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Amino acid metabolism in intestinal bacteria: links between gut ecology and host health

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TABLE OF CONTENTS

1. ABSTRACT

Bacteria in the gastrointestinal (GI) tract play an important role in the metabolism of dietary substances in the gut and extraintestinal tissues. Amino acids (AA) should be taken into consideration in the development of new strategies to enhance efficiency of nutrient utilization because they are not only major components in the diet and building blocks for protein but also regulate energy and protein homeostasis in organisms. The diversity of the AA-fermenting bacteria and their metabolic redundancy make them easier to survive and interact with their neighboring species or eukaryotic host during transition along GI tract. The outcomes of the interactions have important impacts on gut health and whole-body homeostasis. The AA-derived molecules produced by intestinal bacteria affect host health by regulating either host immunity and cell function or microbial composition and metabolism. Emerging evidence shows that dietary factors, such as protein, non-digestible carbohydrates, probiotics, synbiotics and phytochemicals, modulate AA utilization by gut microorganisms. Interdisciplinary research involving nutritionists and microbiologists is expected to rapidly expand knowledge about crucial roles for AA in gut ecology and host health.

2. INTRODUCTION

The digestive tract of humans and animals is colonized by dense and highly complex community of microorganisms composed mainly bacteria, whose total number can exceed 10^{14} cells (1). The activities of these bacteria have potential effects on host nutrition and health through the metabolism of dietary components and interaction with intestinal epithelial cells. Amino acids (AA), which are major nutrients in the diet, not only support the growth of bacteria and their host but also regulate energy and protein homeostasis in the body (2, 3). It is most probable that the interplay between gut bacteria and dietary nitrogenous substances (especially AA) have important impacts not only on the host's nutrition (e.g., AA homeostasis) and health but also on the efficiency of dietary AA supplementation (4-7). Recent development of new molecular techniques and concepts broadened our knowledge about the evolutionary and functional aspects of the complex microbial community in the GI tract (8-11). Therefore, AA metabolism in gut bacteria in complex environments ultimately provides a strategy for their survival and growth in the digestive tract, thereby having both positive and negative effects on the host (6, 12). This review highlights the diversity of intestinal bacteria and

their species-specific AA metabolism with a special emphasis on interactions among dietary composition, gut environments, and host health.

3. DIVERSITY AND ABUNDANCE OF THE AA-FERMENTING BACTERIA IN THE DIGESTIVE TRACT

The utilization of AA is widely distributed among bacteria residing in the digestive tract of humans and animals. Studies over the last decades mainly focused on AA fermentation in gut bacteria and showed the diversity of AA-fermenting bacteria in the digestive tract (Table 1). Among the AA-fermenting bacteria, strains belong to the *Clostridium* clusters (including *Clostridium* spp., *Fusobacterium* spp., *Peptostreptococcus* spp., *Veillonella* spp., *Megasphaera elsdenii*, *Acidaminococcus fermentans*, *Selenomonas ruminantium*) have been extensively investigated. They were identified to be the predominant AA-fermenting microbiota along the digestive tract of humans and animals. It was found that bacteria belonging to *Fusobacterium* spp., *Prevotella* spp. and *Streptococcus* spp. were predominant in the mouth and play important roles in oral health (13). Work on the abundance and diversity of AA-fermenting bacteria in the large intestine of healthy humans indicated that bacteria belonging to the clostridia and peptostreptococci were the most prevalent species using culture-based methods (14). Phylogenetic analysis of the bacterial 16S rRNA gene sequences revealed that bacteria belonging to the *Clostridium* clusters, the *Bacillus-Lactobacillus-Streptococcus* group (including *Streptococcus* spp.) and *Proteobacteria* (including *Escherichia coli* and *Klebsiella* spp.) were abundant in the small intestines of humans and swine (15-21). Such findings suggest that these bacteria might be important in host nutrition and health.

In humans and mono-gastric animals, the numbers of bacteria increase from 10^4 cells per gram digesta in the stomach to 10^{11} cells per gram digesta in the large intestine (19, 22). However, based on the calculations of the existing data, the numbers of AA-fermenting bacteria account for less than 1% of the total bacteria in the large intestine of healthy humans and the rumen (14, 23). Interestingly, the abundance of total bacteria and specific groups of bacteria in different segments of the GI tract could be influenced by the physiological conditions of the gut and dietary factors (1, 14, 22, 24, 25).

