatography.

1. Introduction

Health is always one of the most interesting topics in our life. Using safe substances, derived from natural resources, instead of chemical ones becomes tendency of human life. Lactic Acid Bacteria (LAB) are considered as GRAS (Generally Recognized As Safe). LAB also have been used as probiotics. In addition, LAB are capable of synthesizing useful substances, including bacteriocins, organic acids (like lactic acid and acetic acid), exopolysaccharides… [1-5]. Bacteriocins attract a lot of attention of scientists because bacteriocins are synthesized by ribosome pathway and they possess antibacterial activities [1, 6]. Due to these valuable properties, LAB have been applied in many fields such as in food industry, pharmacy and medicine [1, 7].

In our study, one LAB strain, isolated from the fermented meat of Phu Tho province in Vietnam was used to study some primary characteristics of the LAB strain as well as bacteriocins synthesized by this strain. The LAB strain was classified by using its sequences of 16S rDNA. Also, bacteriocins synthesized by this LAB strain were primarily investigated for their activity unit and the effects of temperature and pH on their activities

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were analyzed as well. In addition, the bacteriocins were primarily purified by using SP FF column (GE Healthcare).

2. Materials and methods

2.1. Materials

Bacterial strains: 5 indicator strains, including *Micrococcus luteus* IFO12708, *Lactococcus lactis* subsp. *lactis* ATCC 1935, *Lactobacillus sakei* subsp. *sakei* JCM1157, *Pediococcus pentosaceus* JCM 5885 and *Enterococcus faecalis* 633093T, were provided by Assoc. Prof. Takeshi Zendo, Laboratory of Microbial Technology, Department of Bioscience & Biotechnology, Faculty of Agriculture Graduate School, Kyushu University (Japan).

Strain UL188 was isolated from the fermented meat of Phu Tho province, Vietnam.

All chemicals were purchased from Sigma Aldrich (USA); LB and MRS media were purchased from LAB (Neogen company, USA).

2.2. Methods

Morphological observation: The morphology of the cell of strain UL188 was observed under the microscope Axio Zeiss.

PCR amplification, sequencing, and phylogenetic analysis: The 16S rDNA was amplified using 27F (AGAGTTTGATCCTGG CTCAG) and 1492R (GGTTACCTTGTTACGACTT) primers. The reaction mixture (50 µl) contained 5 µl of reaction buffer (0.2 M Tris-HCl pH 8.3, 0.25 M KCl, 20 mM $MgCl_2$), 20 nmol of each deoxynucleotide, 50 pmol of each primer, 2.5 U of Taq DNA polymerase, and 1 µl of template DNA. Thermocycles of PCR included 5 minutes of heat shock at 95°C, followed by 30 cycles of 95°C for 30 seconds, 52°C for 30 seconds, and 72°C for 1 minute, and a final extension at 72°C for 7 minutes. The PCR products were then analyzed by electrophoresis on 1% agarose gel, purified with QIA quick gel extraction kit (Qiagen), and the sample was sent to 1st Base company (Singapore) for sequencing.

The 16S rDNA sequences were compared with sequences available on the Gen Bank/EMBL/DDBJ databases by using the BLAST Search tool. The alignment with corresponding sequences was performed by using CLUSTAL_X program, version1.8 [8]. A phylogenetic tree was constructed by the neighbor-joining method [9]. Topography of the constructed tree was evaluated by bootstrap analysis with 1000 replicates [10].

Bacteriocins and Activity unit (AU) determination: The supernatant, obtained from 2 growing days UL188 strain, was put into wells on the plates containing 1 of the 5 indicator strains. Bacteriocins were determined by antibacterial zone around the well. Activity unit of bacteriocins was determined by the following formula:

$$\text{Activity Unit (AU)} = \frac{1}{V} x 2^n x 1000$$

Wherein, V: the sample volume (µl)

2^n: the highest 2-fold dilution at which bacteriocins' activity was remained

Effect of temperature and pH on bacteriocin activities: the pH of the sample was adjusted to 7, 8 and 9 then bacteriocins' activities of the sample at each pH were compared in order to evaluate the effect of pH on bacteriocins' activities. Similarly, the temperature (60°C) at different time intervals (0, 5, 15, 30 and 60 minutes) was used to determine the effect of temperature on activities of the bacteriocins, synthesized by strain UL188.

