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Amino Acid Profiles and Presumptive Nutritional Assessment of Single-Cell Protein from Certain Lactobacilli[1]

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The amino acid profiles, modified essential amino acid (MEAA) indexes, and in vitro pepsin digestibilities were determined for single-cell protein (SCP) from certain industrially important lactobacilli. For the three parameters examined, substantial differences were seen between different *Lactobacillus* species and between strains within a given species. SCP from all of the lactobacilli examined appeared relatively high in MEAA indexes and pepsin digestibility. SCP from *L. acidophilus* 3205 and *L. fermenti* 3954 had the highest MEAA indexes, whereas *L. bulgaricus* 2217 and *L. thermophilus* 3863 had the highest percentage of digestible crude protein. SCP from *L. plantarum* strains had the lowest MEAA indexes. The essential amino acid compositions of SCP from different lactobacilli appear comparable to that of Food and Agriculture Organization reference protein and SCP from other sources.

Lactic acid-producing bacteria of the genus *Lactobacillus* have been widely used in the past for the industrial production of lactic acid from whey, corn, potatoes, sulfite waste liquor, and a number of other materials rich in carbohydrates (1, 7, 8, 12, 13, 17). Lactobacilli are extensively used in the dairy industry for producing yogurt, cheese, buttermilk, and a variety of other fermented milk products, as well as in most pickle fermentations (6, 26). Reddy et al. (20) recently developed a process for the fermentative conversion of whey into a ruminant feed supplement in which approximately 7% of the crude protein is derived from lactic acid bacteria. In spite of the widespread use and importance of lactobacilli in various fermentation processes for the production of food or feed products, sparse data are available regarding the amino acid profiles of single-cell protein (SCP) derived from different lactobacilli, except for one report in 1945 by Camien et al. (5). These workers, by using microbiological assays, determined the essential amino acid contents of three species of lactobacilli. No data were presented on the nutritional quality of the SCP from these organisms. The present study was initiated to determine the amino acid composition of the SCP from 12 strains of industrially important lactobacilli, representing seven species, and to obtain a presumptive assessment of the nutritional value of SCP from these organisms by determining the modified essential amino acid (MEAA) indexes and percent digestibility of the crude protein (total nitrogen × 6.25).

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MATERIALS AND METHODS

Bacteria. *Lactobacillus acidophilus* 3205 and 3532, *L. bulgaricus* 3533, *L. fermenti* 8954 and 3957, *L. plantarum* 3074, and *L. thermophilus* 3863 were obtained from the Institute for Fermentation, Osaka, Japan. *L. casei* 14435 and *L. plantarum* 14431 and 8014 were obtained from the American Type Culture Collection, Rockville, Md. *L. bulgaricus* 2217 was obtained from Chris Hansen Laboratories, Milwaukee, Wis., and *L. delbrueckii* B-443, supplied by R. Costilow, was originally obtained from the Northern Regional Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Ill. All stock cultures were maintained on Trypticase soy agar deeps and transferred once every 4 weeks to tubes of the same medium. All tubes, after inoculation, were incubated at 37°C for 24 h and then stored at 4°C until used.

Preparation of cells. A small amount of growth from 24-h Trypticase soy agar slant culture was transferred aseptically with an inoculating needle to 10 ml of sterile Trypticase soy broth (TSB) in screw-cap tubes (18 by 150 mm) and were incubated at 37°C for 24 h. These TSB cultures were used to inoculate 500 ml of TSB contained in 1-liter, foam-plugged Erlenmeyer flasks which, after incubation as described above, were used to inoculate 3.5 liters of TSB contained in 4-liter, foam-plugged Erlenmeyer flasks.

Harvesting and protein extraction. After 24 h of

incubation at 37°C, cells from the 4-liter cultures were harvested by centrifugation at 45,900 × *g* for 15 min with a centrifuge (Dupont-Sorvall model RC-5) equipped with a continuous-flow centrifuge head. The centrifuged cell paste was washed in 10 volumes of 0.2 M sodium phosphate buffer (pH 7.0) and centrifuged (25,000 × *g*) for 15 min. The supernatant was discarded, and the cell pellet was washed twice in 10 volumes of distilled water and collected by centrifugation as described above. The washed cell pellet was collected in a glass vial, resuspended in 10 volumes of distilled water, shell-frozen, and lyophilized (Cryolizer freeze dryer, model B64, New Brunswick Scientific Co., New Brunswick, N.J.). For cold trichloroacetic acid extraction, about 1.7 g of lyophilized cells was suspended in 100 ml of 5.0% (wt/vol) trichloroacetic acid, incubated for 30 min at 25°C, and centrifuged at 25,000 × *g* for 15 min. The supernatant was discarded, and the pellet was dried (60°C) in a forced-air oven to constant weight. Cold trichloroacetic acid extraction was used for extracting SCP from eight strains of lactobacilli representing six species.

