Amino Acids (2012) 42:1597–1608
DOI 10.1007/s00726-011-0846-x

ORIGINAL ARTICLE

Metabolism of select amino acids in bacteria from the pig small intestine

Zhao-Lai Dai · Xi-Long Li · Peng-Bin Xi · Jing Zhang · Guoyao Wu · Wei-Yun Zhu

Received: 8 November 2010 / Accepted: 8 February 2011 / Published online: 23 February 2011

Abstract This study investigated the metabolism of select amino acids (AA) in bacterial strains (*Streptococcus* sp., *Escherichia coli* and *Klebsiella* sp.) and mixed bacterial cultures derived from the jejunum and ileum of pigs. Cells were incubated at 37°C for 3 h in anaerobic media containing 0.5–5 mM select AA plus [U-^{14}C]-labeled tracers to determine their decarboxylation and incorporation into bacterial protein. Results showed that all types of bacteria rapidly utilized glutamine, lysine, arginine and threonine. However, rates of the utilization of AA by pure cultures of *E. coli* and *Klebsiella* sp. were greater than those for mixed bacterial cultures or *Streptococcus* sp. The oxidation of lysine, threonine and arginine accounted for 10% of their utilization in these pure bacterial cultures, but values were either higher or lower in mixed bacterial cultures depending on AA, bacterial species and the gut segment (e.g., 15% for lysine in jejunal and ileal mixed bacteria; 5.5 and 0.3% for threonine in jejunal mixed bacteria and ileal mixed bacteria, respectively; and 20% for arginine in ileal mixed bacteria). Percentages of AA used for bacterial protein synthesis were 50–70% for leucine, 25% for threonine, proline and methionine, 15% for lysine and arginine and 10% for glutamine. These results indicate diverse metabolism of AA in small-intestinal bacteria in a species- and gut compartment-dependent manner. This diversity may contribute to AA homeostasis in the gut. The findings have important implications for both animal and human nutrition, as well as their health and well-beings.

Keywords Small intestine · Amino acid metabolism · Bacteria · Metabolic fate · Swine

Abbreviations

AA	Amino acids
EAA	Nutritionally essential amino acids
CFU	Colony forming unit
NEAA	Nutritionally nonessential amino acids
SCFA	Short-chain fatty acid

Introduction

Recent studies have identified that the first-pass metabolism of amino acids (AA) in the small intestine plays an important role in regulating AA and nitrogen homeostasis in humans and animals (Bergen and Wu 2009; Fuller and Reeds 1998; Stoll et al. 1998; Wu 1998). Catabolism of some nutritionally nonessential AA (NEAA) by intestinal mucosal cells is now well documented (Flynn et al. 2009; Wu 1998). There is also evidence that nutritionally essential AA (EAA) are extensively degraded by the small intestine (Stoll et al. 1998), but the responsible cell type is not the epithelial absorptive cell (Chen et al. 2007, 2009). This raises the question that luminal bacteria may be primarily responsible for EAA catabolism in the small intestine (Bergen and Wu 2009; Libao-Mercado et al. 2009; Stoll et al. 1998), as reported for the large intestine (Fuller and Reeds 1998; Jensen 1988; Metges et al. 1999a; Torrallardona et al. 2003).

Z.-L. Dai · J. Zhang · W.-Y. Zhu (✉)
Laboratory of Gastrointestinal Microbiology,
Nanjing Agricultural University, 210095 Nanjing, China
e-mail: zhuweiyun@njau.edu.cn

Z.-L. Dai · X.-L. Li · P.-B. Xi · G. Wu (✉)
Department of Animal Science, Texas A&M University,
College Station, TX 77843, USA
e-mail: g-wu@tamu.edu

We recently demonstrated extensive utilization of lysine, threonine, arginine and glutamate and, to a less extent, histidine and branched-chain AA, in bacteria from the pig small intestine (Dai et al. 2010). Interestingly, rates of the AA utilization varied greatly with bacterial species and the gut segment in that *E. coli*, *Klebsiella* sp. and ileal mixed bacteria cultures were more active in utilizing lysine, threonine, arginine and glutamate than other bacterial cultures (Dai et al. 2010). Additionally, Libao-Mercado et al. (2009) reported that more than 90% of valine needed for protein synthesis in the small-intestinal bacteria was derived from the diet and endogenous source rather than from de novo synthesis. Based on these results, we hypothesized that one important metabolic fate of extracellular AA (including EAA) in small-intestinal bacteria may be protein synthesis. Therefore, the present study aimed at investigating the possible metabolic fate of AA in small-intestinal bacteria using pure bacterial strains and mixed bacterial cultures derived from the pig small intestine.

Materials and methods

Chemicals

The following L-[U-^{14}C]-labeled AA were obtained from American Radiolabeled Chemicals (St. Louis, MO, USA): arginine, glutamine, leucine, lysine, methionine, phenylalanine, proline and threonine. These tracers were purified before use, as described by Chen et al. (2009). HPLC-grade water and methanol were purchased from Fisher Scientific (Houston, TX, USA). NCS-II tissue solubilizer was procured from Amersham Biosciences (Piscataway, NJ, USA). Hionic liquid scintillation cocktail was a product of Perkin Elmer (Waltham, MA, USA). Unless otherwise stated, all other chemicals were purchased from Sigma–Aldrich (St. Louis, MO, USA).

Bacterial strains and maintenance

Three bacterial strains including *Streptococcus* sp., *Escherichia coli* and *Klebsiella* sp. were previously isolated from the small intestine of growing pigs as AA metabolizing species (Zhang 2009; Dai et al. 2010). Two mixed bacterial cultures derived from the fifth subculture of the jejunal mixed bacteria or ileal mixed bacteria of growing pigs (Dai et al. 2010) were also used for the present study. Though incapable of reflecting the exact nature of the microbial community (including culturable and uncultural species) in the intestine, these mixed bacterial cultures could to some extent represent the culturable microbial communities that metabolize AA in the jejunum and ileum, respectively. By comparing the differences in AA metabolism in different bacterial strains, jejunal microbiota and ileal microbiota, we could estimate the potential contributions of these bacterial strains in AA utilization by the pig small intestine. All strains and cultures were maintained at 37°C in an anaerobic semi-defined medium (Dai et al. 2010), and the medium was prepared anaerobically as described previously (Dai et al. 2010). Basically, the medium was under a stream of oxygen–free CO_2 by butyl-rubber gassing line, with resazurin as anaerobiosis indicator, and when the medium became colorless reducing agents ($Na_2S{\cdot}9H_2O$ and cysteine-HCl) were added, and after half an hour the medium was dispensed into bottles or tubes, which were then crimp sealed by butyl-rubber stoppers. The pH of the medium was adjusted to 6.5 before autoclave using 1 M HCl.

Subculture of bacteria

Stock cultures of *Streptococcus* sp., *Escherichia coli*, *Klebsiella* sp., jejunal mixed bacteria or ileal mixed bacteria were subcultured in a semi-defined medium at 37°C for 24 h (Dai et al. 2010). The composition of the anaerobic basal medium was similar to the semi-defined medium described previously (Dai et al. 2010) except that casitone and yeast extracts were replaced by AA mixtures (Table 1). Concentrations of most of the AA varied from 0.4 to 2.6 mM based on their physiological levels in the jejunal lumen of growing pigs at 2 h after consuming a corn- and soybean meal-based diet (Wu et al. 1997). Concentrations of the following chemicals were modified from those for the semi-defined medium (Dai et al. 2010): glucose 1 g/L, maltose 0.5 g/L, cellobiose 0.5 g/L, sodium lactate 2 g/L and NH_4Cl 0.02 g/L. The resulting 24-h cultures served as inocula for the experiments of the present study.