Bradford method for total protein determination [11]: 5 ml of dye reagent (100 mg of Coomassie Brilliant Blue G-250 was dissolved in 50 ml of 95% ethanol and 100 ml of 85% (w/v) phosphoric acid) was added to an assay tube, containing 100 µl of sample and then the tube was stayed for 5-10 minutes. The absorbance at 595 nm was measured. A standard curve of albumin with a range of 5 to

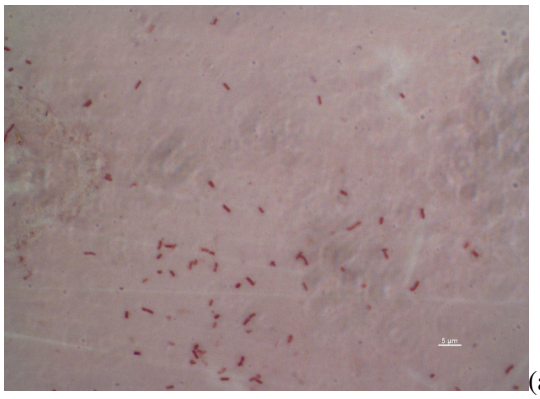
100 micrograms in 100 µl solution was created by albumins' absorbance values at 595 nm. The total protein amount of the sample was calculated based on the standard curve.

Chromatography: The conditions for chromatography was manually performed as follows: Column: HiTrap SP FF (GE Healthcare) 1ml; buffer: 0.02 M sodium acetate, pH=5; flow rate: 1 ml/min; fraction: 0.5 ml; sample volume: 10 ml; wash with 10 ml of 0.02 M sodium acetate, pH=5; elution with 10 ml of 0.02 M sodium acetate, pH=5, containing 0.5M NaCl and then with 10 ml of 0.02 M sodium acetate, pH=5, containing 10 ml of 1 M NaCl. Bacteriocins' activity of each fraction was detected on agar plate containing the indicator strains.

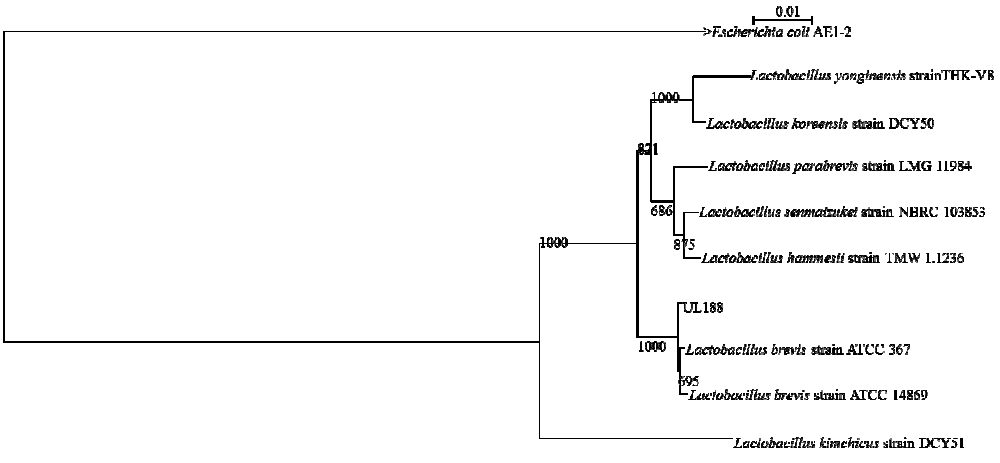
3. Results and discussion

Among 5 picked up LAB strains, isolated from the fermented meat of Phu Tho province, only one strain UL188 possessed β-galactosidase activity (data not shown), therefore this strain was chosen for further study.

3.1. Some characteristics of the bacterial strain UL188



(a)



(b)

Figure 1. Morphology and classification of strain UL188.
(a). Morphology of the cell of strain UL188 under the microscope; (b). Phylogenetic analysis of the strain UL188. Genetic distance is indicated by the scale bar and *Escherichia coli* is used as out group of the tree.

Morphology and classification: After isolation from the fermented meat of Phu Tho province, the morphology of the cell of strain UL188 was observed under the microscope (Fig. 1a). Also, 16S rDNA of the strain was amplified by PCR as shown in the section 2.2.2 (the result was not shown here). Based on the obtained DNA sequences of this 16S rDNA, strain UL188 was classified as *Lactobacillus brevis* with 99% similarity with that of the *Lactobacillus brevis* strain ATCC 367 (Fig. 1b). As its classification, this strain is safe and valuable for application in many fields [12].

