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ORIGINAL ARTICLE

Regulatory role for L-arginine in the utilization of amino acids by pig small-intestinal bacteria

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Abstract We recently reported that bacteria from the pig small intestine rapidly utilize and metabolize amino acids (AA). This study investigated the effect of L-arginine on the utilization of AA by pure bacterial strains (*Streptococcus* sp., *Escherichia coli* and *Klebsiella* sp.) and mixed bacterial cultures derived from the pig small intestine. Bacteria were incubated at 37°C for 3 h in anaerobic AA media containing 0–5 mmol/L of arginine to determine the effect of arginine on the bacterial utilization of AA. Amino acids in the medium plus cell extracts were analyzed by high-performance liquid chromatography. Results indicated concentration-dependent increases in the bacterial utilization of arginine and altered fluxes of arginine into ornithine and citrulline in the bacteria. Net glutamine utilization increased in pure bacterial strains with increased concentrations of arginine. With the addition of arginine, net utilization of threonine, glycine, phenylalanine and branched-chain AA increased ($P < 0.05$) in *Streptococcus* sp. and *Klebsiella* sp., but decreased in *E. coli*. Net utilization of lysine, threonine, isoleucine, leucine, glycine and alanine by jejunal or ileal mixed bacteria decreased ($P < 0.05$) with the addition of arginine. Complete utilization of asparagine, aspartate and serine were observed in pig small-intestinal bacteria after 3 h of incubation. Overall, the addition of arginine affected the metabolism of the arginine-family of AA and the serine- and aspartate-family of AA in small-intestinal bacteria and reduced the utilization of most AA in ileal mixed bacteria. These novel findings indicate that arginine exerts its beneficial effects on swine nutrition partially by regulating AA utilization and metabolism in the small-intestinal microbiota.

Keywords Amino acids · Intestinal bacteria · Nutrition · Small intestine · Swine

Abbreviations

AA	Amino acids
AR	Acid resistance system
EAA	Nutritionally essential amino acids
CFU	Colony forming unit
NO	Nitric oxide

Introduction

L-arginine participates in multi-organ metabolism and plays key roles in reproduction, neonatal growth, wound healing, immune responses, skeletal muscle protein synthesis, and energy metabolism (Wu et al. 2009). The versatile functions of arginine are derived not only from arginine itself but also from its metabolites such as nitric oxide (NO), proline and polyamines (Wu et al. 2009, 2011). Thus, knowledge about arginine metabolism is crucial to improve the well-being of both humans and animals (Blachier et al. 2011; Wu and Morris 1998).

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Increasing evidence suggests that AA metabolism in the small intestine plays an important role in the regulation of the whole-body homeostasis of AA, including the arginine-family of AA (Stoll et al. 1998; Wu 1998). There is an extensive degradation of arginine in enterocytes of post-weaning pigs due to cortisol-mediated induction of arginase (Wu et al. 1996). The major metabolites of arginine in these cells are urea, ornithine and proline (Wu et al. 1996). Interestingly, intramitochondrially generated ornithine and extracellular proline are used for intestinal synthesis of arginine (Wu and Knabe 1995; Wu 1997), constituting extensive arginine recycling in the gut. Furthermore, intestinal arginine metabolism may be affected by dietary intake of arginine (Wu et al. 2009; Wilkinson et al. 2004; Urschel et al. 2007). Till date, the regulation of the metabolism of the arginine-family of AA in the small intestine remains poorly understood due to the complexity of the metabolic networks and the intestinal environment (Marini et al. 2010; Yin et al. 2010).

Recent findings indicate that bacteria in the small intestine may be active in AA metabolism both in vivo and in vitro (Booijink 2009; Dai et al. 2010, 2011a; Fuller and Reeds 1998). The rapid and quantitatively significant utilization and metabolism of nutritionally essential AA (EAA) by pig small-intestinal bacteria helps to explain the relatively low portal balance of dietary AA in mammals. The findings also suggest that the bacterial metabolism of AA in the small intestine plays an important role in host nutrition and nitrogen balance (Stoll et al. 1998; Dai et al. 2010, 2011a; Fuller and Reeds 1998; Bergen and Wu 2009). Moreover, because of the diverse functions of arginine in cells (Wu et al. 2009), this AA may be crucial for the growth and survival of intestinal bacteria both at the species level and across the bacterial communities of different gut compartments (Dai et al. 2010, 2011a). However, the metabolic pathways of arginine in small-intestinal bacteria or its nutritional function are largely unknown. We hypothesized that arginine may regulate the AA metabolism in small-intestinal bacteria, thereby affecting the luminal profiles of nitrogenous compounds and contributing to the regulation of the whole-body AA homeostasis (Fuller and Reeds 1998; Bergen and Wu 2009). The objective of the present study was to test this hypothesis using both pure bacterial strains and mixed bacterial cultures derived from the pig small intestine.

Materials and methods

Chemicals

HPLC-grade water and methanol were purchased from Fisher Scientific (Houston, TX, USA). Unless stated otherwise, all other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Bacterial strains and culture conditions

Bacteria used in this study including *Streptococcus* sp., *Escherichia coli*, *Klebsiella* sp., jejunal mixed bacteria and ileal mixed bacteria were derived from the small intestine of growing pigs (Dai et al. 2010; Zhang 2009). All bacterial cultures were maintained in an anaerobic semi-defined medium described previously (Dai et al. 2011a; Williams et al. 2005) except with following modifications (per liter): 10 g casitone, 2.5 g yeast extract, 2 g soluble starch, 2 g glucose, 1 g maltose, 1 g cellobiose, 5 g sodium lactate, 0.2 g NH_4Cl. The "reducing agent solution" contained 2.05 g $Na_2S{\cdot}9H_2O$ and 2.05 g cysteine-HCl per 100 mL of boiled distilled water. The pH of the medium was adjusted to 6.5 using 1 mol/L hydrochloric acid before autoclave.

Subculture of bacteria

Stock bacterial cultures were subcultured in semi-defined medium at 37°C for 24 h. Aliquots of 1 ml of the 24-h cultures were further subcultured in 9 mL of anaerobic AA basal media twice (each for 24 h). The composition of the anaerobic AA basal media was similar to the semi-defined medium described above except the casitone and yeast extract were omitted and replaced by AA mixtures with arginine concentrations at 0, 0.5, 1, 2 or 5 mmol/L in the media (Table 1). Concentrations of the carbon sources and ammonia in the media were also modified as described previously (Dai et al. 2011a).

In vitro incubation

Anaerobic AA basal media containing doses of arginine (0, 0.5, 1, 2 or 5 mmol/L) were used for the experiment. After the second subculture, 0.1 mL of the 24-h cultures (ca. 10^8 bacteria cells) of *Streptococcus* sp., *E. coli*, *Klebsiella* sp., jejunal mixed bacteria or ileal mixed bacteria were inoculated into tubes containing 0.9 mL of corresponding sterile anaerobic AA basal media. Tubes containing AA mixtures plus doses of arginine but no bacteria served as control. All tubes were incubated at 37°C for 3 h. Tubes were taken before and after the 3-h incubation and put on ice immediately for the enumeration of bacteria and the determination of AA concentrations (Wu et al. 1997, 2007).