Determination of AA oxidation in bacteria

Bacteria were incubated in anaerobic basal media (described above) and contained one of the following L-[U-^{14}C]-labeled AA plus unlabeled tracees at 0.5, 1, 2 or 5 mM (50 dpm/nmol): arginine, glutamine, leucine, lysine, methionine, phenylalanine, proline and threonine. During incubation, media containing AA and bacteria but no labeled AA were run to enumerate bacteria, whereas media containing labeled AA plus unlabeled tracees but no bacteria served as radioactivity blanks for $^{14}CO_2$ production (Flynn et al. 2010). All the tubes were sealed with rubber stoppers that were fixed with center wells placed in 0.5 mL centrifuge tubes, and incubated at 37°C for 3 h. After the 3-h incubation, tubes without labeled AA was removed from the incubator and put on ice immediately for the enumeration of bacteria. Meanwhile, $^{14}CO_2$ produced from

Table 1 Concentrations of amino acids and ammonia in the basal medium for bacterial culture

Amino acids and ammonia	mM
L-Alanine	1.8
L-Arginine[a]	1.0
L-Asparagine[b]	0.7
L-Aspartate	1.5
L-Citrulline	0.1
L-Cystine	0.3
L-Glutamate	2.6
L-Glutamine[a, b]	1.5
Glycine	1.8
L-Histidine	0.5
L-Isoleucine	0.9
L-Leucine[b]	1.7
L-Lysine[b]	1.0
L-Methionine[b]	0.4
L-Ornithine	0.5
L-Phenylalanine[b]	0.8
L-Proline[b]	1.7
L-Serine	1.4
L-Taurine	0.1
L-Threonine[b]	0.8
L-Tryptophan	0.2
L-Tyrosine	0.9
L-Valine	1.0
Ammonia (NH_4Cl)	0.4

Based on physiological concentrations in the jejunal lumen of 60-day-old pigs at 2 h after consuming a corn- and soybean meal-based diet as determined by Wu et al. (1997). NH_4Cl was used to provide 0.4 mM ammonia (NH_3 plus NH_4^+)

[a] Concentrations varied from 0 to 5 mM in some experiments

[b] Asparagine and glutamine were dissolved in anaerobic basal medium to make a 20× solution, sterilized through a 0.22-μm filter, and added to autoclaved asparagine- and glutamine-free media before use

[U-^{14}C]-labeled AA was collected in 0.2 mL NCS-II, and its radioactivity was measured in 4.8 mL organic cocktail using a Packard 1600 TR liquid scintillation counter (Perkin Elmer, Waltham, MA, USA), as described by Chen et al. (2009). Acidified culture media were neutralized by addition of 0.1 mL of 2 M K_2CO_3. The 0-h samples containing labeled AA were processed in the same way as for the 3-h samples.

Determination of AA utilization by bacteria

The supernatant fluid and cell pellet obtained from the above AA oxidation experiment were used to determine AA utilization and protein synthesis in bacteria. Briefly, the supernatant fluid was analyzed for AA using HPLC (Wu et al. 1997, 2007). For determination of the incorporation of AA into bacterial cells, the deproteinized cell pellets in the sediments were washed three times with 10% trichloroacetic acid by centrifugation at 10,000×*g* for 5 min, with removal of the supernatant fluid after each washing. Finally, the cell pellets were dried in room temperature and solubilized in 0.5 mL of 1 M NaOH at 50°C. The radioactivity of the solution was measured by liquid scintillation counter (Dai et al. 2010).

In the aforementioned procedures, the free ^{14}C-labeled AA was completely removed after the incubation and the amount of ^{14}C-labeled AA in the protein pellet represented protein synthesis. In our preliminary study, we found that when the cells were incubated with 1 mM cycloheximide (an inhibitor of protein synthesis), little ^{14}C-labeled AA was detected in the protein pellet. Additionally, the 0-h samples with ^{14}C-labeled AA were placed on ice after sampling and processed in parallel with the 3-h samples. However, little radioactivity was detected in the bacterial protein pellet derived from the 0-h samples.

Enumeration of bacteria

Numbers of bacteria in pure or mixed cultures were determined using the Hungate roll-tube method with the semi-defined media described earlier (liquid media or solidified media containing 1.5% agar) (Eller et al. 1971). The tubes were incubated at 37°C for 24 h before counting the colony. In experiments involving the enumeration of bacteria in the pure culture, the A_{600} value of the culture was converted to bacteria numbers using conversion factors (*Streptococcus* sp., 3.7×10^9 CFU/mL per A_{600} unit; *Escherichia coli*, 1.6×10^9 CFU/mL per A_{600} unit; *Klebsiella* sp., 2.0×10^9 CFU/mL per A_{600} unit, this study).

Calculations and statistic analysis

Rates of $^{14}CO_2$ production from [U-^{14}C]-labeled AA and the incorporation of [U-^{14}C]-labeled AA into bacteria cells were calculated based on the intracellular specific radioactivities (Dai et al. 2010; Wu 1997). The disappearance of AA from the incubation medium was taken to indicate AA utilization by bacteria (Dai et al. 2010). Rates of net utilization of AA after a 3-h incubation period were calculated on the basis of differences in AA concentrations between initial (0 h) and final (3 h) incubation media. The proportions of AA utilized via oxidation and protein synthesis pathways were estimated on the basis of net $^{14}CO_2$ production and the incorporation of [U-^{14}C]-labeled AA into bacteria, respectively. Results were analyzed by one-way ANOVA and the general linear model procedure using SAS (SAS Institute, Cary, NC). P values ≤ 0.05 were taken to indicate significance.

Table 2 CO_2 production from the catabolism of select amino acids by pig small-intestinal bacteria

AA (mM)	CO_2 production, nmol/(10^8 cells · 3 h)				
	Streptococcus sp.	*Escherichia coli*	*Klebsiella* sp.	Jejunal bacteria	Ileal bacteria
L-Arginine					
0.5	1.21 ± 0.03^{eD}	19.6 ± 0.61^{aB}	8.30 ± 0.25^{bC}	1.67 ± 0.08^{deD}	2.44 ± 0.15^{cdC}
1	1.50 ± 0.04^{eC}	20.5 ± 0.67^{aB}	9.00 ± 0.18^{bC}	2.77 ± 0.14^{dC}	4.50 ± 0.31^{cB}
2	1.88 ± 0.06^{dB}	20.9 ± 0.37^{aB}	12.2 ± 0.56^{bB}	4.66 ± 0.24^{cB}	5.52 ± 0.38^{cA}
5	2.11 ± 0.12^{dA}	23.0 ± 0.89^{aA}	15.5 ± 0.86^{bA}	5.89 ± 0.23^{cA}	5.26 ± 0.29^{cAB}
L-Glutamine					
0.5	0.64 ± 0.03^{cC}	6.81 ± 0.60^{aB}	4.32 ± 0.33^{bC}	0.10 ± 0.01^{cB}	0.11 ± 0.01^{cC}
1	0.69 ± 0.02^{bC}	8.38 ± 0.84^{aB}	7.50 ± 0.51^{aB}	0.13 ± 0.01^{bB}	0.11 ± 0.01^{bC}
2	0.94 ± 0.07^{bB}	11.8 ± 0.60^{aA}	11.2 ± 1.10^{aA}	0.26 ± 0.03^{bA}	0.34 ± 0.05^{bB}
5	1.35 ± 0.14^{bA}	13.8 ± 0.89^{aA}	12.4 ± 1.12^{aA}	0.27 ± 0.05^{bA}	0.49 ± 0.06^{bA}
L-Leucine					
0.5	ND	0.02 ± 0.01^{B}	0.02 ± 0.01^{B}	ND	ND
1	0.02 ± 0.01^{bB}	0.07 ± 0.02^{aB}	0.02 ± 0.01^{bB}	ND	ND
2	0.05 ± 0.01^{bB}	0.12 ± 0.02^{aB}	0.10 ± 0.03^{aA}	0.02 ± 0.01^{b}	0.03 ± 0.01^{bB}
5	0.08 ± 0.01^{bA}	0.39 ± 0.08^{aA}	0.11 ± 0.04^{bA}	0.03 ± 0.01^{b}	0.06 ± 0.01^{bA}
L-Lysine					
0.5	4.82 ± 0.14^{bC}	0.11 ± 0.03^{eB}	18.2 ± 0.33^{aD}	0.74 ± 0.04^{dD}	1.54 ± 0.08^{cD}
1	6.49 ± 0.37^{bB}	0.10 ± 0.04^{eB}	34.4 ± 0.78^{aC}	2.84 ± 0.11^{dC}	4.76 ± 0.27^{cC}
2	6.82 ± 0.28^{cAB}	0.15 ± 0.05^{dB}	61.5 ± 1.72^{aB}	5.58 ± 0.35^{cB}	9.18 ± 0.26^{bB}
5	7.66 ± 0.55^{cA}	0.37 ± 0.08^{dA}	72.2 ± 2.98^{aA}	13.1 ± 1.02^{bA}	15.7 ± 1.16^{bA}
L-Methionine					
2	0.23 ± 0.04^{b}	0.51 ± 0.11^{a}	0.47 ± 0.04^{a}	0.05 ± 0.01^{c}	0.05 ± 0.01^{c}
L-Phenylalanine					
2	0.17 ± 0.04^{bc}	0.22 ± 0.04^{b}	0.97 ± 0.10^{a}	0.13 ± 0.03^{bc}	0.04 ± 0.01^{c}
L-Proline					
0.5	0.02 ± 0.01^{cB}	0.25 ± 0.05^{aD}	0.16 ± 0.02^{bC}	0.02 ± 0.01^{cC}	ND
1	0.08 ± 0.02^{bB}	0.52 ± 0.07^{aC}	0.56 ± 0.08^{aB}	0.07 ± 0.01^{bBC}	0.06 ± 0.01^{bB}
2	0.09 ± 0.02^{cB}	0.91 ± 0.11^{aB}	0.62 ± 0.06^{bB}	0.13 ± 0.02^{cB}	0.06 ± 0.01^{cB}
5	0.30 ± 0.05^{bA}	1.48 ± 0.13^{aA}	1.73 ± 0.18^{aA}	0.29 ± 0.04^{bA}	0.13 ± 0.03^{bA}
L-Threonine					
0.5	1.55 ± 0.06^{cC}	12.4 ± 0.46^{aD}	8.09 ± 0.31^{bC}	0.32 ± 0.01^{dD}	0.02 ± 0.01^{dB}
1	3.03 ± 0.25^{cB}	17.8 ± 0.54^{aC}	11.0 ± 0.48^{bB}	0.57 ± 0.03^{dC}	0.06 ± 0.01^{dB}
2	3.56 ± 0.22^{cB}	27.8 ± 0.46^{aB}	12.8 ± 0.82^{bB}	1.14 ± 0.07^{dB}	0.08 ± 0.02^{dB}
5	6.20 ± 0.21^{cA}	54.7 ± 1.44^{aA}	30.9 ± 0.93^{bA}	4.30 ± 0.14^{cdA}	2.45 ± 0.07^{dA}

