Arginine Catabolism in *Lactobacillus sake* Isolated from Meat

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**Lactobacillus sake** isolated from meat can hydrolyze arginine via the arginine deiminase pathway. Two enzymes, arginine deiminase and ornithine transcarbamylase, have been revealed by detection of their reaction products, citrulline and ornithine, respectively. The production of citrulline depends on the concentration of glucose in a synthetic medium; it does not occur when the concentration of glucose is 27.5 mM or higher.

During the anaerobic storage of meat, lactobacilli become the predominant flora. Their growth depends on the use of glucose and arginine (15). Three species, *Lactobacillus carnis*, *L. divergens* (18), and *L. sake* (5), commonly found in fresh meat, can produce NH$_3$ from arginine. However, no extensive study has yet been reported on the degradation pathway of this amino acid by these bacteria.

Microorganisms catabolize arginine via at least six major pathways: arginase, arginine deiminase, arginine succinyl transferase, arginine transaminase, arginine oxidase, oxygenase, and arginine decarboxylase (1). In *Lactobacillus* spp., arginine catabolism occurs via the arginine deiminase pathway, which involves the three enzymes arginine deiminase, ornithine transcarbamylase, and carbamate kinase (1), as follows:

\[
\text{arginine} + H_2O \xrightarrow{\text{arginine deiminase}} \text{citrulline} + \text{NH}_3
\]

\[
\text{citrulline} + P_i \xrightarrow{\text{ornithine transcarbamylase}} \text{ornithine} + \text{carbamylphosphate}
\]

\[
\text{carbamylphosphate} + \text{ADP} \xrightarrow{\text{carbamate kinase}} \text{ATP} + \text{CO}_2 + \text{NH}_3
\]

Assay of arginine deiminase in whole cells. Bacteria were grown on a medium of 2.7 mM glucose, as advised by Hitchener et al. (6). Cells were harvested in the stationary phase (14-h-old cultures) by centrifugation for 10 min at 10,000 × g at 4°C and washed with 0.8% (wt/vol) NaCl. The incubation mixture contained 3 mM arginine and 10$^8$ bacterial cells in 10 mM phosphate buffer, pH 7, in a final volume of 2 ml. After 60 min of incubation at 30°C, the reaction was stopped by adding trichloroacetic acid to 3%. The precipitate was removed by centrifugation. The supernatants (20 μl) and standard amino acids (arginine, citrulline, ornithine; 20 mM in NaHCO$_3$, pH 9) were dansylated with 20 μl of 30 mM dansyl chloride in cold acetone (−18°C). The preparation was incubated at 30°C in darkness for 30 min. The dansylated products were applied to a micropolyamide sheet

When all three enzymes are active, this pathway supplies ATP and may provide a major energy source for cell growth.

The purpose of the present investigation was to detect ornithine transcarbamylase and arginine deiminase in a strain of *L. sake*, a species frequently isolated from vacuum-packed fresh meat (18). In this study, we identified the amino acids produced after growth in a semisynthetic medium and after the incubation of whole cells with arginine or citrulline. Some aspects of the regulation of this pathway were also examined.

**Organism.** *L. sake* INRA 300 was isolated from vacuum-packed beef stored at 4°C for 15 days. It was routinely grown at 30°C on an MRS medium (5).

To detect the end products of arginine degradation, organisms were cultivated on Ledesma medium (9) prepared as previously described (14) with 1.4 mM arginine and 3.2 mM glucose.

Analysis of the amino acid composition of the supernatant before and after growth was performed with a Kontron liqumat analyzer.

Assay of ornithine transcarbamylase. Catabolic ornithine transcarbamylase was assayed as previously reported (11) under the following conditions. Bacterial cells (10$^9$) were suspended in 25 mM acetate buffer, pH 5.8, with 3 mM citrulline and 0.1 mM sodium arsenate. After 60 min of incubation at 30°C, the reaction was stopped by adding trichloroacetic acid to 3%. The remaining citrulline was determined as mentioned above. Activity was expressed as micromoles of citrulline produced in 1 h per milliliter of incubation mixture.

**Evidence for arginine deiminase and ornithine transcarbamylase.** The amino acid compositions of the medium supernatant before and after 15 h of growth were compared. Arginine concentration decreased, whereas ornithine and...
citrulline, which had initially been absent, appeared. Moreover, after incubation of whole cells with arginine or citrulline, dansylation and chromatography revealed the presence of citrulline or ornithine. These results suggested that arginine deiminase and ornithine transcarbamylase were present in the cells.