Enumeration of bacteria

Numbers of bacteria were determined using the Hungate roll-tube method (Eller et al. 1971) with the semi-defined

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

Amino acids and ammonia	mmol/L
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[b]	1.5
Glycine	1.8
L-Histidine	0.5
L-Isoleucine	0.9
L-Leucine	1.7
L-Lysine	1.0
L-Methionine	0.4
L-Ornithine	0.5
L-Phenylalanine	0.8
L-Proline	1.7
L-Serine	1.4
L-Taurine	0.1
L-Threonine	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-d-old pigs at 2 h after consuming a corn- and soybean meal-based diet (Wu et al. 1997; Li et al. 2011). NH_4Cl was used to provide 0.4 mmol/L ammonia (NH_3 plus NH_4^+)

[a] Concentrations varied from 0 to 5 mmol/L 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

media described above (liquid media or solidified media containing 1.5% agar). The tubes were incubated at 37°C for 24 h before counting the colonies. In experiments involving pure bacterial cultures, the A_{600} values of the culture were converted to bacteria numbers using conversion factors based on correlations between bacterial numbers in the culture and the corresponding optical density of the culture (Dai et al. 2011a).

Calculations and statistical analysis

Rates of net utilization/production of AA after the 3-h incubation were calculated on the basis of differences in AA concentrations between initial (0 h) and final (3 h) incubation media. Data were analyzed by one-way ANOVA and the general linear model procedure to evaluate the effects of doses of arginine on the net utilization/production of AA in the media by pig small-intestinal bacteria. Statistical analyses were performed using SAS (SAS Institute, Cary, NC). P values ≤ 0.05 indicate statistical significance.

Results

Bacterial utilization of the arginine-family of amino acids

The pig small-intestinal bacteria utilized arginine in a dose-dependent manner (Table 2). The addition of arginine to the media reduced ($P < 0.05$) citrulline and proline utilization in *Streptococcus* sp. after the 3-h incubation. However, the utilization of glutamine increased ($P < 0.05$) with the addition of arginine in *Streptococcus* sp. and a dose effect was observed only with extracellular arginine at 1 mmol/L and below. The production of ornithine and glutamate increased ($P < 0.05$) in *Streptococcus* sp. with the addition of arginine to the media. In *E. coli*, the addition of arginine to the culture reduced ($P < 0.05$) the bacterial utilization of citrulline and ornithine. Net production of ornithine was observed in *E. coli* with arginine doses at 1 mmol/L and above. The net utilization of glutamine and glutamate increased ($P < 0.05$) in cultures of *E. coli* in the presence of arginine. Net utilization of citrulline decreased ($P < 0.05$) with the increased doses of arginine in *Klebsiella* sp. However, the net utilization of glutamine by cultures of *Klebsiella* sp. increased ($P < 0.05$) with the addition of arginine.

For mixed bacterial cultures, the net utilization of citrulline and ornithine by jejunal mixed bacteria decreased ($P < 0.05$) with the addition of arginine after 3 h of incubation (Table 2). Net production of glutamate was observed in jejunal mixed bacteria with arginine doses at 1 mmol/L and below. In the presence of arginine, the net utilization of ornithine by ileal mixed bacteria decreased ($P < 0.05$). The net utilization of citrulline also decreased ($P < 0.05$) in ileal mixed bacteria with increased doses of arginine and net production of citrulline was observed with arginine doses at 2 mmol/L and above. Similarly, net utilization of glutamate by ileal mixed bacteria decreased with the increased doses of arginine and the production of glutamate was observed with arginine doses at 1 mmol/L and above.

Effects of arginine on the bacterial utilization of the serine- and aspartate-family of amino acids

Complete utilization of asparagine in culture medium was observed in *Streptococcus* sp. and *E. coli* after a 3-h period

Table 2 Effects of L-arginine on the utilization of the arginine-family of AA by pig small-intestinal bacteria

L-Arginine (mmol/L)	*Streptococcus* sp. [nmol/(10^8 cells 3 h)]	*Escherichia coli* [nmol/(10^8 cells 3 h)]	*Klebsiella* sp. [nmol/(10^8 cells 3 h)]	Jejunal bacteria [nmol/(10^8 cells 3 h)]	Ileal bacteria [nmol/(10^8 cells 3 h)]
L-Arginine					
0	ND	ND	ND	ND	ND
0.5	-17.4 ± 1.5^{cC}	-170.9 ± 4.0^{aC}	-66.1 ± 1.6^{bD}	-14.2 ± 1.3^{cD}	-18.6 ± 2.0^{cC}
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^{dC}
2	-54.9 ± 6.0^{cB}	-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 ± 15.3^{bA}	-331.0 ± 4.3^{aA}	-133.1 ± 1.8^{cA}	-58.5 ± 2.5^{dA}
L-Citrulline					
0	-8.1 ± 0.2^{cdA}	-11.3 ± 2.8^{bcAB}	-25.8 ± 0.6^{aA}	-7.2 ± 0.2^{dA}	-13.0 ± 0.2^{bA}
0.5	-2.8 ± 0.5^{dBC}	-13.3 ± 1.5^{bA}	-24.5 ± 0.9^{aA}	-3.4 ± 0.2^{dB}	-10.0 ± 0.6^{cB}
1	-3.7 ± 0.9^{cB}	-6.7 ± 0.7^{bBC}	-16.9 ± 1.3^{aB}	-3.3 ± 0.2^{cB}	-1.5 ± 0.4^{cC}
2	-3.9 ± 0.5^{bcB}	-6.5 ± 1.7^{abBC}	-8.9 ± 0.5^{aC}	-3.2 ± 0.4^{cB}	$+3.3 \pm 0.5^{dD}$
5	-2.1 ± 0.2^{aC}	-3.5 ± 1.7^{aC}	-2.8 ± 0.7^{aD}	-4.4 ± 0.6^{aB}	$+6.2 \pm 1.4^{bE}$
L-Glutamate					
0	$+50.1 \pm 0.7^{dA}$	-23.1 ± 1.6^{aB}	$+172.8 \pm 9.8^{eC}$	$+10.4 \pm 0.6^{cC}$	-9.4 ± 1.3^{bA}
0.5	$+68.1 \pm 3.3^{cB}$	-197.6 ± 10.8^{aA}	$+96.9 \pm 9.0^{dB}$	$+9.7 \pm 0.5^{bC}$	-5.8 ± 1.6^{bA}
1	$+68.9 \pm 6.3^{cB}$	-215.0 ± 16.1^{aA}	$+47.9 \pm 1.8^{cA}$	$+8.6 \pm 1.3^{bC}$	$+22.7 \pm 0.6^{bB}$
2	$+104.0 \pm 9.6^{dC}$	-215.0 ± 20.8^{aA}	$+49.1 \pm 6.0^{cA}$	-6.5 ± 0.4^{bB}	$+27.2 \pm 3.7^{cB}$
5	$+111.4 \pm 4.3^{eC}$	-233.1 ± 13.0^{aA}	$+47.6 \pm 4.0^{dA}$	-10.5 ± 1.6^{bA}	$+27.3 \pm 1.7^{cB}$
L-Glutamine					
0	-67.1 ± 2.8^{cC}	-62.6 ± 7.2^{cB}	-118.9 ± 2.2^{aB}	-73.3 ± 1.1^{c}	-97.4 ± 1.7^{b}
0.5	-110.0 ± 3.9^{cB}	-519.2 ± 3.2^{aA}	-245.2 ± 3.2^{bA}	-69.9 ± 3.5^{e}	-89.4 ± 1.0^{d}
1	-151.8 ± 11.5^{cA}	-510.5 ± 7.7^{aA}	-246.9 ± 4.4^{bA}	-69.3 ± 2.2^{d}	-89.2 ± 3.1^{d}
2	-146.3 ± 4.8^{cA}	-492.7 ± 5.6^{aA}	-233.6 ± 3.7^{bA}	-73.0 ± 4.2^{d}	-85.5 ± 7.9^{d}
5	-154.6 ± 1.0^{cA}	-510.8 ± 9.9^{aA}	-243.2 ± 5.7^{bA}	-67.8 ± 3.2^{d}	-82.6 ± 8.3^{d}
L-Ornithine					
0	$+14.6 \pm 1.5^{dA}$	-43.6 ± 3.9^{aA}	-3.3 ± 0.9^{cA}	-32.4 ± 0.2^{bA}	-44.3 ± 1.1^{aA}
0.5	$+15.3 \pm 1.4^{dA}$	-36.7 ± 2.1^{aA}	-2.9 ± 0.8^{cA}	-27.0 ± 1.2^{bB}	-39.5 ± 0.5^{aB}
1	$+20.0 \pm 2.1^{cB}$	$+19.6 \pm 3.0^{cB}$	-4.3 ± 1.3^{bA}	-28.6 ± 0.6^{aB}	-32.9 ± 1.2^{aC}
2	$+23.5 \pm 2.1^{dB}$	$+16.8 \pm 1.6^{cB}$	-4.9 ± 1.4^{bA}	-28.3 ± 1.7^{aB}	-30.9 ± 2.4^{aC}
5	$+20.6 \pm 2.7^{cB}$	$+21.3 \pm 2.8^{cB}$	$+7.5 \pm 0.8^{bB}$	-27.1 ± 1.1^{aB}	-31.7 ± 1.3^{aC}
L-Proline					
0	-89.4 ± 4.5^{cA}	-162.7 ± 8.3^{aB}	-106.6 ± 4.8^{b}	-25.8 ± 0.2^{d}	-23.7 ± 1.6^{d}
0.5	-81.1 ± 2.5^{cA}	-213.0 ± 3.6^{aA}	-95.5 ± 5.2^{b}	-23.9 ± 0.9^{d}	-22.1 ± 0.8^{d}
1	-52.9 ± 1.8^{cB}	-208.7 ± 5.8^{aA}	-98.7 ± 4.6^{b}	-24.1 ± 2.5^{d}	-19.9 ± 1.1^{d}
2	-45.1 ± 3.5^{cB}	-193.0 ± 2.0^{aA}	-98.8 ± 1.2^{b}	-24.9 ± 1.0^{d}	-18.4 ± 1.0^{e}
5	-46.4 ± 3.3^{cB}	-190.5 ± 8.9^{aA}	-87.8 ± 2.4^{b}	-19.9 ± 2.7^{d}	-18.3 ± 1.3^{d}

