

## Carnobacterium Isolated from Caviar of Sturgeon (*Acipenser ruthenus*) Farmed in Korea

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Sturgeon aqua-cultured in Korea is mainly *Acipenser ruthenus* and its culture began in the early 2000's. In this study, *Carnobacterium* sp. was isolated from unprocessed caviar of aqua-cultured *Acipenser ruthenus*. The 16s rRNA nucleotide sequence obtained from *Carnobacterium* sp. isolate (accession no. KM236206) was deposited with GenBank and homologous with *Carnobacterium divergens* DSM 20623 and NBRC15683 strain. In conclusion, this is first report of isolation of *Carnobacterium* sp. from caviar of *Acipenser ruthenus* aqua-cultured in Korea. In the future, it must be ascertained whether *Carnobacterium* sp. degenerate of caviar or cause diseases in sturgeon.

**Key Words:** Caviar, Sturgeon, *Carnobacterium*

Caviar, the eggs produced by sturgeons, is a desirable food item. The caviars from Beluga (*Huso huso*), Osetra (*Acipenser gueldenstaedtii*) and Sevruga (*Acipenser stellatus*) in the Caspian Sea are known as the most popular (1). In 2008, the global production of farmed caviar was estimated to be 110~120 tons, mostly originating from about 80 fish-farms in 16 countries. In Korea, aqua-culture of mainly *A. ruthenus* began in the early 2000's (2). Caviar of good quality is made from fresh fish roe. It is removed membranes from roe and brined for pickling. Roe holds for 4~6 weeks in salt water which has the concentration of 3.5~5% and pH 4~5, and then is stored at 1 °C (3). In this study, *Carnobacterium* was isolated from unprocessed caviar (with 3% salt) of *A. ruthenus* aqua-cultured in Korea. It 16s

rRNA nucleotide sequence was determined and its' genetic relationship was compared with other various *Carnobacteria* reference strains.

Heterofermentative lactic acid-producing *Carnobacterium* species are gram-positive and facultative anaerobic bacterium (4). They may also act as the primary agents important to the formation of conditions that favor the development of obligate anaerobic bacterium (5). They are found in vacuum-packed meat and even capable of growing at low temperatures, for example, the foods stored under refrigeration (4).

The genus *Carnobacterium* has nine species, but only two of these, *C. divergens* and *C. maltaromaticum*, are prevalent in the foods and natural environment.

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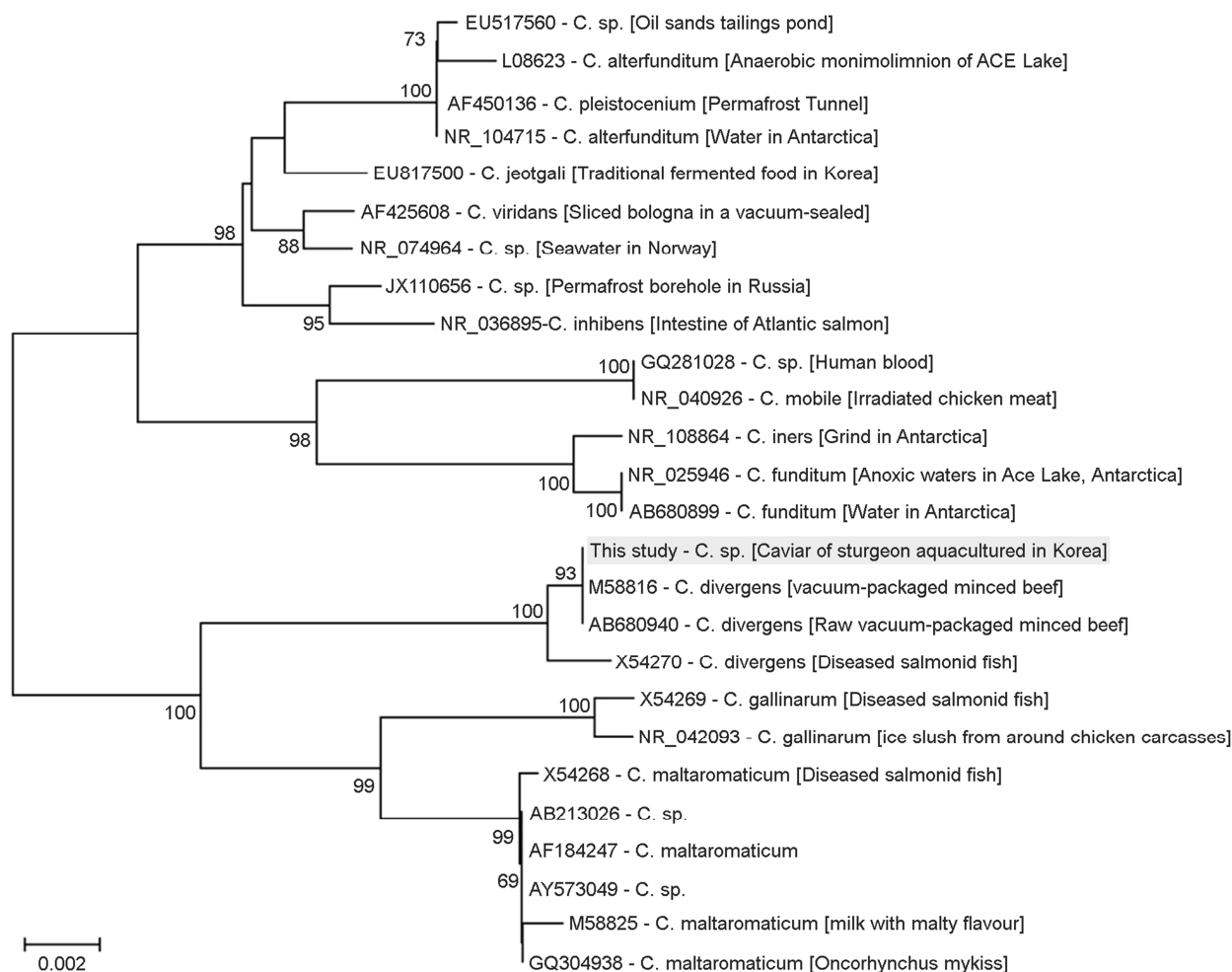
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Two human clinical cases caused with *C. maltaromaticum* and a *Carnobacterium* sp. infection have been reported and the production of the biogenic amine and tyramine from *Carnobacterium* sp. in food may cause migraine headaches but pathogenesis or the presence of virulence factors in *Carnobacterium* is not well documented (6).

