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Predominant Lactic Acid Bacteria from Salted Sea Food

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Abstract

Lactic acid bacteria are dominant microflora in many kinds of fermented foods. In this study, dominant microflora, especially lactic acid bacteria were isolated from salted sea food, and we determined physiological characteristics, and assayed specific property such as bacteriocin activity. The population of lactic acid bacteria as well as aerobic mesophilic counts was at the level of 10^7 cfu /g. Total 17 strains of LAB were isolated from salted sea food sample. The phenotypic characteristics of these strains were determined followed by Bergey's Manual. And genotypic and bacteriocin activity were tested by Schillinger and Lucke[7].

Introduction

Fermented salted sea foods are very traditional seafood products, which can be found in East Asian countries with slightly different ingredients. This kind of products, although very normal in Asia, has not been studied in detail. There are many kinds of salted sea food, moreover they are crude materials for Kimchi(Korean traditional fermented vegetable) as well as fish. The products are made from raw sea foods with traditional methods. In Jeotgal, over 20 percent of salt is contained, also whole sea food or intestines. After that it is spontaneously fermented for at least two months. These fermented products are popular in northeast Asia, especially in Korea and Japan. The aim of this work was to determine and study the predominant microbial group in fermented salted seafood and identify and characterize the dominant lactic acid bacteria from representative products. Also through this work, we make clear functional properties of lactic acid bacteria, which is dominant fermented salted seafood.

Method

Ten gram of fermented salted seafood samples was homogenized with 90ml of 0.85% (w/v) sterile physiological saline in a stomacher lab-blender for 30 seconds and serial diluted (10^{-1}~10^{-8}) in the same diluent. One hundred microliters of the appropriated dilution spread plated on selective media. Violet Red Bile Dextrose Agar (VRBD) (Merck) was used for enumeration of Enterobacteriaceae, which is the most pathogenic and most often encountered organisms in clinical microbiology. Colonies were selected randomly or all sampled if the plate contained less than 10 colonies, according to Leisner et al[3]. Chromosomal DNA was isolated by a modification of the method of Varmanen et al[4]. RAPD-PCR fingerprinting was done using the primer M13 (5'-GAG GGT GCC GGT TCT-3')[18]. PCR amplifications were preformed with a DNA thermal cycler. DNA was amplified in 50$\mu\ell$ volumes containing 100ng template DNA, 5$\mu\ell$ of 250μM dNTPs, 50pM of primer, 1.5U taq polymerase and 1x Taq polymerase buffer within 3mM of $MgCl_2$. The PCR conditions used 40 cycles. PCR products were separated by electrophoresis on 1.8% (w/v) agarose gel using 1x TBE buffer at 48V for 15hr. The gel stained in ethidium bromide and photographed on UV transilluminator. Preliminary Bacteriocin detection and activity were tested by a modification of the method of Ahn et al[2]. The inhibitory potential of lactic acid bacteria cultures and their supernatants were investigated using the modified Agar Well Assay method as described by Shillinger and Lucke [6,7]. The MRS agar was poured into Petri dishes and left to solidify and dry for 1~2 days. Ten cultures of lactic acid bacteria isolated salted sea food were each cultured in MRS broth at 30℃ for 24hr and 10μl of the cultures transferred on MRS agar plate. The cultures inoculated Agar plate incubate at 30℃ for 24hr. 10ml soft agar (0.8%) were prepared by adding indicator strains like S.aureus and E.faecalis, and were gently mixed and poured over the surface of pre spot MRS agar plates. Activity was quantified by measured diameter of clear zone per one spot.

Result

Table 1. Bacteria enumeration from Salted sea Food

Dilution factor	Mesophilic bacterial count (PCA)	LAB count (MRS)	Enterobacteriaceae (VRBD)
1×10^5	>200	151	0
1×10^6	36	4	0

Plates were triplicated and then made the mean value.

There is not Enterbacteriaceae from all fermented salted sea food.

17 strains were isolated from salted sea food.