Values are means ± SEM, $n = 4$. a–e: means in a row with superscripts without a common letter differ, $P < 0.05$; A–E: Means in a column with superscripts without a common letter differ, $P < 0.05$. Bacteria were incubated in the presence of 0–5 mmol/L L-arginine for 3 h. "−" denotes utilization, "+" denotes production

ND not detectable

of incubation regardless of the addition of arginine (Table 3). However, the complete utilization of asparagine in the cultures of jejunal mixed bacteria was only observed with the addition of arginine. Complete utilization of aspartate was observed in the cultures of *E. coli* after 3 h of incubation, while complete utilization of aspartate by *Klebsiella* sp. was observed only with the addition of arginine. After 3 h of incubation, serine was completely utilized in the cultures of *Streptococcus* sp., *E. coli* and *Klebsiella* sp. regardless of the addition of arginine.

When compared with the control group, net utilization of alanine in cultures of *Streptococcus* sp. doubled with

the arginine doses at 1 mmol/L and above (Table 3). The net utilization of glycine and threonine by *Streptococcus* sp. increased ($P < 0.05$) with the increased doses of arginine. In contrast, the net utilization of threonine by *E. coli* decreased ($P < 0.05$) after the 3-h incubation with arginine doses at 0.5 mmol/L and above. Net utilization of alanine, asparagine, glycine and threonine increased ($P < 0.05$) in cultures of *Klebsiella* sp. with the addition of arginine. The addition of arginine to the culture medium reduced the net utilization of alanine, glycine and threonine by jejunal mixed bacteria ($P < 0.05$). After 3 h of incubation, the net utilization of serine by jejunal mixed bacteria increased ($P < 0.05$) with the addition of arginine. In ileal mixed bacteria, the net utilization of asparagine, aspartate and glycine decreased ($P < 0.05$) with the addition of arginine, while reductions in the net utilization of alanine, serine and threonine were observed with arginine doses at 1 mmol/L and above ($P < 0.05$).

Effects of arginine on the bacterial utilization of branched-chain amino acids

The utilization of isoleucine deceased ($P < 0.05$) with the addition of arginine to the cultures of *E. coli*, jejunal mixed bacteria or ileal mixed bacteria, and a dose-dependent effect was observed only in *E. coli* (Table 4). An increase in the net utilization of isoleucine by *Streptococcus* sp. or *Klebsiella* sp. was observed with the addition of arginine. After a 3-h period of incubation, the net utilization of leucine by *E. coli*, jejunal mixed bacteria or ileal mixed bacteria decreased ($P < 0.05$) with the addition of arginine. In contrast, the utilization of leucine by *Streptococcus* sp. or *Klebsiella* sp. increased ($P < 0.05$) with the addition of arginine. The addition of arginine reduced the utilization of valine by *E. coli* or ileal mixed bacteria ($P < 0.05$) after 3 h of incubation. In contrast, the utilization of valine by *Streptococcus* sp. and *Klebsiella* sp. increased ($P < 0.05$) with the addition of arginine.

Effects of arginine on the bacterial utilization of sulfur amino acids

The net utilization of cystine decreased ($P < 0.05$) in *Streptococcus* sp. or ileal mixed bacteria with the addition of arginine (Table 5). Likewise, reduced ($P < 0.05$) net utilization of cystine by *E. coli* or *Klebsiella* sp. was observed with arginine doses at 2 and 5 mmol/L. Similarly, a decrease ($P < 0.05$) in the utilization of methionine by *E. coli* was observed only with arginine doses at 2 mmol/L and above. The utilization of methionine by *Streptococcus* sp. increased ($P < 0.05$) with arginine doses at 1 mmol/L and above.

Effects of arginine on the bacterial utilization of aromatic amino acids

The utilization of phenylalanine by ileal mixed bacteria decreased ($P < 0.05$) with arginine doses at 1 mmol/L and above (Table 6). However, utilization of phenylalanine increased ($P < 0.05$) in *Streptococcus* sp. or *Klebsiella* sp. with the addition of arginine. The addition of arginine reduced ($P < 0.05$) the net utilization of tryptophan in *Klebsiella* sp. after the 3-h incubation. Interestingly, the net utilization of tryptophan by jejunal mixed bacteria increased ($P < 0.05$) with the addition of arginine. Arginine doses at 2 and 5 mmol/L reduced ($P < 0.05$) the net utilization of tyrosine in ileal mixed bacteria. After 3 h of incubation, an increase in the utilization of tyrosine was observed in *Streptococcus* sp., *E. coli* or *Klebsiella* sp. with the addition of arginine.