For hot trichloroacetic acid extraction, cells were harvested as described above, washed with 200 ml of distilled water, and collected by centrifugation at 25,000 × *g* for 15 min. The washed cells were suspended in 200 ml of 5.0% trichloroacetic acid, incubated at 90°C for 30 min, and centrifuged at 25,000 × *g* for 15 min. The supernatant was discarded, and the precipitated protein was suspended in 50 ml of distilled water, shell-frozen, and lyophilized. Hot trichloroacetic acid extraction was used for extracting SCP from four species of lactobacilli, because this procedure is reported to extract nonprotein nitrogen more completely than cold trichloroacetic acid extraction (21, 22). As expected, hot trichloroacetic acid extraction in general resulted in a higher amino acid concentration, as compared with the cold trichloroacetic acid extraction of the same SCP.

Protein hydrolysis. Total nitrogen (TN) was determined on triplicate samples of the SCP from each organism by the micro-Kjeldahl technique (25), with HgO as a catalyst. Crude protein (CP) was defined as TN × 6.25. Two aliquots containing 10 mg of CP from each of the SCP sources were placed in separate glass screw-cap vials. One milliliter of a solution containing 1 μmol each of norleucine and *S*-β-(4-pyridylethyl)-L-cysteine (internal standards) and 9 ml of 6.4 N HCl was added to each glass vial. The vials were flushed with nitrogen, sealed, and heated at 121°C and 1.06-kg/cm² pressure for 24 h. The cooled acid hydrolysates were filtered through a fluted filter paper (Whatman no. 2). The filtrates were evaporated to dryness (60°C) in a Buchler flash evaporator (model PF, A. H. Thomas Co., Philadelphia, Pa.), resuspended in 10 ml of distilled water, and evaporated to dryness two more times. Four milliliters of 0.05 M citrate buffer (pH 2.0) containing 0.3 N LiCl was added to the dried acid hydrolysates, and the solutions were transferred to glass screw-cap vials and stored at −4°C until analyzed for amino acids with an amino acid analyzer (Technicon TSM-1, Technicon Corp., Tarrytown, N.Y.).

In vitro pepsin digestibility. In vitro pepsin digestibility was determined by the procedure of the Association of Official Agricultural Chemists (2), as modified below. Smaller protein digestions were carried out in 50 ml of Oak Ridge-type polycarbonate centrifuge tubes. After pepsin treatment, the indigestible CP was precipitated by treatment with 5% trichloroacetic acid for 30 min at 25°C, followed by centrifugation at 48,200 × *g* for 30 min. TN was determined on duplicate samples of supernatant and pellet.

MEAA index. MEAA index was determined by the procedure for Oser (16), as modified by Mitchell (15), except that tryptophan concentrations were not determined and were not included in the calculations of the MEAA indexes.

RESULTS AND DISCUSSION

Amino acid composition. The results presented in Table 1 show that there are substantial differences in amino acid composition of SCP from different *Lactobacillus* species. SCP from *L. plantarum* and *L. fermenti* 3957 differed from that of the other lactobacilli in that it had a lower amount of lysine. SCP from *L. plantarum* 14431 and 3074 had an approximately three times greater concentration of the essential amino acid, isoleucine, as compared with the concentration of the same amino acid in SCP from *L. plantarum* 8014 as well as in other *Lactobacillus* species. Aspartic acid concentration was quite high in SCP from all lactobacilli, with the exception of that from *L. fermenti* 3957 and *L. plantarum* 14431 and 3074. High levels of aspartic acid have also been reported in rumen bacteria (3).

In Table 1, the amino acid composition of the SCP from lactobacilli is compared with that of the Food and Agriculture Organization (FAO) reference protein (27), certain other SCP (11, 23), and conventional foods (4, 9, 15, 23). The results showed that the concentration of essential amino acids in SCP from most lactobacilli was equal to or greater than those in the FAO reference protein, suggesting that lactobacilli are a good protein source for human adults. However, SCP from *L. plantarum* 8014 was low in leucine. The amino acid profile of the latter strain was substantially different from that of the other two *L. plantarum* strains examined in that it had a lower concentration of leucine and isoleucine and a higher concentration of lysine.