4. AA METABOLISM IN GUT BACTERIA

Nutrients, including dietary and host-derived proteins and AA, are used for the growth and survival of bacteria in the digestive tract (1, 26). Biochemically, proteins are first hydrolyzed into peptides and amino acids by various kinds of host- and bacteria-derived proteases and peptidases (23). The released peptides and AA can be further utilized by both gut bacteria and host, although certain bacteria may not use extracellular AA due to a lack of specific transporters. Studies over the last decades showed that many bacteria such as *Prevotella ruminicola*, *Butyrivibrio fibrisolvens*, *Megasphaera elsdenii*, *Misuokella multiacidas*, *Selenomonas ruminantium* and *Streptococcus bovis* harbor highly active dipeptidyl peptidase and dipeptidase, suggesting that these bacteria might be important for protein digestion and AA absorption in the mammalian digestive tract (23). Indeed, most gut bacteria utilize AA and ammonia as preferred nitrogen source (23). However, compared to AA and ammonia, peptides are the only preferred nitrogen source in *Prevotella ruminicola* (27). It was possible that the peptides are first transported into *P. ruminicola* cells and then utilized by the cells either in the intact form or as free AA derived from peptide degradation (27). Other bacterial species might lack peptide transport systems and thus could only utilize extracellular AA and ammonia (27-31). Overall, peptides, AA and ammonia are either utilized for the synthesis of bacterial cell components or catabolized through different pathways. This diversity of the metabolism of dietary nitrogenous substances in gut bacteria may have either positive or negative effects on the host.

4.1. Incorporation of AA into bacterial cells

The first step of the incorporation of AA into bacterial cells is AA transport. During the early 1980's and 1990's, Russell and colleagues conducted series of research investigating the mechanism of AA transport in rumen bacteria (32). It was found that sodium-dependent transport and, to a lesser extent, facilitated diffusion play important role in AA transport by some of the dominant AA-fermenting bacteria (28, 30, 31). However, the transport of glutamine in *Streptococcus bovis* was likely driven by phosphate-bond energy, i.e. ATP (29). The uptake of arginine and lysine might share the same transporter in *Clostridium bifermentans* strain SR and the conversion of arginine to ornithine by the bacteria facilitates arginine transport (33, 34). When extracellular pH decreased from 7.5 to 5.5, the transport of arginine declined linearly in strain SR (33). The above findings suggest that gut bacteria use different strategies in the utilization of different AA and nitrogen source whereas the extracellular environment might affect the patterns of bacterial AA utilization.

Much evidence shows that the transport and incorporation of certain AA into bacterial cells vary with AA structure. For instance, the proportions of glutamate plus glutamine, aspartate plus asparagine, lysine, BCAA, threonine, serine and glycine were higher than other AA in the cells of both gram positive and gram negative gut bacteria (Table 2). Similar observations were also found in the mixed bacteria, which simulated the real situations in the digestive tract (Table 2). These findings suggest that the above AA might be "essential" for the optimal growth of gut bacteria. It is of great interest that some of these AA are either nutritionally essential or functionally important to the animals (2). Although it is not clear whether the AA utilized for bacterial protein synthesis was mainly from the assimilation of extracellular AA or *de novo* synthesis under physiological conditions, the synthesis of bacterial protein is likely important to host nutrition (35, 36). In ruminants, bacterial proteins synthesized in the rumen are digested and the resultant AA or small peptides are absorbed by the small intestine. This process has nutritional advantages

Table 1. Major AA-fermenting bacteria in the digestive tract of humans and animals