Values are means ± SEM, $n = 8$. Means in a row with superscripts without a common small letter (a–e) differ, $P < 0.05$; means in a column with superscripts without a common capital letter (A–D) differ, $P < 0.05$. Incubation media contained L-[U-^{14}C] labeled AA

ND, not detectable

Results

Catabolism of AA by bacteria from the pig small intestine

Results of our preliminary studies indicated that production of $^{14}CO_2$ from L-[U-^{14}C]-labeled lysine and other AA in both pure and mixed bacteria was linear during a 3-h incubation period (data not shown). Therefore, a 3-h incubation period was adopted for all the experiments of this study. At each concentration of the substrate used, rates of $^{14}CO_2$ production from [U-^{14}C]-labeled lysine, threonine, arginine and glutamine were higher than those for other AA in both pure and mixed bacteria (Table 2). Rates of lysine oxidation were low in *E. coli* but were much higher ($P < 0.05$) in *Klebsiella* sp., *Streptococcus* sp., jejunal mixed bacteria, and ileal mixed bacteria. At 2 mM and lower concentrations, rates of lysine oxidation

Table 3 Incorporation of select amino acids into bacterial cell proteins in pig small-intestinal bacteria

AA (mM)	nmol/(10^8 cells · 3 h)				
	Streptococcus sp.	*Escherichia coli*	*Klebsiella* sp.	Jejunal bacteria	Ileal bacteria
L-Arginine					
1	9.48 ± 0.21^{cB}	27.3 ± 0.82^{bB}	30.7 ± 0.28^{aB}	3.33 ± 0.21^{e}	4.83 ± 0.12^{dB}
2	10.7 ± 0.58^{cA}	34.8 ± 1.36^{aA}	32.4 ± 0.47^{bA}	3.72 ± 0.44^{d}	4.70 ± 0.17^{dB}
5	10.4 ± 0.48^{cA}	36.6 ± 1.84^{aA}	33.9 ± 0.73^{bA}	3.46 ± 0.38^{e}	5.37 ± 0.32^{dA}
L-Glutamine					
1	10.5 ± 0.72^{cB}	29.2 ± 1.20^{bC}	36.5 ± 0.57^{aB}	4.83 ± 0.30^{dB}	10.8 ± 0.24^{c}
2	10.1 ± 0.90^{cB}	35.4 ± 2.20^{bB}	38.6 ± 2.34^{aB}	5.05 ± 0.39^{dB}	11.0 ± 0.38^{c}
5	15.9 ± 1.27^{cA}	54.0 ± 3.14^{aA}	43.5 ± 1.47^{bA}	9.14 ± 0.63^{dA}	11.1 ± 0.47^{d}
L-Leucine					
1	18.0 ± 0.17^{bC}	54.6 ± 1.08^{aB}	53.8 ± 0.41^{aB}	9.23 ± 0.34^{d}	11.6 ± 0.19^{c}
2	25.1 ± 0.35^{cB}	55.4 ± 0.80^{aB}	53.0 ± 0.91^{bB}	9.59 ± 0.30^{e}	13.2 ± 0.87^{d}
5	27.5 ± 0.35^{cA}	67.4 ± 2.44^{aA}	62.8 ± 0.83^{bA}	10.2 ± 0.73^{d}	12.1 ± 0.63^{d}
L-Lysine					
1	10.7 ± 0.37^{bB}	29.6 ± 0.61^{aB}	28.5 ± 0.28^{aC}	7.00 ± 0.24^{c}	6.26 ± 0.13^{cB}
2	10.8 ± 0.43^{cB}	35.8 ± 1.39^{aA}	31.3 ± 0.98^{bB}	7.06 ± 0.33^{d}	6.07 ± 0.11^{dB}
5	13.7 ± 0.82^{bA}	37.5 ± 0.74^{aA}	36.0 ± 0.32^{aA}	7.27 ± 0.50^{c}	8.41 ± 0.35^{cA}
L-Methionine					
2	3.32 ± 0.17^{c}	13.0 ± 0.92^{a}	11.0 ± 0.84^{b}	1.87 ± 0.04^{c}	1.62 ± 0.11^{c}
L-Phenylalanine					
2	8.13 ± 0.37^{c}	30.9 ± 0.70^{a}	24.1 ± 0.50^{b}	5.27 ± 0.18^{d}	6.12 ± 0.17^{d}
L-Proline					
1	7.49 ± 0.26^{cB}	28.4 ± 0.39^{aB}	19.6 ± 0.61^{bC}	4.28 ± 0.12^{dB}	0.70 ± 0.03^{eC}
2	7.73 ± 0.17^{cB}	28.2 ± 0.54^{aB}	24.4 ± 0.43^{bB}	4.04 ± 0.17^{dB}	1.42 ± 0.06^{eB}
5	10.1 ± 0.16^{cA}	34.5 ± 0.80^{aA}	28.2 ± 0.34^{bA}	5.23 ± 0.14^{dA}	3.46 ± 0.23^{eA}
L-Threonine					
1	14.1 ± 0.20^{c}	39.8 ± 0.53^{aB}	25.6 ± 0.28^{bB}	6.62 ± 0.15^{dBC}	5.36 ± 0.13^{eB}
2	14.0 ± 1.09^{c}	40.1 ± 0.79^{aB}	25.5 ± 0.40^{bB}	6.86 ± 0.35^{dAB}	5.13 ± 0.31^{dB}
5	15.6 ± 0.36^{c}	51.8 ± 1.78^{aA}	35.8 ± 1.25^{bA}	8.31 ± 0.76^{dA}	6.80 ± 0.52^{dA}

Values are means ± SEM, $n = 4$. Means in a row with superscripts without a common small letter (a–e) differ, $P < 0.05$; means in a column with superscripts without a common capital letter (A–D) differ, $P < 0.05$. Incubation media contained L-[U-^{14}C] labeled AA

were higher ($P < 0.05$) in ileal mixed bacteria than in jejunal mixed bacteria. Similar results were obtained for arginine at 1 mM and lower concentrations. Rates of threonine and proline oxidation in ileal mixed bacteria were low when present at concentrations less than 2 mM, compared with jejunal mixed bacteria. Interestingly, rates of leucine oxidation were low in both pure and mixed bacteria.

Incorporation of AA into bacterial proteins in bacteria from the pig small intestine

Rates of the incorporation of AA into bacterial proteins were higher ($P < 0.05$) in *E. coli* and *Klebsiella* sp. than in *Streptococcus* sp., jejunal mixed bacteria and ileal mixed bacteria (Table 3). In both pure and mixed bacteria, rates of the incorporation of leucine and glutamine were higher than those for other AA. The rate of incorporation of proline into bacterial proteins was higher ($P < 0.05$) in jejunal mixed bacteria compared with ileal mixed bacteria. Similar results were obtained for threonine at 2 mM and lower concentration. However, compared with that of jejunal mixed bacteria, the incorporation rate of leucine and glutamine into bacterial proteins were significantly higher ($P < 0.05$) in ileal mixed bacteria with the AA concentration at concentrations of 2 mM and below. Similar trend was also found in the 1-mM arginine group. Meanwhile, the incorporation of methionine into bacterial proteins was lower than other AA in both pure and mixed bacteria after 3-h incubation.