Most SCP produced to date are deficient in methionine (8, 11, 24). SCP from lactobacilli examined in this study were also low in methionine, compared with the amount in FAO reference protein. However, lactobacilli SCP were relatively superior to soybean protein and wheat flour in their methionine content. Methionine is somewhat labile to acid hydrolysis (14). Therefore, the values prsented in Table 1 for

TABLE 1. *Amino acid profiles of SCP from lactobacilli, FAO reference protein, and certain SCP and conventional foods*[a]

Protein source[b]	Essential amino acids								Nonessential amino acids								Source
	ILE	LEU	LYS	MET	PHE	THR	VAL	TYR	ALA	ARG	ASP	Half-CYS	GLU	GLY	HIS	PRO	SER	
Cold trichloroacetic acid extraction																		
L. acidophilus 3532	4.3	7.4	10.4	1.9	3.3	4.0	4.9	2.5	9.0	5.1	9.7	0.2	11.1	4.2	2.4	3.5	2.5	
L. bulgaricus 2217	4.5	6.1	9.3	2.2	3.2	4.3	5.8	3.3	6.0	4.5	10.5	0.4	9.1	3.5	2.2	3.6	2.6	
L. bulgaricus 3533	4.2	6.5	7.9	1.9	3.2	3.8	4.9	2.7	7.2	4.0	10.0	0.3	9.8	4.0	1.9	3.0	2.3	
L. casei 14435	5.0	6.8	10.2	2.1	4.0	4.2	5.4	3.2	6.8	5.3	11.0	0.5	12.3	4.4	2.7	2.9	3.1	
L. fermenti 3957	4.4	6.3	7.1	2.0	3.1	3.6	4.9	2.4	8.9	5.1	7.7	0.1	10.3	4.2	2.2	2.6	2.7	
L. plantarum 14431	12.3	5.4	4.5	1.8	2.6	3.4	4.2	2.2	9.3	3.0	7.0	0.0	11.5	3.5	1.4	3.1	2.4	
L. plantarum 3074	13.2	5.7	4.9	1.8	2.9	3.3	4.9	2.5	9.8	3.3	7.8	0.2	11.9	3.4	1.7	2.5	2.5	
L. thermophilus 3863	4.1	6.4	9.0	1.9	3.2	3.7	4.8	2.6	8.4	4.3	10.6	0.2	9.7	4.2	2.2	2.4	2.3	
Hot trichloracetic acid extraction																		
L. acidophilus 3205	6.4	8.2	10.3	2.1	4.2	4.8	6.4	3.5	8.4	4.9	13.3	0.3	12.4	4.4	2.5	3.4	2.8	
L. delbrueckii B-443	5.5	7.6	9.6	1.9	4.2	4.4	5.9	3.3	7.5	4.6	14.6	0.5	14.6	4.3	2.0	3.3	2.8	
L. fermenti 8954	4.6	8.0	10.2	2.2	4.1	6.2	6.7	4.2	7.6	4.3	13.5	0.4	10.9	4.4	2.4	3.1	3.2	
L. plantarum 8014	4.6	1.5	9.0	1.9	3.6	4.3	5.5	3.0	7.2	4.3	11.8	0.3	10.4	3.6	2.2	3.0	2.5	
FAO reference protein	4.0	7.0	5.5	3.5[c]	6.0[d]	4.0	5.0											27
Cellulomonas sp.	4.7	11.2	6.8	1.9	4.4	5.4	10.7	2.7										23
L. plantarum 8014	5.6	5.9	5.2	1.1	2.8	3.8	5.2											5
L. casei 7469	6.2	6.8	7.7	1.1	3.5	4.9	5.8											5
L. fermenti 9338	7.0	7.5	6.9	1.3	4.1	4.9	6.8											5
Saccharomyces cerevisiae	4.6	7.0	7.7	1.7	4.1	4.8	5.3											11
Beef protein	5.3	8.2	8.6	2.5	4.1	4.4	5.5	3.4										9
Whole egg	5.9	8.8	7.8	3.2	5.5	4.9	7.1											15
Soybean protein	5.8	7.6	6.6	1.1	4.8	3.9	5.2	3.2										4
Wheat flour	4.2	7.0	1.9	1.5	5.5	2.7	4.1											23

[a] Amino acid concentrations are expressed as grams of amino acid per 16 g of N.

[b] SCP was extracted with 10% (wt/vol) trichloracetic acid at 25° for organisms under cold trichloroacetic acid. For organisms listed under hot trichloroacetic acid, SCP was extracted with 10% (wt/vol) trichloroacetic acid at 90°C; details are given in Materials and Methods.

[c] Value reported is the sum of values for methionine + cystine.