Bacterial species	Preferred AA substrates	Metabolites	Primary origins	Niches	References
Fusobacteria; Fusobacteria (class); Fusobacteriales; Fusobacteriaceae					
Fusobacterium nucleatum	Arg, Asp, Asn, Gln, Glu, Gly, His, Lys, Orn, Thr	Arg (Orn, urea), Asp (Asn, fumarate, oxaloacetate), Asn (Asp, ammonia), Gln (Glu, ammonia), Glu (acetate, butyrate, ammonia, CO_2), Gly<->Thr, His (urocanate, ammonia, Glu), Lys (3,6-diaminohexanoic acid), Orn (putrescine)	Human	Mouth, small intestine, large intestine	14, 67, 121-123, KEGG Pathway Database
Fusobacterium varium	Glu, His, Lys, Ser	Glu (acetate, butyrate, ammonia, CO_2), His (urocanate, ammonia, Glu), Lys (3,6-diaminohexanoic acid), Ser (acetate, lactate)	Human	Mouth, small intestine, large intestine	122, 124, 125
Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae					
Escherichia coli	Arg, Asn, Asp, Gln, Glu, Gly, Lys, Ser, Thr	Arg (Cit, Orn, putrescine, spermine, agmatine, CO_2), Asn (Asp, ammonia), Asp (Asn, Ala, fumarate, oxaloacetate), Gln (Glu, ammonia), Glu (GABA[1], CO_2, Gln, 2-oxaloacetate), Ser<->Gly<->Thr, Lys (cadaverine, CO_2)	Human, Pig, Ruminant, Horse, Dog, Monkey, Rodent	Stomach, small intestine, colon, feces	72, 122, 126-131, KEGG Pathway Database
Klebsiella pneumoniae	Arg, Asn, Gln, Glu, His, Lys, Met	Arg (Orn, putrescine, spermine), Asn (Asp, ammonia), Glu (Gln), Gln (Glu, ammonia), His (urocanate, ammonia), Lys (cadaverine, CO_2), Met (S-adenosyl-L-methionine)	Human, Pig	Small intestine, colon, feces	122, 129, 131, KEGG Pathway Database
Proteobacteria; delta/epsilon subdivisions; Epsilonproteobacteria; Campylobacterales; Campylobacteraceae					
Campylobacter jejuni	Asn, Asp, Gln, Glu, Pro, Ser	Asn (Asp, ammonia), Asp (fumarate, ammonia), Gln (Glu, ammonia), Glu (Gln), Pro (Glu) Ser (pyruvate, ammonia)	Human, Poultry	Small intestine	71
Firmicutes; Bacilli; Lactobacillales; Streptococcaceae					
Streptococcus bovis	Gln	Pyroglutamate, ammonia	Human, Ruminant, Pig	Rumen, small intestine, large intestine	18, 20, 132
Firmicutes; Clostridia; Clostridiales; Peptostreptococcaceae					
Peptostreptococcus spp.[3]	Gln, Leu, Phe, Ser, Thr	Gln (pyroglutamate, acetate, ammonia, CO_2), Leu (isovalerate, isocaproate, ammonia, H_2), Phe (phenylpropionante, phenyllactate, phenylacetate, ammonia, CO_2), Ser (lactate, malate, acetate, ammonia, CO_2), Thr (propionate, ketobutyrate, butyrate, acetate, lactate, ammonia, CO_2)	Human, Ruminant, Pig	Rumen, small intestine, cecum, colon	14, 34, 122,133-135
Firmicutes; Clostridia; Clostridiales; Clostridiaceae					
Clostridium bifermentans	Ile, Leu, Lys, Pro, Thr Stickland reaction: H+[2] donor (Ala, Val) H+ acceptor (Leu)	Ile and Leu (isovalerate, isocaproate, ammonia, CO_2), Lys, Pro and Thr (propionate, ammonia), stickland reaction (isovalerate, isocaproate, ammonia)	Human, Pig	Large intestine	14, 52, 136,137
Clostridium sporogenes	Ile, Leu, Thr, Trp Stickland reaction: H+ donor (Ala, Ile, Val) H+ acceptor (Gly, Hydroxy-pro, Leu, Pro)	Ile and Leu (acetate, isovalerate, isobutyrate, ammonia), Thr (propionate, n-butyrate, ammonia), Trp (indole, indolepropionic acid, tryptophol, skatole), stickland reaction (isovalerate, isocaproate, ammonia)	Human, Ruminant	Rumen, large intestine	14, 52, 136-140
Clostridium sticklandii[3]	Arg, Ile, Leu, Lys, Thr Stickland reaction: H+ donor (Ser, Arg Orn) H+ acceptor (Gly, Pro)	Arg (Orn, ammonia, Cit, acetate), Ile and Leu (isovalerate, ammonia), Lys (acetate, butyrate, ammonia), Thr (acetate, ammonia), stickland reaction (isovalerate, isocaproate, acetate, ammonia)	Ruminant	Rumen	33, 34, 52, 136, 141, 163
Clostridium difficile	Ile, Leu, Thr Stickland reaction: H+ donor (Ala, Val) H+ acceptor (Leu)	Ile and Leu (isovalerate, ammonia), Thr (propionate, n-butyrate, ammonia), stickland reaction (isovalerate, isocaproate, ammonia)	Human, Pig, Ruminant, Horse, Dog, Poultry, Rabbit, Cat	Small intestine, large intestine, feces	20, 52, 136, 137, 142-145
Clostridium perfringens	Arg, Asp, Asn, Cit, Gln, Glu, GABA, Gly, His, Ser, Thr, Lys, N-methylglycine	Arg (Cit, ammonia, putrescine), Asp (Asn, Ala, fumarate, oxaloacetate), Asn (Asp, ammonia), Cit (Arg), Gln (Glu, ammonia), Glu (Gln, 2-oxaloacetate, GABA), GABA (propylamine, CO_2), His (histamine, CO_2), N-methylglycine (methylamine, CO_2), Ser<->Gly<->Thr, Lys (cadaverine, CO_2)	Human, Ruminant, Pig, Rodent, Dog, Monkey	Stomach, small intestine, cecum	52, 66, 69, 128, KEGG Pathway Database
Clostridium aminophilum[3]	Gln, Glu, His, Ser, Pyroglutamate	Ammonia, acetate, butyrate	Ruminant	Rumen	34, 164, 165, 166, 167
Firmicutes; Clostridia; Clostridiales; Veillonellaceae					
Megasphaera elsdenii	Ser, Thr	Isobutyrate, 2-methylbutyrate, isovalerate	Human, Ruminant, Pig	Rumen, small intestine, large intestine, feces	14, 18, 146, 147, 148
Acidaminococcus fermentans[3]	Asp, Cit, Gln, Glu, His, Orn, Ser, Thr	Asp (fumarate, oxaloacetate), Cit (Arg), Gln (Glu, ammonia), Glu (Gln, acetate, butyrate, CO_2), His (urocanate, ammonia), Orn (Cit, Arg, putrescine, spermine), Ser<->Gly<->Thr	Human, Ruminant, Pig	Small intestine, large intestine, feces	123, 148, 149, KEGG Pathway Database
Selenomonas ruminantium	Arg, Gln, Glu, Lys, Orn	Arg (agmatine, putrescine, CO_2), Gln (Glu, ammonia), Glu (Gln, 2-ketoglutarate, ammonia), Lys (cadaverine, CO_2), Orn (putrescine)	Ruminant, Pig	Rumen, cecum	95, 134, 150, 151
Bacteroidetes/Chlorobi group; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae					
Veillonella spp.	Lys, Orn	Lys (cadaverine, CO_2), Orn (putrescine)	Human, Pig,	Rumen, stomach,	20, 122, 152, 153