Effects of arginine on the bacterial utilization of lysine and histidine

The net utilization of lysine by *Streptococcus* sp., jejunal mixed bacteria or ileal mixed bacteria decreased with the addition of arginine (Table 7). The net utilization of lysine increased ($P < 0.05$) in *E. coli* cultures with the arginine doses at 2 and 5 mmol/L. The net utilization of histidine decreased in the cultures of *E. coli* with the arginine dose at 1 mmol/L and above. In contrast, net utilization of histidine increased ($P < 0.05$) in *Streptococcus* sp. with the increased doses of arginine.

Discussion

Little is known about arginine metabolism or function in small-intestinal bacteria. Here, we report that the utilization of arginine was dose-dependent in different bacterial species and mixed bacterial cultures derived from pig small intestine (Table 2). The results are consistent with our previous study which showed rapid utilization of arginine by mixed small-intestinal bacteria during the 24-h incubation (Dai et al. 2010). Furthermore, this study indicated that rates of arginine utilization varied greatly among bacterial species and mixed bacterial cultures. The large amount of arginine utilized by *E. coli* and *Klebsiella* sp. suggests that although the numbers of *E. coli* and *Klebsiella* sp. are low in the small intestine of healthy pigs, these bacteria may contribute substantially to AA metabolism in the lumen of the small intestine. Although the populations or activities of bacteria and composition of individual species in the mixed bacterial culture may change during in vitro incubation with different AA and over time (Russell 1991, 1993; Rychlik et al. 2002), bacteria responsible for

Table 3 Effects of L-arginine on the utilization of the serine- and aspartate-family of AA by pig small-intestinal bacteria

L-Arginine (mmol/L)	*Streptococcus* sp. [nmol/(10^8 cells 3 h)]	*Escherichia coli* [nmol/(10^8 cells 3 h)]	*Klebsiella* sp. [nmol/(10^8 cells 3 h)]	Jejunal bacteria [nmol/(10^8 cells 3 h)]	Ileal bacteria [nmol/(10^8 cells 3 h)]
L-Alanine					
0	-15.6 ± 0.9^{dB}	-75.5 ± 6.2^{aB}	-20.2 ± 1.8^{dB}	-39.3 ± 1.3^{cA}	-54.4 ± 1.4^{bA}
0.5	-12.8 ± 1.7^{eB}	-166.2 ± 3.8^{aA}	-42.3 ± 4.0^{cA}	-33.4 ± 2.0^{dB}	-51.5 ± 1.5^{bA}
1	-27.3 ± 4.1^{cA}	-165.0 ± 6.3^{aA}	-42.6 ± 1.7^{bA}	$-32.9 \pm 2.0b^{cB}$	-44.2 ± 4.4^{bB}
2	-31.5 ± 4.2^{bA}	-158.9 ± 6.2^{aA}	-38.6 ± 4.6^{bA}	-31.7 ± 0.9^{bB}	-40.5 ± 2.1^{bB}
5	-29.3 ± 0.7^{cA}	-150.3 ± 0.7^{aA}	-39.9 ± 2.5^{bA}	-32.0 ± 1.4^{cB}	-39.5 ± 1.5^{bB}
L-Asparagine					
0	-122.4 ± 0.0^{1b}	-520.3 ± 0.0^{1a}	-53.2 ± 3.7^{cC}	-34.6 ± 0.8^{eB}	-47.1 ± 2.2^{dA}
0.5	-125.7 ± 0.0^{1b}	-473.5 ± 0.0^{1a}	-92.0 ± 4.0^{cB}	-75.1 ± 0.0^{2dA}	-48.8 ± 0.6^{eA}
1	-132.4 ± 0.0^{1b}	-483.3 ± 0.0^{1a}	-106.9 ± 2.6^{cA}	-74.8 ± 0.0^{1dA}	-33.2 ± 4.0^{eB}
2	-127.3 ± 0.0^{1b}	-454.3 ± 0.0^{1a}	-110.5 ± 3.6^{cA}	-76.2 ± 0.0^{2dA}	-33.2 ± 3.1^{eB}
5	-118.7 ± 0.0^{1b}	-437.8 ± 0.0^{1a}	-115.0 ± 4.2^{bA}	-73.3 ± 0.0^{1cA}	-37.1 ± 2.1^{dB}
L-Aspartate					
0	-17.0 ± 1.5^{dB}	-1043.9 ± 0.0^{1a}	-218.2 ± 2.2^{bD}	$+12.5 \pm 1.2^{e}$	-54.4 ± 4.4^{cA}
0.5	-22.0 ± 0.6^{dB}	-995.2 ± 0.0^{1a}	-262.1 ± 0.0^{1bC}	$+14.7 \pm 0.9^{e}$	-40.0 ± 0.6^{cB}
1	-39.1 ± 2.3^{cA}	-983.7 ± 0.0^{1a}	-285.0 ± 0.0^{1bB}	$+11.5 \pm 0.9^{e}$	-29.4 ± 3.5^{dB}
2	-40.2 ± 5.7^{cA}	-959.3 ± 0.0^{1a}	-284.4 ± 0.0^{1bB}	$+11.6 \pm 3.6^{d}$	-30.1 ± 4.3^{cB}
5	-46.4 ± 2.0^{cA}	-941.2 ± 0.0^{1a}	-302.0 ± 0.0^{1bA}	$+15.0 \pm 1.9^{e}$	-31.2 ± 1.5^{dB}
Glycine					
0	-9.1 ± 1.8^{dB}	-174.0 ± 8.6^{a}	-56.6 ± 2.6^{bB}	-37.2 ± 1.4^{cA}	-41.5 ± 1.7^{cA}
0.5	-13.0 ± 2.5^{dB}	-178.8 ± 4.5^{a}	-111.1 ± 4.0^{bA}	-34.3 ± 1.3^{cAB}	-20.1 ± 0.9^{cB}
1	-56.6 ± 4.1^{cA}	-194.2 ± 7.6^{a}	-125.9 ± 5.5^{bA}	-33.1 ± 1.4^{dB}	-22.4 ± 1.9^{dB}
2	-61.9 ± 2.0^{cA}	-188.2 ± 8.8^{a}	-127.2 ± 5.8^{bA}	-31.4 ± 1.3^{dBC}	-20.2 ± 0.8^{dB}
5	-58.7 ± 2.7^{cA}	-194.2 ± 8.0^{a}	-121.4 ± 2.0^{bA}	-27.8 ± 0.5^{dC}	-23.2 ± 1.1^{dB}
L-Serine					
0	-216.7 ± 0.0^{1c}	-979.3 ± 0.0^{1a}	-348.4 ± 0.0^{1b}	-23.4 ± 0.7^{eC}	-55.2 ± 2.5^{dA}
0.5	-224.3 ± 0.0^{1c}	-883.5 ± 0.0^{1a}	-342.9 ± 0.0^{1b}	-33.9 ± 1.0^{eB}	-51.2 ± 1.5^{dA}
1	-244.6 ± 0.0^{1c}	-814.3 ± 0.0^{1a}	-351.8 ± 0.0^{1b}	-30.4 ± 0.6^{dB}	-20.2 ± 2.0^{eB}
2	-239.6 ± 0.0^{1c}	-779.5 ± 0.0^{1a}	-322.8 ± 0.0^{1b}	-35.7 ± 1.0^{dB}	-19.3 ± 0.4^{eB}
5	-228.7 ± 0.0^{1c}	-753.8 ± 0.0^{1a}	-340.8 ± 0.0^{1b}	-39.2 ± 0.9^{dA}	-19.5 ± 1.0^{eB}
L-Threonine					
0	-13.4 ± 1.3^{cC}	-288.1 ± 13.9^{aA}	-39.6 ± 1.4^{bB}	-24.7 ± 0.6^{bcA}	-42.6 ± 2.0^{bA}
0.5	-44.0 ± 4.4^{cB}	-299.5 ± 4.5^{aA}	-94.6 ± 2.4^{bA}	-20.4 ± 0.5^{dB}	-38.2 ± 0.4^{cA}
1	-79.7 ± 11.8^{bA}	-234.3 ± 12.3^{aB}	-97.3 ± 1.2^{bA}	-19.6 ± 1.5^{cB}	-18.8 ± 2.8^{cB}
2	-86.5 ± 7.4^{bA}	-158.1 ± 7.7^{aC}	-93.5 ± 1.9^{bA}	-20.6 ± 1.6^{cB}	-21.1 ± 0.4^{cB}
5	-82.1 ± 10.5^{bA}	-130.3 ± 18.6^{aC}	-92.3 ± 1.7^{bA}	-21.4 ± 0.7^{cB}	-17.6 ± 1.1^{cB}