[d] Value reported is the sum of values for phenylalanine + tyrosine.

methionine underestimate the actual methionine concentration in the SCP of the lactobacilli.

Lysine is the most limiting essential amino acid in cereals, but it is present in higher concentration in animal protein sources such as beef and egg. The lysine concentration in SCP from *L. thermophilus*, *L. bulgaricus* 2217, *L. acidophilus* 3532 and 3205, *L. delbrueckii*, *L. fermenti* 3954, *L. plantarum* 8014, and *L. casei* strains surpassed that in beef or whole egg and was approximately twice as much as that present in *L. plantarum* 14431 and 3074.

Camien et al. (5) previously reported the essential amino acids of *L. plantarum* 8014 and 8041, *L. casei* 7469, and *L. fermenti* 9338. Defatted lactobacilli were acid-hydrolyzed by refluxing cells for 20 h in 10 volumes of 8 N HCl, and the amino acid content was determined by microbiological assay. The procedures used were substantially different from ours, and this may account for the small differences observed in essential amino acids reported by Camien et al. These workers suggested that proteins in different strains of microorganisms do not differ markedly in amino acid composition. Our results indicated differences in amino acid composition between SCP from different species of lactobacilli and among strains within a given species. Similar variations in amino acid compositions of SCP from different organisms have been reported by other investigators (19, 24).

In vitro pepsin digestibility and MEAA index. The amino acid concentrations present in a protein source may have no relationship to the biological availability of these amino acids (18). To make a preliminary assessment of the nutritional value of the SCP from lactobacilli, the MEAA index and in vitro pepsin digestibility were determined for each SCP type (Table 2). The MEAA index is a measure of the levels of all the essential amino acids in the test protein versus those in whole egg protein (18). Considerable differences in MEAA indexes and digestible crude protein (CP) for the SCP from different lactobacilli were observed. The SCP from *L. acidophilus* 3205 and *L. fermenti* 3954 appeared to be relatively superior to other lactobacilli, with MEAA indexes of 86 and 85, respectively. SCP from *L. bulgaricus* 2217 and *L. thermophilus* 3863 had the highest digestible CP values, 89.2 and 88.1, respectively, but had relatively low MEAA indexes, 76 and 69, respectively. MEAA indexes and digestible CP values were relatively high for *L. casei* 14435 and *L. delbrueckii* B-443. SCP from *L. plantarum* strains had lower MEAA indexes and digestible CP values, suggesting that they are lower-quality protein sources than casein and the SCP from other lactobacilli.

TABLE 2. *MEAA index and percent digestible CP of SCP from certain lactobacilli*

SCP source	MEAA index	Digestible CP (%)
Casein	91	98.5[a] ± 0.2[b]
L. acidophilus 3532	73	79.3 ± 0.5
L. acidophilus 3205	86	83.7 ± 0.5
L. bulgaricus 2217	76	89.2 ± 0.1
L. bulgaricus 3533	69	81.3 ± 0.4
L. casei 14435	80	82.3 ± 0.2
L. delbrueckii B-443	80	82.5 ± 0.2
L. fermenti 3954	85	86.5 ± 0.5
L. fermenti 3957	69	81.6 ± 0.5
L. plantarum 14431	59	79.9 ± 0.8
L. plantarum 8014	62	80.6 ± 0.3
L. thermophilus 3863	69	88.1 ± 0.3

[a] Calculated from reference 10. Values giving standard deviation greater than 1.0 were not included in the calculation of the mean percent digestible crude protein.

[b] Standard error of the mean.

Lactobacilli are commonly used in industry for the production of fermented food or feed products. The lactobacilli SCP in these fermented foods generally constitutes only a small percentage of the total protein. However, in any one of these processes, it would be more desirable to use a strain of *Lactobacillus* that has a higher quality SCP than a strain with a SCP of poorer quality, other considerations being equal. Reddy et al. (20) recently used *L. bulgaricus* 2217 for the fermentation of whey and produced a product called fermented ammoniated condensed whey (FACW), which is rich in CP (55% CP) and was shown to be an efficacious nitrogen supplement for cattle. Approximately 7% of the CP in FACW comes from *L. bulgaricus*, 17% is from whey proteins, and 70% is from ammonium lactate. Considering the quantity of SCP from *L. bulgaricus* 2217 in this product and its relatively superior amino acid profile, MEAA index, and percent digestible CP, it appears possible that *L. bulgaricus* SCP and whey proteins (mostly lactalbumin, which has a high biological value for humans) could be separated from ammonium lactate in FACW and used as a source of protein for humans. These studies are currently in progress in our laboratory.

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