			Ruminant, Horse, Poultry, Rodent	small intestine, large intestine	
Bacteroidetes/Chlorobi group; Bacteroidetes; Bacteroidia; Bacteroidales; Bacteroidaceae					
Bacteroides fragilis	Asn, Asp, Cit, Gln, Glu, GABA, Gly, putrescine, Ser, Thr, His	Asn (Asp, ammonia), Asp (Asn, Ala, fumarate, oxaloacetate), Cit (Arg), Gln (Glu, ammonia), Glu (Gln, 2-oxaloacetate), GABA (propylamine, CO_2), putrescine (pyrrolidine), Ser<->Gly<->Thr, His (urocanate, ammonia)	Human, Pig	Small intestine, large intestine	20, 53, 69, 154, KEGG Pathway Database

[1] GABA, gamma-aminobutyric acid [2] H+, proton [3] Potential hyperammonia-producing bacteria. Standard abbreviations of amino acids are used.

Table 2. Composition of proteins and AA biosynthesis in some intestinal bacteria

Bacterial species	Predominant AA[1] / AA biosynthesis	References
Gram positive bacteria		
Staphylococcus aureus	Gly, Ala, Glu[2], Asp[3], Lys, Val, Leu, Ser, Thr, Ile	38
Streptococcus spp.	Arg, Glu/ +Glu[4], Asp/ +Asp, Lys, Ile/ +Ile, Gly/ +Gly, Leu/ +Leu, Pro/ +Pro, Phe/ +Phe, Ala/ +Ala, Tyr/ +Tyr, Val/ +Val, Thr/ +Thr, Ser/ +Ser	21, 27, 35
Gram negative bacteria		
Escherichia coli	Glu, Asp, Ala, Gly, Leu/ +Leu, Val/ +Val, Lys/ +Lys, Ile/ +Ile, Arg, Thr, Ser, Pro, Tyr	21, 38, 155, KEGG Pathway Database
Klebsiella spp.	Asp, Glu, Ala, Gly, Leu, Val, Lys, Ser, Thr, Arg, Ile	21, 38
Selenomonas ruminantium	Lys, Glu/ +Glu, Asp/ +Asp, Leu/ +Leu, Ile/ +Ile, Val/ +Val, Ala/ +Ala, Arg, Phe/ +Phe, Thr/ +Thr, Pro/ +Pro, Ser/ +Ser, His, +Gly. +Tyr	27, 35, 156
Megasphaera elsdenii	Glu, Asp, Lys, Leu, Val/ +Val, Ala, Ile, Gly, Arg	156, 157
Prevotella spp.	Glu/ +Glu, Asp/+Asp, Lys, Leu/ +Leu, Ile/ +Ile, Val/ +Val, Ala/ +Ala, Gly/ +Gly, Tyr/+Tyr, Phe/ +Phe, Thr/ +Thr, Arg, +Pro, +Ser	27, 35, 156-158
Bacteroides spp.	Glu, Asp, Lys, Leu/ +Leu, Gly, Ala, Ile, Arg, Thr, Phe	158, 159
Clostridium spp.[5]	Pro, Asp, Ser, Leu, Ala/ +Ala, Thr, Val/ +Val, +Lys	160
Mixed bacteria		
Ruminal bacteria[6]	Glu, Asp/ +Asp, Ala, Gly, Leu/ +Leu, Lys/ +Lys, Val, Thr, Ser/ +Ser, Ile, Phe	161, 162
Small intestinal bacteria	Asp, Glu/ +Glu, Gly/ +Gly, Ala/ +Ala, Val/ +Val, Arg, Leu, Ile, Lys/ +Lys, Ser, Thr/ +Thr, Pro/ +Pro, +His	6, 21
Colonic bacteria	Ser, Asp, Arg, Thr, Gly, Leu, Glu, Cysteine, Pro, +Val, +Phe, +Met	14