Values are means ± SEM, $n = 4$. a–e: Means in a row with superscripts without a common letter differ, $P < 0.05$; A–D: means in a column with superscripts without a common letter differ, $P < 0.05$. Bacteria were incubated in the presence of 0–5 mmol/L L-arginine for 3 h. "−" denotes utilization, "+" denotes production

[1] Complete utilization of the corresponding amino acid was observed after 3 h of incubation

the utilization and metabolism of AA may be adapted and enriched during in vitro incubation. Thus, the notion of low number and high activity for bacterial metabolism should be considered (Wallace 1996). Except for growth and ATP generation, the function of the extensive utilization of arginine in pig small-intestinal bacteria, especially the potential pathogenic bacteria, is largely unknown, although a role for arginine in acid resistance and production of virulence factors has been proposed (Richard and Foster 2004; Dong and Schellhorn 2009). Thus, the current work focused on the regulatory role of arginine on AA utilization in bacteria isolated from the pig small intestine.

Results from this study indicate that the arginine-dependent reduction in the net utilization or the stimulation

Table 4 Effects of L-arginine on the utilization of branched-chain AA by pig small-intestinal bacteria

L-Arginine (mmol/L)	*Streptococcus* sp. [nmol/(10^8 cells 3 h)]	*Escherichia coli* [nmol/(10^8 cells 3 h)]	*Klebsiella* sp. [nmol/(10^8 cells 3 h)]	Jejunal bacteria [nmol/(10^8 cells 3 h)]	Ileal bacteria [nmol/(10^8 cells 3 h)]
L-Isoleucine					
0	-2.4 ± 0.3^{dC}	-77.3 ± 1.3^{aA}	-18.2 ± 2.8^{cB}	-17.2 ± 1.1^{cA}	-35.2 ± 1.8^{bA}
0.5	-8.2 ± 0.7^{dB}	-62.5 ± 6.5^{aAB}	-23.9 ± 2.1^{cAB}	-11.8 ± 1.5^{dB}	-34.7 ± 0.6^{bA}
1	-13.1 ± 1.8^{cA}	-49.5 ± 1.7^{aBC}	-35.0 ± 5.3^{bA}	-11.1 ± 0.9^{cB}	-18.7 ± 2.9^{cB}
2	-14.4 ± 1.4^{cA}	-46.6 ± 5.7^{aC}	-32.6 ± 1.6^{bA}	-12.9 ± 1.2^{cB}	-21.5 ± 1.4^{cB}
5	-13.7 ± 0.5^{cA}	-24.8 ± 6.8^{bD}	-32.3 ± 1.1^{aA}	-13.5 ± 0.8^{cB}	-20.5 ± 1.3^{bcB}
L-Leucine					
0	-3.0 ± 1.4^{dB}	-97.1 ± 7.7^{aA}	-17.2 ± 5.1^{cB}	-27.5 ± 2.3^{cA}	-54.4 ± 1.6^{bA}
0.5	-9.8 ± 2.7^{dA}	-98.1 ± 6.0^{aA}	-26.0 ± 4.1^{cB}	-18.7 ± 2.6^{cdB}	-44.9 ± 2.6^{bB}
1	-9.6 ± 1.6^{cA}	-64.7 ± 3.3^{aB}	-62.3 ± 5.0^{aA}	-17.9 ± 1.1^{cB}	-28.5 ± 1.4^{bC}
2	-11.7 ± 1.5^{dA}	-66.4 ± 2.7^{aB}	-57.5 ± 3.6^{bA}	-18.7 ± 1.5^{dB}	-29.1 ± 1.8^{cC}
5	-10.2 ± 0.7^{cA}	-66.8 ± 2.7^{aB}	-65.4 ± 2.0^{aA}	-20.9 ± 2.5^{bB}	-24.2 ± 1.8^{bC}
L-Valine					
0	-9.7 ± 1.4^{cB}	-102.1 ± 9.7^{aA}	-22.8 ± 1.9^{bcB}	-18.4 ± 1.4^{c}	-33.6 ± 1.1^{bA}
0.5	-14.0 ± 1.7^{cB}	-114.6 ± 5.5^{aA}	-29.6 ± 2.4^{bB}	-14.6 ± 0.6^{c}	-31.1 ± 1.3^{bA}
1	-23.2 ± 6.4^{cA}	-108.2 ± 17.1^{aA}	-49.2 ± 6.3^{bA}	-13.0 ± 1.9^{c}	-18.5 ± 3.0^{cB}
2	-23.6 ± 3.7^{bA}	-64.7 ± 14.8^{aB}	-48.0 ± 1.7^{aA}	-15.3 ± 1.6^{b}	-18.7 ± 3.2^{bB}
5	-23.0 ± 1.0^{bA}	-60.4 ± 8.9^{aB}	-50.1 ± 1.2^{aA}	-15.4 ± 1.5^{b}	-20.1 ± 1.9^{bB}

Values are means ± SEM, $n = 4$. a–e: Means in a row with superscripts without a common letter differ, $P < 0.05$; A–D means in a column with superscripts without a common letter differ, $P < 0.05$. Bacteria were incubated in the presence of 0–5 mmol/L L-arginine for 3 h. "−" denotes utilization

Table 5 Effects of L-arginine on the utilization of sulfur AA by pig small-intestinal bacteria