3.2. Activity units of bacteriocins and the effects of temperature and pH on the bacteriocins' activities

3.2.1. Activity unit

Activity unit of bacteriocins, synthesized by strain UL188, against 5 indicator strains was presented in the Table 1. Among 5 used indicators, the bacteriocin synthesized by strain UL188 was against only *Micrococcus luteus* IFO12708 with 4267 AU.

Table 1. Activity units of bacteriocins, synthesized by strain UL188

Indicator strains	Activity unit (AU/ml)
Micrococcus luteus IFO12708	4267
Lactococcuslactis subsp. lactis ATCC 1935	0
Lactobacillus sakei subsp. sakei JCM 1157	0
Pediococcus pentosaceus JCM 5885	0
Enterococcus faecalis 633093T	0

3.2.2. The effect of temperature on bacteriocins' activities

The result showed that after 5 minutes treated at 60°C, the bacteriocins lost most of their activity (Fig. 2). According to Neha Gautam, bacteriocin of *L. brevis* had the highest activity at 40- 50°C in 10 minutes [12]. However, some heat stable bacteriocins of *L. brevis* probably remained their activities at 100°C or 121°C, but some other sensitive bacteriocins lost gradually their activities at the temperature higher than 50°C. The reason is the nature of bacteriocin being protein, therefore the conformation of some bacteriocins can be destroyed at high temperature [12]. Based on the results here, it could be said that bacteriocins synthesized by the strain UL188 were not heat stable.

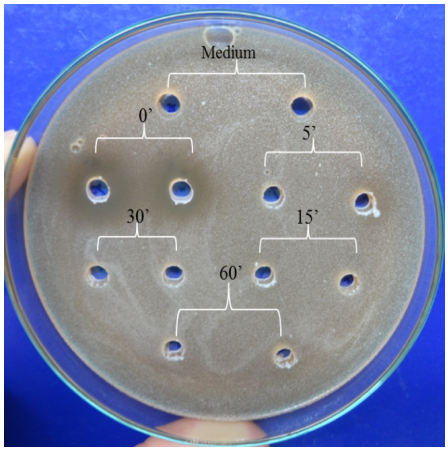


Figure 2. The effect of temperature on bacteriocins' activities.
Medium: MRS medium only;0', 5',15',30', 60': the sample treated at 60°C for 0 minute, 5 minutes, 15 minutes, 30 minutes and 60 minutes, respectively.

3.2.3. The effect of pH on bacteriocins' activity

The result showed that bacteriocins' activities of the strain UL188 were decreased when pH was increased. The highest diameter of antibacterial activities was 1.2 mm of the original sample (pH around 5.5), whereas at pH7 this diameter was 0.5 mm (decreased 41.6%). In the research of Gautam N, bacteriocins of the *L. brevis* had the highest activity at neutral pH (pH 6 and pH 7) [12]. In our case, the activities of the bacteriocins of the strain UL188 were decreased at pH 7. Generally, the bacteriocins of the strain UL188 did not work well at pH higher than 7.

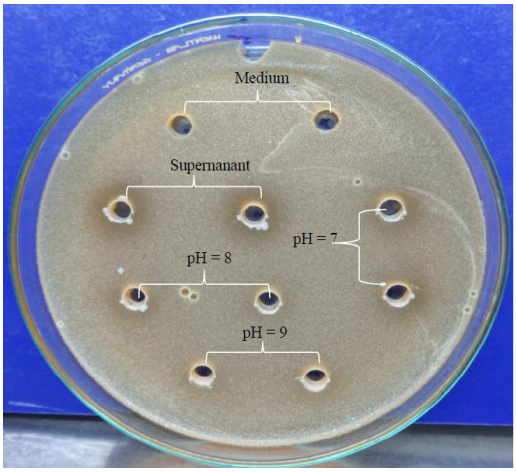


Figure 3. The effect of pH on bacteriocins' activities.
Medium: MRS medium only; Supernatant: sample without adjusting pH; pH 7, pH 8, pH 9: the sample with adjusted pH to 7, 8 and 9, respectively.