[1] In the order of the proportion of individual AA to the AA pool, approximately 50% of the cell dry weight is crude protein, varied between references [2] Glutamate plus glutamine [3] Aspartate plus asparagine [4] "+" denote production of amino acid [5] Based on the AA utilization data [6] AA composition of the bacterial cell wall, exclude data from AA biosynthesis. Standard abbreviations of amino acids are used.

especially in the re-assimilation of ammonia derived from the breakdown of proteins and AA (23). However, in humans and mono-gastric animals, the proteins in gut bacteria mainly come from the assimilation of dietary and host-derived AA. Because the small intestine is the major site for nutrient digestion and absorption and because the absorption of AA is limited in the large intestine (6, 37), AA utilization by bacteria in the GI tract represents an event of nutritional waste in humans and mono-gastric animals. Therefore, studies on the mechanism of AA transport and incorporation in the small intestinal bacteria are essential to the development of new strategies for improving the efficiency of utilization of dietary protein and AA.

AA composition varied among different bacteria strains (Table 2). In gram positive bacteria, alanine proportion was higher in *Staphylococcus aureus* cells. Proportions of arginine, proline and phenylalanine were higher in the cells of *Streptococcus* spp. (Table 2). Compared to gram positive bacteria, proportions of lysine and histidine were higher in cells of *Selenomonas ruminantium* and proline proportion was higher in the cells of *Clostridium* spp. (Table 2). This result suggests that the "essential" AA for the growth of gut bacteria might be species specific. The conservation and variation in the AA composition of bacterial cell proteins in different bacterial species might partially reflect the evolutionary relationship of different organisms (38). Therefore, it is possible that certain AA could be utilized as biological markers to monitor the gut bacteria composition and activity especially for potential pathogens.

4.2. Biosynthesis of AA

As mentioned above, the AA incorporated into bacterial cells also derive from *de novo* synthesis using various substrates. Studies with pure bacteria cultures showed that ammonia might be the preferred nitrogen source and support the fastest growth for *Escherichia coli* (39). Ruminal bacteria, such as *Prevotella bryantii*, *Selenomonas ruminantium* and *Streptococcus bovis*, also synthesize various AA using ammonia as the sole nitrogen source *in vitro* (35, Table 2). However, the addition of peptides or AA mixtures decreased the *de novo* synthesis of AA in a dose dependent manner especially for proline and aromatic AA (phenylalanine and tyrosine) (35). It was estimated that, in ruminal bacteria, approximately 70% of the bacterial AA nitrogen was derived from ammonia in the presence of physiological concentrations of peptides (35). Similar observations were found for mixed bacteria which obtained 65% and 35% of their nitrogen from peptides and ammonia, respectively, when both were available (40, 41). It seems that the *de novo* synthesis of AA might be important for bacteria in the rumen.

Some investigators suggested that the biosynthesis of AA by bacteria in the digestive tract of mono-gastric animals may regulate AA homeostasis in the

host (5, 6, 42). When pigs were fed a diet containing $^{15}NH_4Cl$ and [U-^{14}C]-polyglucose, the incorporation of ^{14}C into essential AA and of ^{15}N into lysine was detected (43). It was found that the small intestine was the major site for the absorption of microbial derived essential AA especially lysine even if the isotopic ^{15}N enrichment of microbial lysine was higher in the large intestine (37). Similar observations were also obtained from the study with human subjects (44). Studies on the preferred nitrogen source for the bacterial *de novo* synthesis of lysine showed that the retention of ^{15}N from $^{15}NH_4Cl$ was higher than from [^{15}N]-urea (44, 45). The possibilities are a) the small-intestinal bacteria have a low activity of urease and thus unable to utilize urea efficiently (5); b) [^{15}N]-urea could be diluted by unlabeled urea from both systematic and local (enterocytes) metabolism of AA and ammonia (5, 46). Additionally, the AA required for the synthesis of bacterial protein might partially derive from the AA synthesized *de novo* (Table 2). Because extensive nitrogen recycling occurs in the gut (7), it would be important to determine the nitrogenous precursors for microbial AA synthesis, the proportion of the microbial AA to account for first-pass intestinal AA metabolism, the metabolic fate of AA in microbial proteins, and factors that regulate these processes (5, 42).

The contributions of the *de novo* synthesized microbial AA to the AA requirements of humans and mono-gastric animals are still uncertain (5, 6, 37, 42). Quantitative estimations showed that the *de novo* synthesized microbial lysine account for about 10% to 100% of the daily lysine requirement in pigs and the microbial lysine enrichment in the ileum account for 44% of lysine in the plasma of human subjects (43, 44). However, the above estimations ignored the net loss of AA during the first-pass intestinal AA metabolism (47). It is now known that there is substantial catabolism of AA by small-intestinal mucosal cells and small-intestinal bacteria (7, 21, 26, 48-50). Because nonruminants exhibit a negative nitrogen balance when fed an AA- or protein-free diet, bacteria do not make a significant contribution of AA to the host.