L-Arginine (mmol/L)	*Streptococcus* sp. [nmol/(10^8 cells 3 h)]	*Escherichia coli* [nmol/(10^8 cells 3 h)]	*Klebsiella* sp. [nmol/(10^8 cells 3 h)]	Jejunal bacteria [nmol/(10^8 cells 3 h)]	Ileal bacteria [nmol/(10^8 cells 3 h)]
L-Cystine					
0	-6.8 ± 0.7^{cA}	-103.2 ± 5.4^{aA}	-33.7 ± 1.4^{bA}	-4.2 ± 0.7^{c}	-9.8 ± 0.7^{cA}
0.5	-5.0 ± 1.7^{cAB}	-99.3 ± 5.4^{aA}	-30.3 ± 1.0^{bA}	-3.5 ± 0.8^{c}	-6.2 ± 1.0^{cB}
1	-2.0 ± 0.5^{cB}	-97.0 ± 5.5^{aA}	-31.0 ± 1.3^{bA}	-5.0 ± 0.5^{c}	-6.6 ± 1.2^{cB}
2	-3.4 ± 0.7^{bB}	-87.6 ± 5.0^{aAB}	-10.5 ± 0.7^{bB}	-3.8 ± 0.6^{b}	-7.2 ± 1.2^{bB}
5	-2.1 ± 0.7^{cB}	-77.7 ± 4.5^{aB}	-12.3 ± 1.7^{bB}	-4.0 ± 0.9^{c}	-5.3 ± 0.6^{cB}
L-Methionine					
0	-5.4 ± 1.1^{dB}	-68.4 ± 2.4^{aA}	-10.2 ± 1.5^{cC}	-9.2 ± 0.4^{cd}	-14.7 ± 0.4^{bA}
0.5	-6.2 ± 1.3^{dB}	-79.9 ± 2.9^{aA}	-23.1 ± 1.0^{bB}	-8.4 ± 0.6^{d}	-14.9 ± 0.5^{cA}
1	-13.3 ± 1.0^{cA}	-70.6 ± 3.3^{aA}	-36.2 ± 0.9^{bA}	-7.2 ± 0.8^{d}	-9.1 ± 0.8^{cdB}
2	-13.6 ± 2.0^{cA}	-44.9 ± 1.6^{aB}	-25.5 ± 1.0^{bB}	-8.3 ± 0.6^{d}	-9.3 ± 0.8^{dB}
5	-10.7 ± 0.8^{cA}	-42.3 ± 7.3^{aB}	-26.7 ± 1.0^{bB}	-7.7 ± 1.0^{c}	-9.4 ± 0.4^{cB}

Values are means ± SEM, $n = 4$. a–e: Means in a row with superscripts without a common letter differ, $P < 0.05$; A–D: means in a column with superscripts without a common letter differ, $P < 0.05$. Bacteria were incubated in the presence of 0–5 mmol/L L-arginine for 3 h. "−" denotes utilization

of the net production of ornithine and citrulline in small-intestinal bacteria (Fig. 1) might occur through the upregulation of the metabolic fluxes from arginine to ornithine and citrulline (Fernández and Zúñiga 2006). It was previously reported that the arginase or arginine deiminase pathway was the main arginine catabolic pathway in lactic acid bacteria, leading to the production of ornithine, ammonia and CO_2 (Fernández and Zúñiga 2006). However, in *E. coli* and *Klebsiella aerogenes*, two pathways initiated by arginine decarboxylase and arginine succinyltransferase may be important for the catabolism of arginine (Shaibe et al. 1985; Schneider et al. 1998). The arginine

Table 6 Effects of L-arginine on the utilization of aromatic AA by pig small-intestinal bacteria

L-Arginine (mmol/L)	*Streptococcus* sp. [nmol/(10^8 cells 3 h)]	*Escherichia coli* [nmol/(10^8 cells 3 h)]	*Klebsiella* sp. [nmol/(10^8 cells 3 h)]	Jejunal bacteria [nmol/(10^8 cells 3 h)]	Ileal bacteria [nmol/(10^8 cells 3 h)]
L-Phenylalanine					
0	-0.7 ± 1.1^{cB}	-35.0 ± 4.4^{a}	-12.4 ± 1.2^{bB}	-11.5 ± 0.3^{b}	-30.3 ± 1.4^{aA}
0.5	-1.0 ± 0.8^{dB}	-52.1 ± 5.5^{a}	-25.8 ± 2.0^{bA}	-11.6 ± 0.9^{c}	-31.2 ± 0.4^{bA}
1	-16.2 ± 1.6^{cA}	-53.4 ± 4.4^{a}	-26.3 ± 2.2^{bA}	-11.8 ± 1.3^{c}	-17.4 ± 0.7^{cB}
2	-16.8 ± 1.7^{cA}	-53.2 ± 3.4^{a}	-25.3 ± 1.6^{bA}	-11.2 ± 0.7^{c}	-17.0 ± 2.4^{cB}
5	-16.4 ± 2.1^{bcA}	-47.3 ± 8.7^{a}	-28.1 ± 1.8^{bA}	-13.0 ± 0.5^{c}	-17.8 ± 1.9^{bcB}
L-Tryptophan					
0	-2.2 ± 0.4^{d}	-35.7 ± 2.7^{a}	-24.4 ± 1.5^{bA}	-2.3 ± 0.8^{dC}	-11.4 ± 1.4^{c}
0.5	-2.3 ± 0.4^{c}	-27.7 ± 7.6^{a}	-15.6 ± 0.3^{bB}	-5.4 ± 0.5^{bcB}	-9.9 ± 0.5^{bc}
1	-3.7 ± 1.1^{b}	-26.1 ± 9.1^{a}	-8.5 ± 0.5^{bCD}	-4.9 ± 0.5^{bB}	-9.6 ± 0.5^{b}
2	-4.1 ± 0.6^{b}	-24.6 ± 10.1^{a}	-9.1 ± 1.1^{bC}	-5.1 ± 0.5^{bB}	-8.9 ± 0.4^{b}
5	-3.7 ± 0.5^{b}	-24.0 ± 8.7^{a}	-5.6 ± 1.4^{bD}	-8.2 ± 0.8^{bA}	-9.4 ± 0.3^{b}
L-Tyrosine					
0	-14.8 ± 0.4^{bB}	-31.4 ± 7.2^{aB}	-8.1 ± 1.2^{bD}	-13.7 ± 2.2^{b}	-19.6 ± 2.4^{bA}
0.5	-18.4 ± 1.6^{bB}	-95.6 ± 4.0^{aA}	-16.4 ± 2.2^{bC}	-20.0 ± 3.8^{b}	-18.3 ± 0.8^{bA}
1	-36.5 ± 2.4^{bA}	-93.3 ± 6.7^{aA}	-31.7 ± 3.3^{bB}	-20.5 ± 3.4^{c}	-16.7 ± 1.6^{cA}
2	-33.2 ± 1.4^{bA}	-102.5 ± 11.4^{aA}	-29.8 ± 1.9^{bB}	-24.0 ± 4.4^{bc}	-11.9 ± 0.6^{cB}
5	-33.3 ± 2.8^{cA}	-102.6 ± 1.6^{aA}	-75.0 ± 1.4^{bA}	-22.3 ± 2.4^{d}	-11.9 ± 0.9^{eB}

Values are means ± SEM, $n = 4$. a–e: Means in a row with superscripts without a common letter differ, $P < 0.05$; A–D: means in a column with superscripts without a common letter differ, $P < 0.05$. Bacteria were incubated in the presence of 0–5 mmol/L L-arginine for 3 h. "−" denotes utilization

Table 7 Effects of L-arginine on the utilization of lysine and histidine by pig small-intestinal bacteria