Production of arginine catabolism enzymes during growth. Figure 1 shows the changes in medium composition and enzyme activities during growth of L. sake. In the first 8 h, the pH fell from 7 to 5.8 and then rose to 7. These variations in pH were correlated with glucose consumption and with NH₃ production as revealed by citrulline accumulation. Arginine degradation began when glucose was exhausted. The highest levels of arginine deiminase and ornithine transcarbamylase activities were measured in early-stationary-phase cells. These activities decreased rapidly with older cultures (Fig. 1b).

Effect of glucose. The increase in glucose concentration in the medium resulted in an increased cell yield (Fig. 2). Citrulline production was maximum at 0.6 mM glucose in the medium but was inhibited at a concentration up to 1 mM. When glucose 6-phosphate, pyruvate, malate, or glycerol was added to the medium instead of glucose, growth was impaired but citrulline production was still high. On the other hand, no citrulline was detected with 1.2% ribose.

Effect of arginine. An arginine concentration in the medium in the range of 1.4 to 14 mM enhanced the growth yield but did not affect the arginine deiminase activity of early-stationary-phase cells.

In conclusion, detection of citrulline and ornithine as end products of arginine degradation indicates that arginine deiminase and catabolic ornithine transcarbamylase are involved in the catabolism of this amino acid by L. sake. This species belongs to the Streptobacterium subgroup of the genus Lactobacillus. This is the first time that these enzymes have been reported in this subgroup. In fact, according to Manca De Nadra and Pesce De Ruiz Holgado (13), enzymes of this pathway are absent in Streptobacterium species, but they occur in the subgenus Betabacterium and in some species of the subgenus Thermobacterium in which only the first enzyme, arginine deiminase, is generally detected. Nevertheless, Jonsson et al. (7) reported that a strain of L. plantarum isolated from fish was able to degrade arginine to ornithine.

In L. sake, the arginine deiminase activity seems to be modulated by glucose concentration. This regulation has been frequently reported (7, 10). Hitchener et al. (6) also pointed out that some homofermentative strains of lactobacilli isolated from meat produced NH₃ from arginine only with 0.05% glucose. Kandler and Weiss (8) reported that L. sake does not produce NH₃, but the strain may have been cultivated in a medium with an unsuitable glucose concentration. Moreover, a low glucose concentration is required for arginine transport in L. buchneri cells (12).

The arginine deiminases are generally induced by arginine as described in Streptococcus faecalis (19), Pseudomonas putida (16), and L. leichmanii (10), but in Bacillus licheniformis (4) this induction occurs only when O₂ is scarce. The enzymes of L. buchneri and of a strain of L. plantarum isolated from fish are not induced by this amino acid, although it increases their activity (7, 11).

Whether arginine induced any enzymatic activity was difficult to prove in L. sake because this strain is auxotrophic for arginine (14). Similar difficulties have been encountered for Mycoplasma hominis (17). The results reported here suggest that this pathway may provide energy for these bacteria, but further studies are needed to determine whether ATP is really produced via carbamate kinase, the last enzyme of the arginine deiminase pathway.

Arginine degradation probably plays a significant role in

![Graph](https://example.com/graph1.png)

**FIG. 1.** Changes in medium composition (a) and in enzyme activities (b) during growth of L. sake on Hitchener medium. Symbols: ◆, pH; •, 10 log optical density (OD) at 660 nm; □, citrulline (micromoles per milliliter); ■, glucose (micromoles per milliliter); □, arginine deiminase activity; □, ornithine transcarbamylase activity.

![Graph](https://example.com/graph2.png)

**FIG. 2.** Effect of glucose concentration on yield and citrulline production in Ledesma medium. Growth was assessed by measuring the optical density (OD) at 660 nm. Symbols: ◆, optical density after 15 h of culture; ■, citrulline production (micromoles per milliliter of supernatant) measured in the corresponding supernatant.
the growth of this species on meat, in which the arginine content is very high whereas glucose is about 0.55 mM. It will be useful to demonstrate the presence of these enzymes in other strains of lactobacilli isolated from meat, mainly in *L. carnis* and *L. divergens*. Indeed, according to Shaw and Harding (18), only 18% of these strains use arginine as an energy source, although 82% are able to produce NH₃. Some strains may only possess the first enzyme, as Manca De Nadra and Pesce De Ruiz Holgado (13) noted.

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**LITERATURE CITED**