4.3. Catabolism of AA and production of important metabolites

Not all the AA ingested can be absorbed and enter the blood stream. As one of the metabolic fates, quantitatively significant amounts of dietary AA are catabolized by enterocytes during first-pass metabolism (7). Both *in vitro* and *in vivo* studies showed that pig small-intestinal mucosal cells degrade many non-essential AA, while the oxidation of essential AA by enterocytes is limited *in vitro* (47-50). Compared to enterocytes, AA catabolism in ruminal bacteria as well as microorganisms in the large intestine of humans and animals were extensive (51, Table 1). Theoretically, gut bacteria harbor the ability to catabolize almost all kinds of AA. However, AA such as glutamine/glutamate, asparagine/aspartate, lysine, serine, threonine, arginine, glycine, histidine and branched-chain AA might be the preferred substrates for degradation by gut bacteria (Table 1). The major pathways for microbial AA catabolism are deamination and decarboxylation. The fermentation products of AA include ammonia, short-chain and branched-chain fatty acids (e.g., acetate, propionate, butyrate, valerate, isobutyrate, and isovalerate), phenolic and indolic compounds (metabolites from phenylalanine and tryptophan), organic acids (lactate, formate, succinate, and oxaloacetate), gaseous compounds (mainly carbon dioxide, hydrogen, hydrogen sulfide and methane), and amines (e.g., agmatine, putrescine, spermidine, and spermine) (12, 51-53, Table 1). Gut bacteria such as clostridia could also obtain energy from AA catabolism through the Stickland reaction and the major metabolites are branched-chain fatty acids and ammonia (52, Table 1). In the gut, all of these microbial AA metabolites form a highly complex reservoir that has a potential impact on the physiology of the gut epithelium (12). Because the overall activity of bacteria in the small intestine is similar to that in the large intestine, it would be of great nutritional importance to determine whether AA catabolism in gut bacteria affects growth and health in the host.

Studies revealed that some of the metabolites from the AA catabolism by bacteria in the large intestine might be crucial for the physiopathology of the colon disease (12). Gaseous compounds (hydrogen sulfide and nitric oxide), nitrogenous products (e.g., ammonia, polyamines and nitrite), branched-chain fatty acids, as well as phenolic and indolic substances may serve as biomarkers for evaluating bacterial fermentation of dietary components and risks of colon cancer. The details of these AA metabolites and their impact on the physiopathology of the colon epithelium are summarized elsewhere (12). Substrates for the production of the detrimental AA metabolites by bacteria in the large intestine may be mainly derived from the small intestine. Therefore, it is important to define the factors and mechanisms that modulate nutrient flows and metabolic interactions in the small intestine as well as their impact on the physiology and micro-ecology of the large intestine (19).

4.4. Compartmental AA metabolism in gut bacteria

AA metabolism in the gut lumen might be compartment specific due to the differences in microbial community composition along the digestive tract (1, 14, 18, 20, 21, 24). For instance, proportions of arginine and proline were higher in the cells of small-intestinal bacteria and colonic bacteria when compared with ruminal bacteria (Table 2). However, proportions of serine and cysteine were higher in colonic bacteria than small-intestinal bacteria (Table 2). Furthermore, changes in the AA composition of the bacterial community might reflect the composition, abundance and activity of certain bacteria in specific gut segments.

Changes in the microbial ecology along the digestive tract will rather affect the compartment-wise AA metabolism in the lumen of intestine. Recent studies suggested that the bacterial AA utilization might mainly for the synthesis of bacterial protein in the small intestine while catabolism dominant the AA metabolism in the large intestine especially under low carbohydrate conditions (26, 21, 14). Meanwhile, it was found that in the pig small intestine, the utilization of AA by jejunal microbiota might be different from that of ileal microbiota *in vitro* (21). The

study also suggested that the utilization/metabolism of specific AA in small intestinal bacteria might be dependent on the cross-feeding of different bacterial species (21). As consequences, this might regulate the AA and nitrogen metabolism in the gut of the host in a multi-compartmental manner (62).

4.5. Metabolic interactions among gut bacteria and host

Early studies with germ free and conventionalized mice revealed that the gut microbiota altered the distributions of free AA in different regions of the GI tract (54). This finding led to an interesting and important hypothesis that the gut bacteria may play an important role in host AA homeostasis and health through multiple pathways. Such metabolic interactions exist among different bacterial species in the GI tract or between bacteria and the host at the local level (mucosal cells) and systemic level (whole body) and even within different compartments of the same intestinal section.