Table 4 Utilization of select amino acid in the media by pig small-intestinal bacteria

AA (mM)	nmol/(10^8 cells · 3 h)				
	Streptococcus sp.	*Escherichia coli*	*Klebsiella* sp.	Jejunal bacteria	Ileal bacteria
L-Arginine					
1	49.3 ± 5.4^{cB}	214.7 ± 3.8^{aB}	98.2 ± 1.9^{bC}	23.6 ± 3.9^{dC}	21.6 ± 1.6^{dB}
2	54.9 ± 5.9^{cAB}	228.1 ± 3.0^{aB}	134.0 ± 6.8^{bB}	51.5 ± 1.9^{cB}	27.5 ± 2.1^{dB}
5	71.7 ± 7.3^{dA}	287.0 ± 1.5^{bA}	331.0 ± 4.3^{aA}	133.1 ± 1.8^{cA}	58.8 ± 2.5^{dA}
Net utilization (%)[a]	12.2 ± 1.3^{c}	16.9 ± 0.2^{b}	22.6 ± 1.1^{a}	25.1 ± 0.9^{a}	9.9 ± 0.8^{c}
L-Glutamine					
1	85.3 ± 5.9^{cB}	397.1 ± 2.9^{aB}	206.2 ± 0.4^{bC}	33.8 ± 2.4^{dC}	66.9 ± 1.3^{eB}
2	83.7 ± 2.9^{dB}	650.7 ± 7.6^{aA}	329.5 ± 3.6^{bB}	46.8 ± 3.8^{eB}	109.2 ± 5.6^{cA}
5	107.7 ± 2.5^{cA}	667.6 ± 8.9^{aA}	447.9 ± 6.2^{bA}	104.4 ± 2.6^{cA}	108.8 ± 4.6^{cA}
Net utilization (%)	21.6 ± 0.7^{c}	44.3 ± 0.5^{b}	52.7 ± 0.6^{a}	21.9 ± 1.8^{c}	39.7 ± 2.0^{b}
L-Leucine					
1	30.8 ± 2.1^{c}	98.8 ± 9.8^{aB}	66.4 ± 3.7^{bC}	22.6 ± 1.0^{c}	16.3 ± 0.7^{c}
2	34.2 ± 1.6^{c}	111.4 ± 5.8^{aAB}	78.8 ± 3.5^{bB}	26.9 ± 2.0^{cd}	22.0 ± 3.5^{d}
5	34.4 ± 2.7^{c}	130.2 ± 11.4^{aA}	92.7 ± 3.6^{bA}	27.2 ± 0.9^{c}	21.7 ± 1.8^{c}
Net utilization (%)	8.3 ± 0.4^{b}	7.7 ± 0.4^{b}	13.3 ± 0.6^{a}	12.2 ± 0.9^{a}	7.3 ± 1.2^{b}
L-Lysine					
1	44.4 ± 0.7^{cC}	121.5 ± 2.4^{bC}	190.1 ± 3.6^{aC}	34.8 ± 1.5^{dC}	39.1 ± 3.5^{cdC}
2	56.2 ± 1.1^{dB}	198.4 ± 4.2^{bB}	297.0 ± 4.2^{aB}	42.2 ± 2.1^{eB}	64.4 ± 3.9^{cB}
5	68.0 ± 1.6^{dA}	275.3 ± 6.0^{bA}	403.9 ± 4.8^{aA}	49.6 ± 2.6^{eA}	89.8 ± 4.3^{cA}
Net utilization (%)	13.3 ± 0.2^{c}	14.0 ± 0.2^{c}	45.5 ± 0.6^{a}	18.3 ± 0.8^{b}	19.5 ± 0.9^{b}
L-Methionine					
2	17.3 ± 1.7^{c}	70.0 ± 3.3^{a}	36.0 ± 0.9^{b}	7.20 ± 0.8^{d}	5.50 ± 0.8^{d}
Net utilization (%)	3.0 ± 0.2^{b}	5.2 ± 0.2^{a}	6.1 ± 0.1^{a}	3.5 ± 0.4^{b}	3.4 ± 0.3^{b}
L-Phenylalanine					
2	16.0 ± 1.6^{c}	53.0 ± 4.0^{a}	42.0 ± 2.3^{b}	10.9 ± 1.3^{c}	17.4 ± 0.8^{c}
Net utilization (%)	3.7 ± 0.4^{b}	3.9 ± 0.3^{b}	4.4 ± 0.4^{b}	5.8 ± 0.6^{a}	6.5 ± 0.3^{a}
L-Proline					
1	20.8 ± 0.5^{cB}	114.0 ± 8.5^{aB}	53.7 ± 3.2^{bC}	10.8 ± 0.72^{c}	12.2 ± 0.8^{cB}
2	25.9 ± 2.2^{cA}	109.3 ± 4.3^{aB}	68.8 ± 5.6^{bB}	12.9 ± 1.7^{d}	15.9 ± 1.5^{cdAB}
5	27.9 ± 0.9^{cA}	155.5 ± 5.0^{aA}	98.9 ± 2.7^{bA}	14.9 ± 1.7^{d}	16.6 ± 1.2^{dA}
Net utilization (%)	5.8 ± 0.1^{c}	10.1 ± 0.1^{b}	13.1 ± 0.3^{a}	6.1 ± 0.5^{c}	5.6 ± 0.1^{c}
L-Threonine					
1	51.8 ± 2.7^{b}	245.6 ± 9.6^{aC}	64.5 ± 2.6^{bC}	19.5 ± 1.2^{c}	18.6 ± 1.2^{cB}
2	50.9 ± 4.7^{c}	287.7 ± 8.9^{aB}	116.3 ± 3.8^{bB}	20.4 ± 2.3^{d}	25.6 ± 1.8^{dA}
5	49.9 ± 6.8^{c}	329.8 ± 8.3^{aA}	168.1 ± 5.1^{bA}	21.4 ± 3.3^{d}	32.7 ± 2.5^{dA}
Net utilization (%)	13.8 ± 0.9^{b}	19.4 ± 0.5^{a}	18.8 ± 0.5^{a}	8.6 ± 0.8^{c}	9.2 ± 0.3^{c}

Values are means ± SEM, $n = 4$. Means in a row with superscripts without a common small letter (a–e) differ, $P < 0.05$; means in a column with superscripts without a common capital letter (A–D) differ, $P < 0.05$

[a] Data were calculated for bacteria incubated in the presence of 2 mM AA for 3 h. The percentage of the net AA utilization was calculated as follows: [(AA concentration in the culture at 3 h-AA concentration in the culture at 0 h)/AA concentration in the culture at 0 h] × 100%

Net utilization of AA by pig small-intestinal bacteria

Generally, the proportions of the utilization of glutamine (22–53%), lysine (13–46%), arginine (10–25%) and threonine (9–20%) to their respective AA pools in the culture were relatively high after 3-h incubation (Table 4). The AA utilization in the cultures of *E. coli* and *Klebsiella* sp. was faster than in other cultures. In both pure and mixed bacteria cultures, the disappearance rates of glutamine, lysine, arginine and threonine after 3-h incubation were higher than those of other AA. Compared with that of ileal mixed bacteria culture, the utilization of arginine in jejunal mixed