Seven aqua-cultured sturgeons (*A. ruthenus*) weighing an average of 12 kg were collected in the Fishery Institute Freshwater Fishery Development Center in Chungnam, Korea. The weight of the produced caviar from the sturgeons was about 1.3 kg. Caviar samples were treated with

3% salt and immediately stored at 1°C in a refrigerated storage room until analysis. Twenty five grams of sample in 225 ml phosphate buffered saline were homogenized using a Bagmixer (Interscience, Saint Nom, France) and cultured for 24 h in tryptic soy agar (TSA) at 35°C. The identification of the colonies were performed by the sequence analysis of 16s rRNA. The isolate was transferred several times on TSA to obtain a pure culture. Next, colony PCR was performed with PCR Pre-Mix (SolGent, Daejeon, Korea) with primer sets (27F primer; 5'-AGA GTT TGA TCC TGG CTC AG-3', 1492R primer; 5'-ACG GTT ACC



**Figure 1.** Phylogenetic analysis based on 16S rRNA sequences of *Carnobacterium* sp. from caviar of sturgeon (*Acipenser ruthenus*) farmed in Korea with reference strains. Nucleotide sequences were analyzed by the neighbor-joining method. The numbers at the branches indicate the bootstrap values for 1000.

TTG TTA CGA CTT-3'). After purification, the PCR product was sequenced by using a BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. The reaction mixtures were analyzed with a PRISM 3730XL automated DNA analyzer system (Applied Biosystems). The 16S rRNA sequence obtained was compared with all sequences of *Carnobacterium* from the GenBank database at the National Center for Biotechnology Information. The genotype of the isolate was determined as the highest scoring strain using the GenBank Basic Local Alignment Search Tool (BLAST). The 16S rRNA nucleotide sequence of *Carnobacterium* sp. isolate in this study was compared with the reference sequences using MegAlign software (DNASTAR, Madison, WI, USA) (7). Phylogenetic relationships among the 16S rRNA nucleotide sequences of the isolate in this study and reference strains were determined using MEGA software v.4.0. Maximum Composite Likelihood was used as the substitution method, while the neighbor-joining method was used to reconstruct the phylogenetic tree (8). The reliability of the phylogenetic tree was determined by bootstrap re-sampling of 1,000 replicates.

*Carnobacterium* sp. was isolated from caviar produced from aqua-cultured sturgeon in Korea and the nucleotide sequence of its 16s rRNA gene was determined. As the result, it showed the highest similarities (99~100%) with *C. divergens*. However, it had 97~98% nucleotide similarities with *C. maltaromaticum* and *C. piscicola*. These low nucleotide divergences (< 3%) with the sequences of reference strains had limitation for identifying *Carnobacterium* sp. So, the isolate of this study were presented as *Carnobacterium* sp. The 16s rRNA sequence was analyzed by phylogenetic relationship with 25 *Carnobacteria* reference strains from the public data libraries (Fig. 1) and deposited with GenBank under accession number KM236206.

The 16s rRNA nucleotide sequence was homologous with *C. divergens* DSM 20623 strain (accession no. M58816) isolated from vacuum-packaged grounded beef and NBRC 15683 strain (accession no. AB680940) isolated from raw vacuum-packaged grounded beef. It also showed 4.3% nucleotide divergence from *C. jeotgali* isolated from jeotgal

in Korea.

*Carnobacterium* sp. have been isolated frequently from the food and environment (6). *C. mobile* has been rarely isolated from vacuum packed turkey (9), cooked modified atmosphere-packed shrimp in brine (10), traditional Korean fermented food (11), live Arctic char (12) and Atlantic cod (13). *C. funditum* has been isolated rarely from polar lakes or sea, the intestines of live fish and marine sponge, but never from food. *Carnobacteria* are not known to be not present in the human gastrointestinal tract (6).

The presence of virulence factors in *Carnobacteria* associated with human is not well known, so far. *C. viridans*, however, shows beta-haemolytic activity on sheep blood agar (14) and *C. maltaromaticum* may be a fish pathogen.

In conclusion, to our knowledge, this is first report of isolation of *Carnobacterium* sp. from caviar of *A. ruthenus* aqua-cultured in Korea. In the future, it must be ascertained whether *Carnobacterium* sp. degenerate of caviar or cause diseases in sturgeon.

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