Metabolic interactions among gut bacteria may occur directly through the digestion of substrates (e.g. cross-feeding) and indirectly via selective pressure such as competition, selection and adaptation (9). As for the AA metabolism in gut bacteria, it is most likely that the ammonia produced from AA deamination could serve as a nitrogen source for the biosynthesis of bacterial AA when carbohydrates are available. Recent *in vitro* study showed that cross-feeding might exist in the catabolism of aromatic AA by colonic *Bifidobacterium longum* and *Clostridium clostridioforme* (55). After the depletion of glucose, the hydroxyphenyllactic acid produced from the degradation of phenylalanine by *B. longum* could be further degraded by *C. clostridioforme* (55). In the large intestine, one bacterial species might also affect the nutrient metabolism of its "neighbors" indirectly. When germ-free mice were colonized with *Eubacterium rectale* and *Bacteriodes thetaiotaomicron* (members belonging to the two dominant bacterial phyla present in the human distal gut microbiota), *E. rectale* adapted to the presence of *B. thetaiotaomicron* by reducing the production of its glycan-degrading enzymes, increasing expression of select AA and sugar transporters, and synthesizing glutamine/glutamate and D-alanine (56). However, the importance of the microbe-microbe interactions (e.g., feedback loops or molecular cross-talk) in intestinal AA metabolism is unknown.

Microbial metabolites may modulate gene expression in bacteria and their production of enzymes related to AA metabolism in the digestive tract. Compared to the laboratory medium, when the probiotic bacteria *Lactobacillus plantarum* pass through the GI tract of mice, expression of the genes involved in the acquisition and synthesis of AA increased dramatically (57). For instance, glutamate-5-semialdehyde dehydrogenase participates in the conversion of glutamate to proline and argininosuccinate synthase converts citrulline to arginine (57). Further studies showed that several genes (e.g. argininosuccinate synthase) displayed intestinal compartment-specific activity (small intestine > colon) (58). However, proteomic studies on the interactions between *Lactobacillus fermentum* and intestinal epithelium cells indicated that, compared to laboratory medium, *L. fermentum* reduced the production of proteins related to arginyl-tRNA synthetase (protein syhthesis) and aspartate-semialdehyde dehydrogenase (glycine, serine and threonine metabolism and lysine biosynthesis) when exposed to the lumen of the rabbit jejunum (59). The contradictory results may be due to the physiological characteristics of different *Lactobacillus* species, different animal models or diets used. Recent work on the functionality of the human ileostomy effluent microbiota using the metatranscriptomics approach revealed that about 25% of the sequences retrieved from the mRNA enriched cDNA-amplified fragment length polymorphism profiles were related to metabolism and about 20% of these metabolism-related genes encoded proteins participating in transport and metabolism of AA (20). Examples include alanine dehydrogenase produced from bacteria belonging to the order of Bacteriodales, aspartate kinase from the order of Clostridiales, glutamate synthase and AA transporters from the order of Lactobacillales, and succinylglutamate desuccinylase from the order of Enterobacteriales (20). These results indicate that bacteria in the small intestine are active in the metabolism of nitrogenous compounds and have developed adaptive strategies to survive and propagate during the co-evolution with its host.

Increasing evidence indicates that the gut microbiota plays an important role in the physiology and metabolism of enterocytes, thereby regulating the nitrogen recycling within the gut and the whole body nitrogen metabolism. For example, during *in vitro* incubation with *L. fermentum,* Caco-2 cells increased the production of proteins that were beneficial for gut integrity, including voltage-dependent anion channel 1, glutathione transferase, and heat shock protein gp96 (59). Glutathione transferase family enzymes detoxify xenobiotics and, therefore, reduce the oxidative stress of cells (60). These enzymes can also remove toxic metabolites and may interact with ATP-binding cassette transporters (60, 61). These findings suggest that enterocytes could respond and adapt to the gut microbiota either by regulating the transport of nutrients or by transforming diet- or gut bacteria-derived substances to sustain mucosal integrity and function. Therefore, enterocytes likely develop strategies to maintain their protein balance by controlling nitrogen cycling within the gut, while benefiting its bacterial ecosystem. The anabolism and catabolism of AA by both enterocytes and perhaps gut bacteria limit the availability of dietary AA to extraintestinal tissues (44, 47). Meanwhile, nitrogenous substances (e.g., digestive enzymes, bile, mucins, cell debris and urea) secreted into the gut lumen are subjected to digestion or fermentation in the small intestine and the large intestine (5, 7). It is likely that gut bacteria utilize the ammonia generated from the catabolism of extracellular and intracellular proteins, AA, or urea for the synthesis of bacterial protein (5, 6, 7, 37, 43, 44). This could be regarded as a form of nitrogen recycling, which is of nutritional importance especially for animals fed a low-protein diet. Although the absorption of AA is limited in the large intestine (44), AA synthesized in this segment of the gut may provide AA to luminal bacteria (7, 62).