Table 5 Metabolic fate of select amino acids in pig small-intestinal bacteria

AA	% of net utilization of corresponding AA in 3 h				
	Streptococcus sp.	*Escherichia coli*	*Klebsiella* sp.	Jejunal bacteria	Ileal bacteria
L-Arginine					
Protein synthesis	19.4 ± 0.75^{bB}	15.3 ± 0.43^{dB}	24.2 ± 0.25^{aB}	7.21 ± 0.60^{eB}	17.1 ± 0.44^{cB}
CO_2 production[a]	3.42 ± 0.11^{cC}	9.17 ± 0.16^{bC}	9.08 ± 0.42^{bC}	9.04 ± 0.47^{bB}	20.1 ± 1.38^{aB}
Other metabolic routes	77.2 ± 0.76^{bA}	75.6 ± 0.36^{bA}	66.7 ± 0.48^{cA}	83.7 ± 0.96^{aA}	62.8 ± 1.68^{dA}
L-Glutamine					
Protein synthesis	12.1 ± 0.77^{aB}	5.43 ± 0.24^{cB}	11.7 ± 0.51^{aB}	10.8 ± 0.59^{abB}	10.1 ± 0.25^{bB}
CO_2 production	1.12 ± 0.08^{cC}	1.81 ± 0.26^{bC}	3.40 ± 0.33^{aC}	0.56 ± 0.07^{dC}	0.31 ± 0.14^{dC}
Other metabolic routes	86.8 ± 0.78^{cA}	92.8 ± 0.28^{aA}	84.9 ± 0.51^{dA}	88.6 ± 0.60^{bA}	89.6 ± 0.26^{bA}
L-Leucine					
Protein synthesis	73.4 ± 1.04^{aA}	49.8 ± 0.72^{dA}	67.2 ± 1.15^{bA}	35.7 ± 1.11^{eB}	59.8 ± 3.97^{cA}
CO_2 production	0.85 ± 0.22^{C}	0.62 ± 0.17^{B}	0.69 ± 0.26^{C}	0.33 ± 0.10^{C}	0.76 ± 0.22^{C}
Other metabolic routes	25.7 ± 1.04^{eB}	49.6 ± 0.72^{bA}	32.1 ± 1.15^{dB}	64.0 ± 1.11^{aA}	39.4 ± 3.97^{cB}
L-Lysine					
Protein synthesis	19.4 ± 0.78^{aB}	18.6 ± 0.72^{abB}	10.5 ± 0.33^{cC}	17.5 ± 0.81^{bB}	9.84 ± 0.18^{cC}
CO_2 production	12.3 ± 0.5^{bC}	0.08 ± 0.03^{cC}	20.6 ± 1.62^{aB}	13.8 ± 0.87^{bC}	14.9 ± 0.41^{bB}
Other metabolic routes	68.3 ± 0.78^{cA}	81.3 ± 0.72^{aA}	68.9 ± 0.33^{cA}	68.7 ± 0.81^{cA}	75.3 ± 0.18^{bA}
L-Methionine					
Protein synthesis	21.3 ± 1.08^{bB}	16.0 ± 1.14^{cB}	27.1 ± 2.06^{aB}	24.5 ± 0.47^{abB}	28.2 ± 1.93^{aB}
CO_2 production	1.48 ± 0.27^{aC}	0.64 ± 0.13^{bC}	1.15 ± 0.09^{abC}	0.72 ± 0.16^{bC}	0.79 ± 0.19^{bC}
Other metabolic routes	77.2 ± 1.08^{bA}	83.3 ± 1.14^{aA}	71.7 ± 2.06^{cA}	74.8 ± 0.47^{bcA}	71.0 ± 1.93^{cA}
L-Phenylalanine					
Protein synthesis	58.4 ± 2.64^{aA}	55.6 ± 1.27^{aA}	46.8 ± 0.97^{bB}	47.2 ± 1.59^{bB}	36.0 ± 0.99^{cB}
CO_2 production	1.19 ± 0.30^{aC}	0.39 ± 0.06^{bC}	1.89 ± 0.19^{aC}	1.16 ± 0.25^{abC}	0.22 ± 0.05^{bC}
Other metabolic routes	40.4 ± 2.64^{cB}	44.0 ± 1.27^{cB}	51.3 ± 0.97^{bA}	51.6 ± 1.59^{bA}	63.8 ± 0.99^{aA}
L-Proline					
Protein synthesis	29.9 ± 0.66^{bB}	25.8 ± 0.49^{cB}	35.5 ± 0.62^{aB}	31.4 ± 1.29^{bB}	8.91 ± 0.35^{dB}
CO_2 production	0.35 ± 0.08^{bC}	0.83 ± 0.10^{abC}	0.90 ± 0.09^{abC}	0.99 ± 0.15^{aC}	0.41 ± 0.05^{bC}
Other metabolic routes	69.7 ± 0.66^{cA}	73.3 ± 0.49^{bA}	63.6 ± 0.62^{dA}	67.6 ± 1.29^{cA}	90.7 ± 0.35^{aA}
L-Threonine					
Protein synthesis	28.4 ± 2.21^{bB}	14.0 ± 0.28^{dB}	23.2 ± 0.36^{cB}	33.4 ± 1.71^{aB}	20.4 ± 1.22^{cB}
CO_2 production	7.21 ± 0.44^{cC}	9.74 ± 0.16^{bC}	11.7 ± 0.75^{aC}	5.54 ± 0.33^{dC}	0.31 ± 0.10^{eC}
Other metabolic routes	64.4 ± 2.21^{bA}	76.2 ± 0.28^{aA}	65.1 ± 0.36^{bA}	61.0 ± 1.71^{bA}	79.3 ± 1.22^{aA}

Values are means ± SEM, $n = 4$, and were calculated for bacteria incubated in the presence of 2 mM AA. Means in a row with superscripts without a common small letter (a–d) differ, $P < 0.05$; means in a column with superscripts without a common capital letter (A–C) differ, $P < 0.05$

[a] Calculations are based on the assumption that the decarboxylation of selected AA was mainly from the elimination of the alpha-carboxyl group (–COOH) of the corresponding amino acid

bacteria culture was significantly higher ($P < 0.05$) at concentrations of 2 mM and above. Similar results were obtained for leucine utilization. Meanwhile, the utilization of lysine in ileal mixed bacteria culture was higher ($P < 0.05$) than in jejunal mixed bacteria culture. Similarly, compared with that of jejunal mixed bacteria culture, the utilization of glutamine and proline were higher in ileal mixed bacteria.

Metabolic fate of AA in pig small-intestinal bacteria

Details regarding proportions of the CO_2 produced from AA oxidation and the incorporation of AA into bacterial proteins to the net AA utilization by pig small-intestinal bacteria are summarized in Table 5. Proportions of AA oxidation to the net AA utilization were higher for lysine, threonine and arginine than other AA. Except for negligible

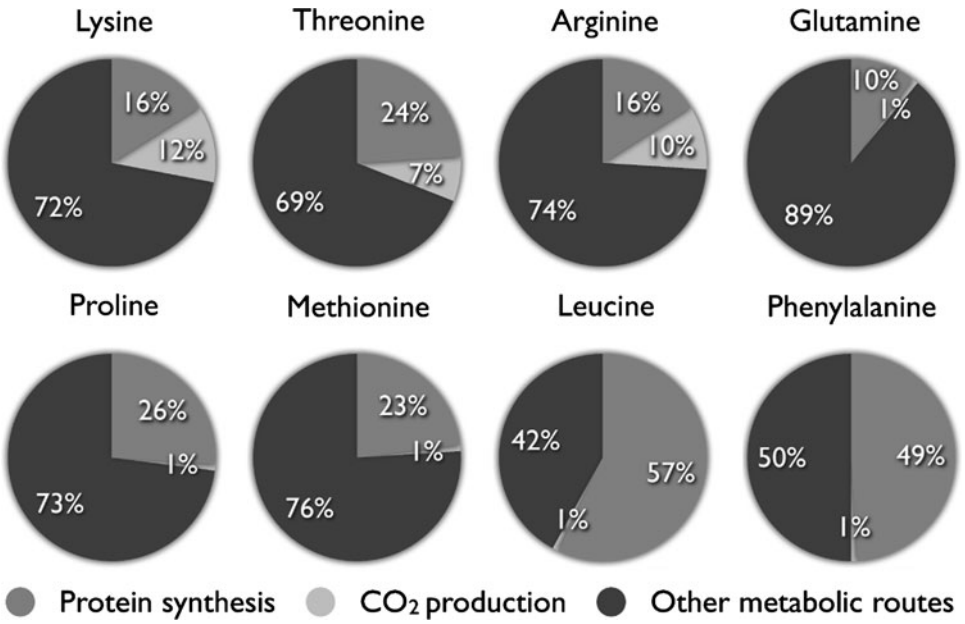

Fig. 1 Distribution of AA metabolic routes and the proportions to AA utilization for protein synthesis and oxidation in pig small-intestinal bacteria. Values are the mean values calculated from the data in Table 5

oxidation of lysine in *E. coli* culture, about 20% of the utilized lysine was oxidized in *Klebsiella* sp. culture, and this rate was higher ($P < 0.05$) than that in cultures of *Streptococcus* sp., jejunal mixed bacteria and ileal mixed bacteria where oxidation accounted for 12–15% of the lysine utilization. The oxidation of threonine was higher ($P < 0.05$) in cultures of *Klebsiella* sp. and *E. coli*, which accounted for 10% of the threonine utilization by the bacteria. Meanwhile, the proportion of threonine oxidation to threonine utilization was lower ($P < 0.05$) in ileal mixed bacteria culture than in the jejunal mixed bacteria culture (Table 2). Proportion of arginine oxidation to arginine utilization was higher ($P < 0.05$) in ileal mixed bacteria culture (20%) than in the cultures of jejunal mixed bacteria and pure bacteria (Table 5). Interestingly, only 3% of the arginine utilized by *Streptococcus* sp. was oxidized to CO_2.