Table 2. Physiology test

Isolate No.	Morphology	CO_2 from glucose	Catalase	Gram	10℃	45℃	pH 3.9	pH 9.6	NaCl 6.5%	Arginine hydrolysis
HK 2	Ovoid	+	−	+	+	−	+	−	+	−
HK 4	Ovoid	+	−	+	+	−	+	+	+	−
HK 5	Ovoid	+	−	+	+	−	+	+	+	−
HK 6	Short rod	−	−	+	−	−	+	−	−	−
HK 7	Short rod	−	−	+	−	−	+	−	−	−
HK 8	Ovoid	−	−	+	+	−	+	+	−	−
HK 9	Short rod	−	−	+	−	−	+	−	−	−
HK10	Ovoid	−	−	+	−	−	+	+	+	−
HK11	Ovoid	−	−	+	+	−	+	+	+	−
HK13	Ovoid	−	−	+	−	−	+	−	+	−
HK15	Ovoid	−	−	+	−	+	+	+	D	−
HK18	Ovoid	−	−	+	+	−	+	+	+	−
HK20	Ovoid	D	−	+	−	−	+	+	−	−

17 strains were done physiology test.

+ : Growth positive, −: No growth D: doubtable.

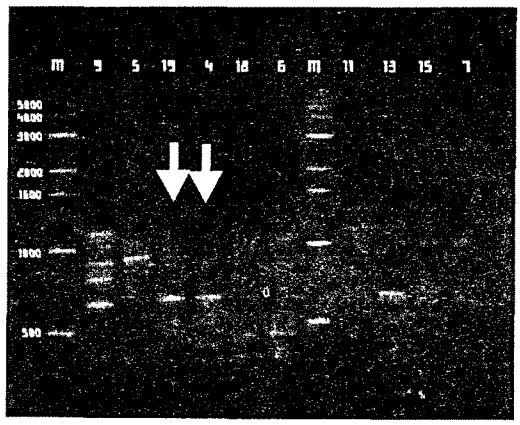
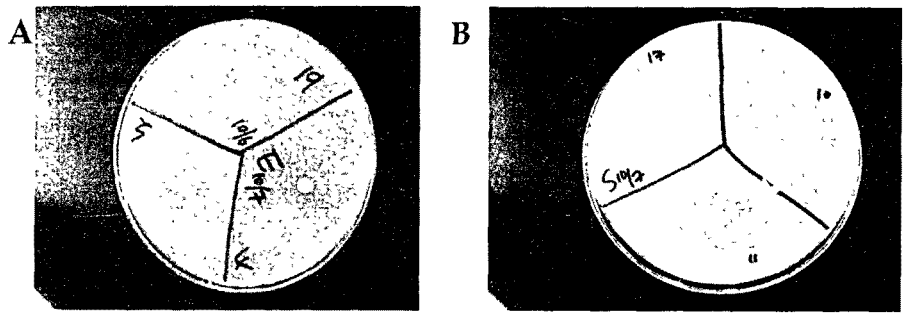


Fig. 1. RAPD-PCR performed using M13 primer. Lanes m is molecular mass markers (4000 bp, 3000 bp, 2000 bp, 1600 bp, 1000 bp, 500 bp).

Band pattern of HK1and HK4 (white arrow) are almost same; same strains. HK18 and HK6 are similar band pattern. We have to test comparison more specific.



A: *E. faecalis* overlay B: *S. aureus* overlay

Fig. 2. Preliminary bacteriocin activity test

Bacteriocin activity was detected by the deferred inhibition assay. White arrow indicated inhibitory region. *S. aureus* and *E. facalis* are used as indicator strains.

Table 3. Inhibitory diameter by LAB

Strain No.	Against *S. aureus*	Against *E. faecalis*
HK 4	2.2cm	1.8cm
HK 5	1.7cm	1.8cm
HK 8	0.6cm	1.0cm
HK11	1.2cm	1.5cm
HK19	None	1.8cm

Plates are duplicated and then make the mean value.

HK4 is the best strain against both of indicators.

HK19, especially had inhibitory diameter only against *E. faecalis*.

Table 4. Measurement of pH and OD600 after 24 and 48hrs

O.D	24hr	48hr	pH	24hr	48hr
HK18	1.97	2.09	HK18	5.44	5.22
HK17	1.59	1.44	HK17	5.81	5.54
HK16	2.96	2.97	HK16	4.53	4.45
HK15	0.92	1.29	HK15	6.01	5.75
HK11	2.75	3.04	HK11	4.33	4.34
HK10	2.06	2.51	HK10	5.64	5.29
HK 8	2.39	2.52	HK 8	4.87	4.74
HK 5	2.78	3.11	HK 5	4.40	4.38
HK 4	2.83	>3.00	HK 4	4.32	4.34

Conclusion

We isolated predominant lactic acid bacteria from salted sea food in grocery market. Physiological and genotypic characteristics were determined using isolated 17 strains. Some of them had inhibitory region against *E. faecalis* and *S. aureus*. The sequencing using 16S-rRNA and species-specific PCR are under way for the further identification.

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