L-Arginine (mmol/L)	*Streptococcus* sp. [nmol/(10^8 cells 3 h)]	*Escherichia coli* [nmol/(10^8 cells 3 h)]	*Klebsiella* sp. [nmol/(10^8 cells 3 h)]	Jejunal bacteria [nmol/(10^8 cells 3 h)]	Ileal bacteria [nmol/(10^8 cells 3 h)]
L-Histidine					
0	-4.2 ± 0.1^{cD}	-164.5 ± 14.3^{aA}	-43.6 ± 2.8^{b}	-17.3 ± 0.2^{c}	-21.8 ± 0.6^{c}
0.5	-9.8 ± 2.0^{dC}	-154.9 ± 5.5^{aA}	-47.4 ± 1.5^{b}	-18.2 ± 0.7^{c}	-24.9 ± 1.1^{c}
1	-22.1 ± 1.1^{cdB}	-106.9 ± 2.3^{aB}	-48.0 ± 2.3^{b}	-17.2 ± 0.3^{d}	-23.7 ± 1.1^{c}
2	-24.8 ± 1.0^{cB}	-106.2 ± 4.4^{aB}	-50.4 ± 5.0^{b}	-17.7 ± 0.5^{c}	-24.1 ± 1.5^{c}
5	-39.0 ± 2.9^{cA}	-106.6 ± 5.2^{aB}	-48.4 ± 1.4^{b}	-18.1 ± 1.1^{d}	-23.8 ± 2.7^{d}
L-Lysine					
0	-91.4 ± 0.9^{bA}	-79.7 ± 4.6^{cB}	-185.5 ± 1.4^{a}	-39.2 ± 0.6^{eA}	-57.0 ± 1.8^{dA}
0.5	-52.6 ± 1.7^{cB}	-89.2 ± 7.9^{bB}	-184.7 ± 4.6^{a}	-32.9 ± 0.4^{dB}	-54.9 ± 1.9^{cA}
1	-48.4 ± 2.2^{cB}	-82.9 ± 2.7^{bB}	-184.1 ± 2.2^{a}	-32.4 ± 1.4^{dB}	-22.9 ± 2.6^{eB}
2	-48.9 ± 2.7^{cB}	-128.7 ± 2.3^{bA}	-186.3 ± 2.7^{a}	-33.8 ± 2.3^{dB}	-18.8 ± 1.8^{eB}
5	-47.5 ± 2.3^{cB}	-131.2 ± 5.0^{bA}	-184.4 ± 7.0^{a}	-33.8 ± 1.5^{dB}	-19.5 ± 1.7^{eB}

Values are means ± SEM, $n = 4$. a–e: Means in a row with superscripts without a common letter differ, $P < 0.05$; A–D: means in a column with superscripts without a common letter differ, $P < 0.05$. Bacteria were incubated in the presence of 0–5 mmol/L L-arginine for 3 h. "−" denotes utilization

decarboxylation pathway produces urea and putrescine, while the arginine succinyltransferase pathway generates glutamate from the transfer of the amino group to succinyl-CoA (Shaibe et al. 1985; Schneider et al. 1998). The reduced utilization of citrulline and increased production of ornithine in *Streptococcus* sp., *E. coli* and *Klebsiella* sp. in response to elevated levels of extracellular arginine (Table 2) suggest that arginase and arginine deiminase pathways exist in the bacteria. It is now known that the nitric oxide (NO) produced from arginine by intestinal mucosal cells can kill pathogenic bacteria (Wu et al. 2009; Deitch et al. 1995; Witthöft et al. 1998; Resta-Lenert and Barrett 2002). Therefore, arginine metabolism and the production of corresponding metabolites in the luminal

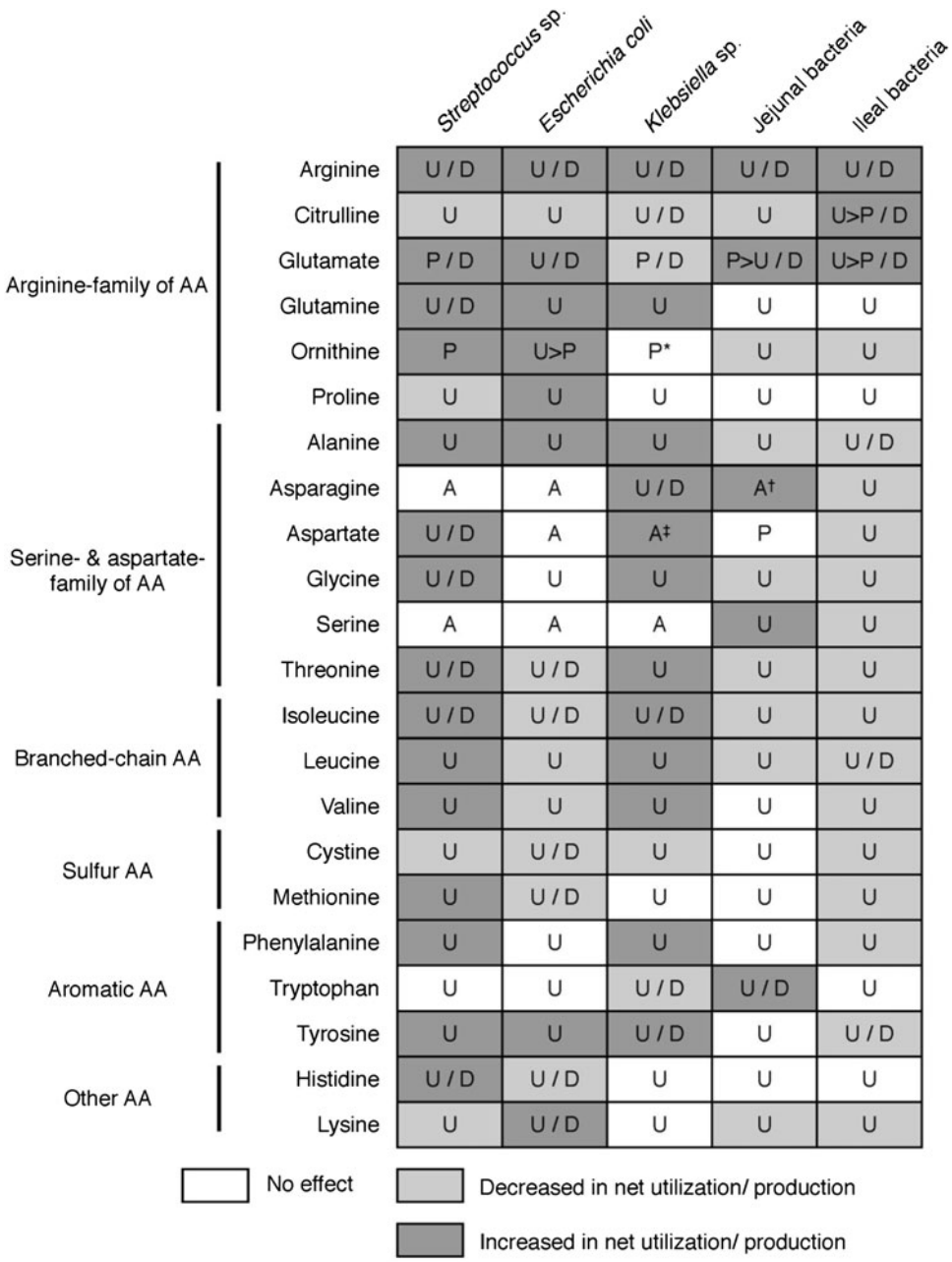

Fig. 1 Overall effects of L-arginine on the utilization or production of amino acids in small-intestinal bacteria. *A* complete utilization, *D* dose dependent, *P* net production, *U* net utilization; U > P/P > U, shifts between net utilization and net production over time; *production of ornithine in *Klebsiella* sp. was observed only in the presence of 5 mmol/L L-arginine after 3 h of incubation, †complete utilization of asparagine in culture of jejunal mixed bacteria was only observed in the presence of arginine after 3 h of incubation, ‡complete utilization of aspartate in cultures of *Klebsiella* sp. was observed only in the presence of arginine after 3 h of incubation

bacteria might reduce arginine availability for the synthesis of NO and regulate the metabolism of the arginine-family of AA in small intestinal mucosal cells, thereby indirectly affecting NO synthesis. Thus, the metabolism of arginine by small-intestinal bacteria not only plays a crucial role in the growth of the bacteria but also may be regarded as a surviving strategy for their colonization in the small intestine (Dai et al. 2011b).