The proportions of leucine and phenylalanine used for bacterial protein synthesis relative to total utilization were higher than those of other AA (Table 5). Leucine used by *Streptococcus* sp. for protein synthesis was higher ($P < 0.05$) than by *Klebsiella* sp. and *E. coli*, which comprised about 70% of leucine utilization. Meanwhile, compared with total utilization, leucine used for bacterial protein synthesis was higher ($P < 0.05$) in ileal mixed bacteria than in jejunal mixed bacteria. About 55% of the phenylalanine utilized by *Streptococcus* sp. or *E. coli* was accounted for protein synthesis, and this rate was higher ($P < 0.05$) than that accounted by *Klebsiella* sp. (47%). Proportion of the leucine used for bacterial protein synthesis to leucine utilization was higher in jejunal mixed bacteria than ileal mixed bacteria.

Proportions of threonine, proline and methionine used for protein synthesis to their total utilization were similar among the pig small-intestinal bacteria with small variations (Table 5). About 15% of the threonine utilized by *E. coli* was accounted for protein synthesis. Compared with ileal mixed bacteria, proportion of the threonine used for protein synthesis to total threonine utilization was higher ($P < 0.05$) in jejunal mixed bacteria. Similarly, proportion of the proline used for protein synthesis to total utilization was higher ($P < 0.05$) in jejunal mixed bacteria than ileal mixed bacteria. Protein synthesis represented 24% of the arginine utilized by *Klebsiella* sp. and this rate was higher ($P < 0.05$) than that in either *Streptococcus* sp. or *E. coli*. In ileal mixed bacteria, 17% of arginine utilization was accounted for protein synthesis, which was higher ($P < 0.05$) than that in jejunal mixed bacteria. In contrast, proportion of the lysine used for protein synthesis to total lysine utilization was higher in jejunal mixed bacteria than in ileal mixed bacteria. Proportions of glutamine used for protein synthesis to total glutamine utilization were relatively low in both pure and mixed bacteria.

Protein synthesis represented approximately 57% of the leucine and 50% of the phenylalanine utilized by small intestinal bacteria (Fig. 1). About 25% of the proline, threonine and methionine utilized by these cells were recovered in proteins, as compared with 15% for lysine and arginine and 10% for glutamine (Fig. 1). Oxidation to CO_2 accounted for approximately 10% of the utilization of lysine, threonine and arginine. For other AA, oxidation was negligible compared with their net utilization.

Discussion

The crucial role of AA in the nutrition and health of humans and animals depends on their metabolism within the body (Wu 2009). For example, the first-pass metabolism of AA in the small intestine plays an important role in maintaining gut function and health, but could result in a decreased efficiency of nutrient utilization (Stoll et al. 1998; Wu 1998). Work with milk-protein fed pigs showed that 30–60% of EAA were extracted by the portal drained viscera in the first-pass metabolism, and only 10% of the extracted AA was used for the synthesis of mucosal protein (Stoll et al. 1998). It is now known that among EAA, only branched-chain AA can be catabolized by gut mucosal cells; the metabolic fate of other EAA in the small intestine is not clear (Chen et al. 2007, 2009). Using microbial subculture approach, we demonstrated that pig small intestinal bacteria could extensively utilize arginine, glutamate and EAA (e.g., lysine and threonine) and this result could provide an alternative account for the first-pass metabolism of both NEAA and EAA in the small intestine (Dai et al. 2010). However, in the previous study (Dai et al. 2010), the incubation time was relatively long (24 h), the concentrations of AA (10 mM each) were much higher than their normal concentrations in the small intestine, and rates of AA oxidation in bacteria were not determined. Thus, the present study was conducted by employing a 3-h incubation approach and using medium AA concentrations similar to those in the lumen of the pig small intestine. The results indicated high rates of utilization of glutamine, lysine, arginine and threonine, with 36, 22, 17 and 15% being extracted from the cultures after 3-h incubation (Table 4). These results support the view that AA metabolism by bacteria in the small-intestinal lumen could contribute greatly to the first-pass intestinal AA metabolism.

Results from this study indicate that protein synthesis, as an important metabolic fate of AA in pig small-intestinal bacteria, occurred in an AA-specific manner. For example, protein synthesis represented half of the leucine and phenylalanine or 25% of the threonine, proline and methionine utilized by small-intestinal bacteria (Fig. 1). This partially agreed with the findings of the previous studies that the content of leucine, threonine and proline was high, but the proportions of phenylalanine and methionine were relatively low, in the proteins of both gram-positive and gram-negative bacteria (Dai et al. 2010; Sorimachi 1999). Low abundance of AA in protein can explain the low rates of microbial utilization of phenylalanine and methionine compared with other AA (Tables 3, 4). These results suggest that the de novo synthesis of branched chain AA (especially leucine), phenylalanine, threonine, proline and methionine is limited in pig small-intestinal bacteria. In other words, the availability of these AA from the diet may greatly affect protein synthesis in intestinal bacteria (Libao-Mercado et al. 2009). Meanwhile, relatively low proportions of lysine, arginine and glutamine were used for protein synthesis compared with total utilization by bacteria (Fig. 1). However, because the rates of total utilization of these AA were high in the small-intestinal bacteria (Tables 3, 4), relatively large amounts of lysine, arginine and glutamine were used for protein synthesis in these cells compared with phenylalanine and methionine (Dai et al. 2010). These findings suggest that AA requirements (type and amount) for the synthesis of bacterial protein are well conserved during evolution, and the variations in the proportions of protein synthesis to the total AA utilization reflect the diversification of AA metabolism in the intestinal bacteria.

An interesting finding of the present study is that catabolism of AA in the small-intestinal bacteria might dominate the first-pass metabolism of some AA (lysine, arginine and threonine) in the gut. As one of the major catabolic routes, oxidative decarboxylation eliminates the carboxyl group (–COOH) from the carbon skeleton of AA and thus plays an important role in modulating the pool of precursors for biosynthesis in both small-intestinal bacteria and mucosal cells. As the deamination and transamination of AA are rapid and generally exist in both gut bacteria and mucosal cells (Barker 1981; Metges et al. 1999b; Wu 1998), quantification of these biochemical events cannot be simply measured by the net production of ammonia. Compared with AA deamination, determination of [^{14}C]-AA oxidation may be better used for the estimation of AA catabolism in the small-intestinal bacteria. Using this approach, we found that a considerable amount of lysine and threonine utilized by pig small-intestinal bacteria was catabolized through oxidation to CO_2 (Table 5). This result supports the proposition that small-intestinal bacteria can oxidize lysine and threonine, thereby playing an important role in their first-pass intestinal metabolism (Chen et al. 2007, 2009; Stoll et al. 1998).

Results of the current work also indicate that metabolic routes of AA other than protein synthesis and catabolism (production of CO_2) may be quantitatively important in the bacteria of the small intestine (Table 5; Fig. 1). Although we cannot identify the detailed metabolic pathways in each of the small-intestinal bacteria studied herein, several pathways for AA metabolism could be taken into consideration in future investigation. For instance, as noted previously, the deamination of AA may occur for AA, especially for glutamine. Additionally, studies with bacteria in the rumen and the large intestine have demonstrated that some bacterial species harbor the ability to catabolize various AA and produce ammonia and short-chain fatty acid (SCFA) as end products (Rychlik et al. 2002; Smith and Macfarlane 1997, 1998; Wallace 1986, 1996). This

might help to explain the elevation of ammonia in the portal vein of animals (e.g., pigs) after feeding (Stoll et al. 1998). Moreover, predominant SCFA production from AA catabolism varies markedly with the type of AA and the species of gut bacteria (Smith and Macfarlane 1997; Wallace 1986). These authors found that the predominant SCFA produced from branched-chain AA by bacteria were branched-chain fatty acids (e.g., isobutyrate and isovalerate), while propionate and butyrate were the predominant end products of the bacterial catabolism of threonine (Smith and Macfarlane 1997; Wallace 1986). Furthermore, amines and polyamines are generated in parallel with the decarboxylation of AA in bacteria residing in the large intestine (Blachier et al. 2007; Smith and Macfarlane 1996). For example, the decarboxylation of arginine and ornithine form agmatine and putrescine, respectively (Dai et al. 2011), and both products are precursors for the synthesis of spermidine and spermine (Wu et al. 2009). Of particular note, studies with *E. coli* revealed that the bacterial metabolism of AA and SCFA may closely relate to each other, especially with the coordination of polyamine production and the regulation of SCFA metabolism (Jenkins and Nunn 1987; Lioliou and Kyriakidis 2004; Lioliou et al. 2005). The metabolic versatility may reflect the multiple functions of the intestinal bacteria in nutrition, growth, and adaptation (Kyriakidis and Tiligada 2009). It should be borne in mind that the microbial environment in the small intestine is different from that in the rumen and large intestine. Further studies are required to investigate how AA metabolic pathways in small-intestinal bacteria links to the homeostasis of the microbial ecosystem in the gut.