3.3. Primary purification of bacteriocins

At first, bacteriocins synthesized by strain UL188 were primarily purified by using Sephadex gel filtration. However, the result was not obtained. Then, the purification was performed by using ion exchange chromatography. In this case, the column was HiTrap SP FF (GE Healthcare) and the chromatography condition was showed in the section 2.2.6. The profile of chromatography was presented in Figure 4.

The result showed that bacteriocins synthesized by strain UL188 were primarily purified as many proteins unbound to the column and a part of proteins eluted at 0.5 M and 1 M NaCl were removed. According to the chromatography profile, there are still some questions about the detail of the bacteriocins, synthesized by the strain UL188. In order to answer these questions, other experiments should be performed.

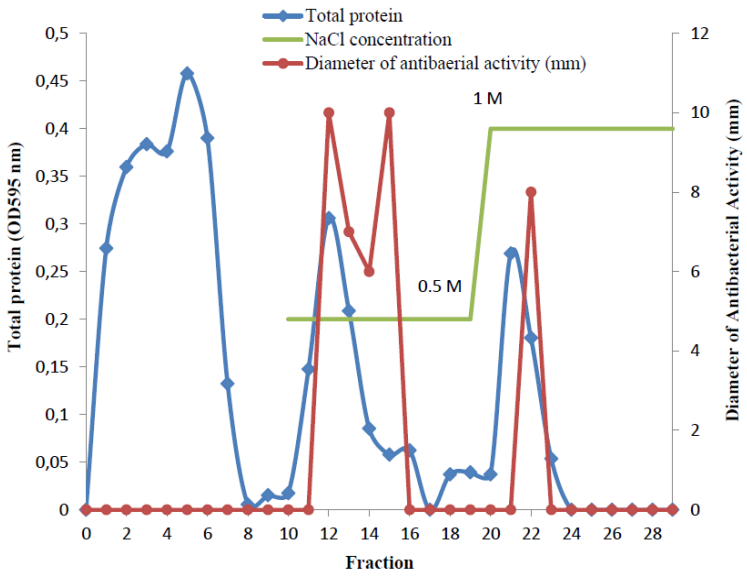


Figure 4. Chromatography profile of bacteriocins synthesized by strain UL188.

Column: HiTrap SP FF (GE Healthcare) 1ml; buffer: 0.02 M sodium acetate, pH 5; flow rate: 1 ml/min; fraction: 0.5 ml; sample volume: 10 ml; wash with 10 ml of 0.02 M sodium acetate, pH 5; elution with 10 ml of 0.02 M sodium acetate, pH 5, containing 0.5M NaCl and then with 10 ml of 0.02 M sodium acetate, pH 5, containing 10 ml of 1 M NaCl.

4. Conclusion

Strain UL188, isolated from fermented meat of Phu Tho province, Vietnam was classified as *Lactobacillus brevis.* Bacteriocins synthesized by the strain UL188 were not stable to high temperature and did not work well at pH higher than 7. Bacteriocins synthesized by strain UL188 were primarily purified by using the SP Fast Flow column (GE Healthcare).

Acknowledgments

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Nghiên cứu chủng vi khuẩn UL188 phân lập từ thịt chua của tỉnh Phú Thọ, Việt Nam

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Tóm tắt: Vi khuẩn lactic (Lactic Acid Bacteria - LAB) và bacteriocin do chúng sinh ra đã và đang thu hút sự quan tâm của các nhà khoa học bởi các đặc tính ưu việt của chúng. Chính vì thế mà LAB và bacteriocin được ứng dụng trong nhiều lĩnh vực như công nghiệp thực phẩm, dược phẩm và y tế. Trong nghiên cứu này, chủng vi khuẩn lactic UL188 phân lập từ thịt chua của Phú Thọ đã được sử dụng làm đối tượng nghiên cứu. Một số đặc điểm cơ bản của chủng này như hình thái và phân loại cũng như khả năng sinh tổng hợp bacteriocin bởi chủng này được bước đầu nghiên cứu. Cụ thể là, hoạt độ (AU) của bacteriocin và một số yếu tố ảnh hưởng như nhiệt độ và pH đối với hoạt độ bacteriocin của chủng này đã được xác định. Ngoài ra, bằng phương pháp sắc ký trao đổi ion, đã bước đầu tinh sạch được bacteriocin do chủng UL188 sinh ra.

Từ khóa: Lactic acid bacteria (LAB), bacteriocins, hoạt độ (activity unit, AU), sắc ký.