A novel and important observation of this study is that arginine affected the metabolism of glutamate and glutamine in small-intestinal bacteria. The increases in net utilization of glutamine and net production of glutamate in *Streptococcus* sp. with increased doses of arginine suggest that arginine stimulates the glutaminase pathway for the conversion of glutamine to glutamate in the bacteria (Fernández and Zúñiga 2006). Additionally, the increases in net production of glutamate with increased arginine doses in *Streptococcus* sp. indicated the presence of the arginine succinyltransferase pathway in the bacteria (Schneider et al. 1998). Moreover, the increase in net utilization of glutamate in *E. coli* and the decrease in net production of glutamate in *Klebsiella* sp. suggest that other factors such as carbohydrate concentration and nitrogen source availability may affect arginine catabolism in the two bacterial species (Schneider et al. 1998). The catabolism of arginine and glutamate/glutamine and the

production of CO_2 and ammonia by intestinal bacteria may function as a strategy for these cells to survive in low pH (Fernández and Zúñiga 2006). For example, it has been reported that two acid resistance systems (AR) namely the AR2 and AR3 in *E. coli* can be induced by low pH (Richard and Foster 2004). The AR2 requires extracellular glutamate, while the AR3 requires extracellular arginine (Richard and Foster 2004). Therefore, our results suggest that the AR2 and AR3 may coordinate to regulate the transport and catabolism of AA such as glutamate and arginine in response to acid stress. Regarding the importance of arginine and glutamine/glutamate to the nitrogen balance in intestinal bacteria, the metabolism of the arginine-family of AA may also be related to nitrogen metabolism in the gut. However, till date, it remains unknown whether the metabolism of the arginine-family of AA in small-intestinal bacteria contributes to nitrogen cycling in the small intestine (Fuller and Reeds 1998; Bergen and Wu 2009).

Another novel finding of this study is the high requirements of serine and aspartate in small-intestinal bacteria (Table 3). Our results support the previous findings that serine was rapidly metabolized by *E. coli* and that the growth of *Streptococcus* sp. or *Klebsiella* sp. was stimulated in the presence of serine (Prüß et al. 1994; Chaussee et al. 2003; Vining and Magasanik 1981). The major products from the catabolism of serine were pyruvate and ammonia, which could serve as energy and nitrogen sources for the growth of bacteria (Fernández and Zúñiga 2006; Chaussee et al. 2003; Vining and Magasanik 1981; Sawers 1998). Of particular note, a recent study revealed that the utilization of serine and aspartate in pathogenic bacteria *Staphylococcus aureus* might be related to the production of phosphopeptides which contributed to the virulence of the bacteria (Burnside et al. 2010). Interestingly, in the present work, we found that complete utilization of aspartate was observed only in *E. coli* or *Klebsiella* sp. but not in *Streptococcus* sp., possibly due, in part, to differences in both rates of transport by the cell membrane and physiology between gram-positive and gram-negative bacteria. These findings indicate that serine and perhaps aspartate may be "essential" for the bacteria not only by serving as building blocks of cell components but also through participating in the synthesis of secretory molecules that may be important for the bacterial adaptation and colonization in the small intestine as well as interaction with the host (Lyte et al. 2011).

A salient and intriguing observation of this study is the high requirement of asparagine by *E. coli* and *Streptococcus* sp. (Table 2). The possible explanation is that the high usage of asparagine in *E. coli* may result, in part, from the high requirement of aspartate by the bacteria. The asparagine was catalyzed by the bacterial asparaginase to form aspartate (Fernández and Zúñiga 2006). A similar mechanism may also exist in *Streptococcus* sp. Thus, asparagine rather than aspartate may be the preferred substrate for the synthesis of bacterial components or secretory molecules. At present, the detailed biochemical mechanism is not known and further studies are warranted. Additionally, the utilization of asparagine and glutamine by *Klebsiella* sp. was enhanced in response to increased doses of arginine. This result suggests that the metabolism of asparagine and glutamine is closely related with each other and contributes to the production of aspartate in the bacteria (Fernández and Zúñiga 2006). Further studies are required to define the physiological role for the coordinated metabolism of arginine, asparagine and glutamine in small-intestinal bacteria.

Finally, our current results indicate that arginine regulates the compartmental metabolism of AA in the small intestine. In general, compared to jejunal mixed bacteria, the net utilization of most AA decreased in ileal mixed bacteria with the addition of arginine (Fig. 1). Thus, the bacterial utilization of most AA was suppressed by arginine in the ileal microbiota. In other words, the growth of the ileal microbiota may depend on arginine as a major source of nitrogen. Although the utilization of citrulline and ornithine decreased with the addition of arginine in both jejunal and ileal mixed bacteria, net production of citrulline was observed in ileal mixed bacteria (Table 2). Interestingly, in response to increased doses of arginine, the net utilization of glutamate increased in jejunal mixed bacteria yet the net production of glutamate was observed in ileal mixed bacteria. This is consistent with important roles for glutamate in regulating nitrogen balance in bacteria (Dai et al. 2011b). Thus the compartmental variations in the bacterial metabolism of glutamate plus citrulline in the small intestine may reflect the differences in the luminal pools of nitrogenous compound between the jejunum and the ileum, as well as the regulation of nitrogen recycling in the small intestine (Fuller and Reeds 1998; Claus et al. 2008). Also, increased utilization of asparagine and serine in jejunal mixed bacteria in the presence of elevated levels of arginine suggests that arginine plays an important role in supporting the growth and activity of asparagine- and serine-consuming bacteria (e.g. *Streptococcus* sp. and *E. coli*).

Collectively, results of the present study indicate variations in AA utilization and metabolism in porcine small-intestinal bacteria. The arginine regulation of AA metabolism in small-intestinal bacteria is dependent on bacterial species abundance and community composition. Extracellular concentrations of arginine affect the metabolic network of small-intestinal bacteria, which leads to alterations in the rates of AA utilization until a new balance is established for the optimal structure and function of the

microbial community (Almaas et al. 2004; Almaas 2007; Samal 2008). Furthermore, changes of AA metabolites in the intestinal lumen brought about by increased arginine availability would affect the transcriptome and proteome of the intestinal mucosal cells, thereby modulating the nutrition and physiology of mucosal cells and extraintestinal tissues. This view is supported by the findings that dietary supplementation with arginine improves small-intestinal morphology, function and growth, as well as whole-body weight gain in young pigs (Kim and Wu 2004; Tan et al. 2009; Wu et al. 2010). In vivo studies are required to uncover the metabolic interplay between the small-intestinal mucosa and the luminal microbiome with special emphasis on the functional aspects of dietary AA and gut microbiome (Bergen and Wu 2009; Wu 2009; Egert et al. 2006; He et al. 2009). This new knowledge will aid in the development of new strategies for the prevention of small intestine-related disorders and the improvement in nutrition and health of both humans and animals.

In conclusion, arginine plays an important role in modulating the metabolism of the arginine-family of AA as well as the serine- and aspartate-family of AA in pig small-intestinal bacteria in a species- and gut compartment-dependent manner. These novel findings suggest that supplemental dietary arginine could reduce the irreversible catabolism of dietary AA in the first-pass metabolism of the small intestine and affect the luminal pool of bacterial nitrogenous compounds in the gut. Thus, arginine can regulate nitrogen recycling in the intestine to benefit nutrition and health of the organisms.

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Conflict of interest The authors declare that they have no conflict of interest.

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