Intestinal catabolism of AA has important implications for nutrition. For example, adaptive lysine metabolism in the small intestine in response to low intake of dietary protein (van Goudoever et al. 2000) may partially result from the action of its luminal bacteria. Also, the extensive catabolism of arginine in small-intestinal bacteria may serve as an alternative pathway for the synthesis of proline and polyamines (putrescine, spermidine, and spermine). In the large intestine, amines produced from the bacterial decarboxylation of AA are known to regulate the physiology of the gut mucosal cells (Blachier et al. 2007; Smith and Macfarlane 1996). Similarly, arginine-derived polyamines in the small intestine play important roles in the physiology of small intestinal mucosal cells and the postnatal maturation of the gut immune system (Flynn et al. 2009; Pérez-Cano et al. 2010; Wu et al. 2000, 2009). Furthermore, deamination was a major metabolic fate of glutamine in enterocytes (Windmueller 1982; Windmueller and Spaeth 1975) and bacteria (Dai et al. 2010). The ammonia and glutamate produced from glutamine likely participate in many pathways for the synthesis of multiple AA which may enter the portal circulation for use by extraintestinal tissues. Collectively, the extensive catabolism of lysine, arginine and glutamine by the bacteria in the lumen of the small intestine not only affects AA and nitrogen cycling in the gut but also plays an important role in the modulation of intestinal physiology and function through the production of important metabolites (Bergen and Wu 2009; Blachier et al. 2007; Burrin and Davis 2004; Fuller and Reeds 1998; Wu 1998).

Another important finding of this study was that the rates and pathways of AA metabolism in small-intestinal bacteria are dependent on bacterial species and gut compartment. This might have important implications in AA nutrition and gut health. First, AA absorption and metabolism in enterocytes may be differentially affected by the composition and abundance of bacteria along the length of the small intestine (Claus et al. 2008). For example, the proportions of lysine, threonine and proline utilized for protein synthesis were higher in jejunal mixed bacteria than in ileal mixed bacteria. This may be due to the fast growth of bacteria in the jejunum and the rapid incorporation of these AA into bacteria (Dai et al. 2010; Sorimachi 1999). Therefore, less amounts of AA will be available for the absorption by jejunal mucosal cells. Furthermore, a higher proportion of AA utilized for oxidation in ileal mixed bacteria may result from the bacterial response to high concentrations of acidic fermentation products (e.g., lactate and volatile fatty acids) in the lower part of the small intestine (Richard and Foster 2004). Meanwhile, shifts in arginine metabolism and the formation of its products in the lumen of small intestine might have potential impact on the metabolic flux of the urea cycle and nitrogen recycling in the small intestine, especially in the ileum (Bergen and Wu 2009; Claus et al. 2008; Fuller and Reeds 1998). Second, the present study may aid in explaining the health and growth-promoting effects of dietary interventions by inhibiting the growth of potential pathogenic gut bacteria, thereby improving AA absorption in the small intestine (Kong et al. 2009). Compared with *Streptococcus* sp. (commensal bacteria in the pig small intestine, Konstantinov et al. 2006), our current study revealed high requirements of AA and high metabolic activities in the two potentially pathogenic bacteria *E. coli* and *Klebsiella* sp. Therefore, reducing the numbers of the AA-fermenting bacteria in the small intestine through strategies, such as dietary interventions, could modulate the temporal and spatial metabolic phenotypes of the small-intestinal microbiota, leading to improvement in intestinal AA absorption and health (Martin et al. 2009). Third, understanding the mechanisms responsible for the regulation of AA metabolism in small-intestinal bacteria could help to develop new methods to enhance the availability of dietary AA to extraintestinal tissues in both humans and animals

(Kyriakidis and Tiligada 2009). To some extent, enterocytes and the luminal bacteria contribute to the high metabolic costs for maintenance in the small intestine. Therefore, variations in the compartmental AA metabolism should not be ignored. Additionally, both intestinal and microbial factors should be taken into consideration in development of new means to modulate intestinal AA metabolism.

Overall, our work supports the view that oxidation of AA and protein synthesis in small-intestinal bacteria play an important role in the first-pass metabolism of AA and nitrogen recycling in the small intestine (Bergen and Wu 2009; Fuller and Reeds 1998; Metges 2000; Stoll et al. 1998). Diversification of AA metabolism in different bacterial species is partially dependent on the surrounding environments (Richard and Foster 2004). The long-term adaptation of the bacteria to the intestinal environment during their co-evolution with eukaryotic neighbors shapes their niches within the gut and at the same time exerts their influences on host nutrition and physiology (Ley et al. 2006; Schaible and Kaufmann 2005). Furthermore, the microbial community composition and the metabolic interactions within the community along the small intestine affect compartmental AA metabolism in the lumen of small intestine. Appreciation of these characteristics of AA metabolism in small-intestinal bacteria as well as the metabolic interactions within the microbial community and between microbes and gut mucosal cells may help better understand the physiopathological ecology of the small intestine (Blachier et al. 2007; Claus et al. 2008; Martin et al. 2009). This will ultimately lead to successful development of new strategies to improve the nutrition and health of the host.

In conclusion, the results of the present study indicate rapid and extensive utilization of glutamine, lysine, arginine, threonine and leucine in the pig small-intestinal bacteria. Protein synthesis was an important metabolic fate for AA (particularly leucine and threonine) metabolism in the bacteria. The catabolism of lysine, threonine, arginine and glutamine by small-intestinal bacteria play important role in the first-pass metabolism of AA in the small intestine. The metabolic fates of AA varied among bacterial species, with the rates of utilization being higher in *E. coli* and *Klebsiella* sp. than in *Streptococcus* sp. These results support the notion that bacterial AA metabolism in the small intestine is highly compartmentalized and differs between jejunal mixed bacteria and ileal mixed bacteria. The findings provide fundamental knowledge necessary for development of new strategies to improve AA nutrition and gut health in humans and animals.

Acknowledgments This work was supported by the Natural Science Foundation of China (30810103909), the National Basic Research Program of China (2004CB117500-4), National Research Initiative Competitive Grants from the Animal Growth & Nutrient Utilization Program (2008-35206-18764) of the USDA National Institute of Food and Agriculture, and Texas AgriLife Research Hatch Project (H-8200). We are grateful to Dr. Jo-Ann Fleming, Dr. Haijun Gao, and Dr. Junjun Wang for technical assistance and helpful discussion. ZL Dai thanks the China Scholarship Council for support of his study at Texas A&M University between 17 February 2009 and 28 February 2010.

References

Barker HA (1981) Amino acid degradation by anaerobic bacteria. Ann Rev Biochem 50:23–40

Bergen WG, Wu G (2009) Intestinal nitrogen recycling and utilization in health and disease. J Nutr 139:821–825

Blachier F, Mariotti F, Huneau JF, Tomé D (2007) Effects of amino acid-derived luminal metabolites on the colonic epithelium and physiopathological consequences. Amino Acids 33:547–562

Burrin DG, Davis TA (2004) Proteins and amino acids in enteral nutrition. Curr Opin Clin Nutr Metab Care 7:79–87

Chen LX, Yin YL, Jobgen WS, Jobgen SC, Knabe DA, Hu W, Wu G (2007) In vitro oxidation of essential amino acids by jejunal mucosal cells of growing pigs. Livest Sci 109:19–23

Chen LX, Li P, Wang JJ, Li XL, Gao HJ, Yin YL, Hou YQ, Wu G (2009) Catabolism of nutritionally essential amino acids in developing porcine enterocytes. Amino Acids 37:143–152

Claus SP, Tsang TM, Wang Y, Cloarec O, Skordi E, Martin F-P, Rezzi S, Ross A, Kochhar S, Holmes E, Nicholson JK (2008) Systemic multicompartmental effects of the gut microbiome on mouse metabolic phenotypes. Mol Syst Biol 4:219

Dai ZL, Zhang J, Wu G, Zhu WY (2010) Utilization of amino acids by bacteria from the pig small intestine. Amino Acids 39:1201–1215

Dai ZL, Zhu WY, Wu G (2011) Amino acid metabolism in intestinal bacteria: links between gut ecology and host health. Front Biosci 16:1768–1786

Eller C, Crabill MR, Bryant MP (1971) Anaerobic roll tube media for nonselective enumeration and isolation of bacteria. Appl Microbiol 22:522–529

Flynn NE, Bird JG, Guthrie AS (2009) Glucocorticoid regulation of amino acid and polyamine metabolism in the small intestine. Amino Acids 37:123–129

Flynn NE, Patryak M, Seely J, Wu G (2010) Glycine oxidation and conversion into amino acids in *Saccharomyces cerevisiae* and *Candida albicans*. Amino Acids 39:605–608

Fuller MF, Reeds PJ (1998) Nitrogen cycling in the gut. Annu Rev Nutr 18:385–411

Jenkins LS, Nunn WD (1987) Regulation of the ato operon by the atoC gene in *Escherichia coli*. J Bacteriol 169:2096–2102

Jensen BB (1988) Effect of diet composition and virginiamycin on microbial activity in the digestive tract of pigs. In: Buraczewska L, Buraczewska S, Pastuzewska B, Zebrowska T (eds) Digestive physiology in the pig. Polish Acad Sci, Jabònna, Poland, pp 392–400

Kong XF, Yin YL, He QH, Yin FG, Liu HJ, Li TJ, Huang RL, Geng MM, Ruan Z, Deng ZY, Xie MY, Wu G (2009) Dietary supplementation with Chinese herbal powder enhances ileal digestibilities and serum concentrations of amino acids in young pigs. Amino Acids 37:573–582

Konstantinov SR, Awati AA, Williams BA, Miller BG, Jones P, Stokes CR, Akkermans ADL, Smidt H, de Vos WM (2006) Postnatal development of the porcine microbiota composition and activities. Environ Microbiol 8:1191–1199

Kyriakidis DA, Tiligada E (2009) Signal transduction and adaptive regulation through bacterial two-component systems: the *Escherichia coli* AtoSC paradigm. Amino Acids 37:443–458

Ley RE, Peterson DA, Gordon JI (2006) Ecological and evolutionary forces shaping microbial diversity in the human intestine. Cell 124:837–848

Libao-Mercado AJO, Zhu CL, Cant JP, Lapierre H, Thibault J-N, Sève B, Fuller MF, de Lange CFM (2009) Dietary and endogenous amino acids are the main contributors to microbial protein in the upper gut of normally nourished pigs. J Nutr 139:1088–1094

Lioliou EE, Kyriakidis DA (2004) The role of bacterial antizyme: from an inhibitory protein to AtoC transcriptional regulator. Microb Cell Fact 3:8

Lioliou EE, Mimitou EP, Grigoroudis AI, Panagiotidis CH, Panagiotidis CA, Kyriakidis DA (2005) Phosphorylation activity of the response regulator of the two-component signal transduction system AtoSC in *Escherichia coli*. Biochim Biophys Acta 1725:257–268

Martin F-PJ, Sprenger N, Yap IKS, Wang Y, Bibiloni R, Rochat F, Rezzi S, Cherbut C, Kochhar S, Lindon JC, Holmes E, Nicholson JK (2009) Panorganismal gut microbiome-host metabolic crosstalk. J Proteome Res 8:2090–2105

Metges CC (2000) Contribution of microbial amino acids to amino acid homeostasis of the host. J Nutr 130:1857S–1864S

Metges CC, El-Khoury AE, Henneman L, Petzke KJ, Grant I, Bedri S, Pereira PP, Ajami AM, Fuller MF, Young VR (1999a) Availability of intestinal microbial lysine for whole body lysine homeostasis in human subjects. Am J Physiol Endocrinol Metab 277:597–607

Metges CC, Petzke KJ, El-Khoury AE, Henneman L, Grant I, Bedri S, Regan MM, Fuller MF, Young VR (1999b) Incorporation of urea and ammonia nitrogen into ileal and fecal microbial proteins and plasma free amino acids in normal men and ileostomates. Am J Clin Nutr 70:1046–1058

Pérez-Cano FJ, González-Castro A, Castellote C, Franch A, Castell M (2010) Influence of breast milk polyamines on suckling rat immune system maturation. Dev Comp Immunol 34:210–218

Richard H, Foster JW (2004) *Escherichia coli* glutamate- and arginine-dependent acid resistance systems increase internal pH and reverse transmembrane potential. J Bacteriol 186:6032–6041

Rychlik JL, LaVera R, Russell JB (2002) Amino acid deamination by ruminal *Megasphaera elsdenii* strains. Curr Microbiol 45:340–345

Schaible UE, Kaufmann SHE (2005) A nutritive view on the host–pathogen interplay. Trends Microbiol 13:373–380

Smith EA, Macfarlane GT (1996) Studies on amine production in the human colon: enumeration of amine-forming bacteria and physiological effects of carbohydrate and pH. Anaerobe 2:285–297

Smith EA, Macfarlane GT (1997) Dissimilatory amino acid metabolism in human colonic bacteria. Anaerobe 3:327–337

Smith EA, Macfarlane GT (1998) Enumeration of amino acid fermenting bacteria in the human large intestine: effects of pH and starch on peptide metabolism and dissimilation of amino acids. FEMS Microbiol Ecol 25:355–368

Sorimachi K (1999) Evolutionary changes reflected by the cellular amino acid composition. Amino Acids 17:207–226

Stoll B, Henry J, Reeds PJ, Yu H, Jahoor F, Burrin DG (1998) Catabolism dominates the first-pass intestinal metabolism of dietary essential amino acids in milk protein-fed piglets. J Nutr 128:606–614

Torrallardona D, Harris CI, Fuller MF (2003) Pigs' gastrointestinal microflora provide them with essential amino acids. J Nutr 133:1127–1131

van Goudoever JB, Stoll B, Henry JF, Burrin DG, Reeds PJ (2000) Adaptive regulation of intestinal lysine metabolism. Proc Natl Acad Sci USA 97:11620–11625

Wallace RJ (1986) Catabolism of amino acids by *Megasphaera elsdenii* LC1. Appl Environ Microbiol 51:1141–1143

Wallace RJ (1996) Ruminal microbial metabolism of peptides and amino acids. J Nutr 126(4 Suppl):1326S–1334S

Windmueller HG (1982) Glutamine utilization by the small intestine. Adv Enzymol 53:201–237

Windmueller HG, Spaeth AE (1975) Intestinal metabolism of glutamine and glutamate from the lumen as compared to glutamine from blood. Arch Biochem Biophys 171:662–672

Wu G (1997) Synthesis of citrulline and arginine from proline in enterocytes of postnatal pigs. Am J Physiol Gastrointest Liver Physiol 272:G1382–G1390

Wu G (1998) Intestinal mucosal amino acid catabolism. J Nutr 128:1249–1252

Wu G (2009) Amino acids: metabolism, functions, and nutrition. Amino Acids 37:1–17

Wu G, Davis PK, Flynn NE, Knabe DA, Davidson JT (1997) Endogenous synthesis of arginine plays an important role in maintaining arginine homeostasis in postweaning growing pigs. J Nutr 127:2342–2349

Wu G, Flynn NE, Knabe DA (2000) Enhanced intestinal synthesis of polyamines from proline in cortisol-treated piglets. Am J Physiol Endocrinol Metab 279:395–402

Wu G, Collins JK, Perkins-Veazie P, Siddiq M, Dolan KD, Kelly KA, Heaps CL, Meininger CJ (2007) Dietary supplementation with watermelon pomace juice enhances arginine availability and ameliorates the metabolic syndrome in Zucker diabetic fatty rats. J Nutr 137:2680–2685

Wu G, Bazer FW, Davis TA, Kim SW, Li P, Rhoads JM, Satterfield MC, Smith SB, Spencer TE, Yin Y (2009) Arginine metabolism and nutrition in growth, health and disease. Amino Acids 37:153–168

Zhang J (2009) Isolation and identification of amino acid utilizing bacteria from theporcine small intestine. Thesis, Nanjing Agricultural University, Nanjing, China