At the whole-body level, the modulation of host metabolism by gut microbiota may occur in multiple tissues and cell types (Figure 1). Studies aimed at comparing the multi-compartmental metabolic profiles of conventional mice and its germ-free counterparts showed that, in the presence of gut microbiota, concentrations of creatinine in urine, of hypotaurine in the liver, of alanine and creatine in the small intestine were substantially reduced (62). However, the presence of the gut microbiota resulted in increased levels of oxidized glutathione in the liver, of tyrosine, glutamate, alanine and aspartate in the small intestine, and of creatine, glutamine, and aspartate in the colon (62). It is well known that creatinine and creatine are closely related to muscle mass and arginine metabolism (63). Also, glutamine/glutamate, aspartate, and glutathione play important role in the regulation of nitrogen and energy balance in tissues and at the whole body, including the fluxes of the citric acid cycle and the urea cycle, as well as protein synthesis and degradation (2, 3, 48, 62, 64, 65). Therefore, it could be concluded that nitrogen and AA metabolism in the gut microbiota and the underlying metabolic interactions with the host may be important in the regulation of host protein and energy balance. Based on marked differences in intestinal microbial AA metabolism among subjects, the concept of personalized requirements of dietary proteins should not be neglected (8). However, the linkage between the multi-compartmental metabolic profiles and host health/disease is still not clear. The development of certain biomarkers and databases will help to better understand, predict, prevent and treat life-threatening metabolic diseases.

5. FUNCTIONAL ASPECTS OF AA METABOLISM IN GUT BACTERIA

Apart from the nutritional importance of nitrogenous substances to the growth of gut bacteria, AA metabolism in gut bacteria may serve as an important "survival strategy" for the bacteria to adapt and survive in the gut and also "cross-talk" to their neighboring species and eukaryotic host. Therefore, it is important to define the role of AA metabolism in the survival of gut bacteria as well as the impact to their surrounding environments and the consequences on host health and disease.

5.1. Adaptation and survival

Gut bacteria have many mechanisms to cope with harsh, variable, rapidly changing environment of the digestive tract. Stresses, such as low pH, nutrient limitation, and starvation, are now taken into consideration (66). It was found that bacteria in the dental plaque ecosystems maintained the neutral pH of their environment (acid-neutralizing activity) by the catabolism of different AA thus showed a beneficial effect to the prevention of caries. For instance, in *Fusobacterium nuleatum,* rates of glutamate and aspartate fermentation as well as ammonia production were higher at pH 5.0 than at pH 5.5 (67). In *Streptococcus gordonii*, the expression of arginine deiminase was induced by low pH, which resulted in elevated production of ammonia (68). These findings can aid in development of new strategies to prevent caries and improve oral health (67, 68).

In the GI tract, a low pH can be induced by either hydrochloric acid secreted from the stomach epithelium or short chain fatty acids (formerly known as volatile fatty acids, VFA) from the bacterial fermentation of carbohydrates. This may result in acid stress in gut bacteria, therefore affecting the survival and gut transition of potential pathogenic bacteria. Early studies demonstrated that the decarboxylation of gamma-aminobutyric acid (GABA) and putrescine by *Bacteroides fragilis* was optimal under acidic conditions (pH 6.0) (69). When the pH of the medium was changed from 7.0 to 6.0, the preferred AA for decarboxylation in *Clostridium perfringens* shifted from arginine and GABA to lysine and putrescine (69). In *Escherichia coli*, two acid resistance systems have been discovered namely the acid resistance system 2 and 3 (AR2 and AR3), depending on the catabolism of AA (70). The AR2 and AR3 require extracellular glutamate and arginine, respectively. However, both AA can be decarboxylated to alter the membrane potential from a net inside negative to a net inside positive charge, leading to an increase in internal pH (Δ pH) (70).

The intestinal metabolism of AA may also be important for the survival of gut bacteria under conditions of nutrient limitation and starvation during gut transition. When oxygen is limiting, the production of aspartase increased in *Campylobacter jejuni* (71). As a consequence, the conversion of aspartate to fumarate and ammonia was augmented with subsequent increases in fumarate utilization and bacterial growth (71). In *C. jejuni*, aspartate is one of the key substances in the metabolic pathway that controls the utilization (including catabolism) of AA and ATP production. Therefore, aspartase is critical for the survival of *C. jejuni* in the lumen of the small intestine under anaerobic conditions (71). In the large intestine, low levels of luminal nutrients can trigger the starvation and death of bacteria especially in the presence of high concentrations of organic acids. It was shown that *Escherichia coli* increased its lysine decarboxylation activity under phosphate-deficient conditions (72). These findings suggest that, in response to nutrient restriction and acid stress, gut bacteria develop multiple strategies to survive in the intestine by modulating their AA metabolic pathways.

5.2. Production of important molecules

Studies over the last decades have shown that gut bacteria respond to their environments and affect the physiology and metabolism of their neighboring species and eukaryotic host through the synthesis of a wide variety of AA and their metabolites. It is generally regarded that the peptidoglycan produced from gram positive bacteria and lipopolysaccharide generated from gram negative bacteria plus some bacteria-derived macromolecules (e.g., glycan ligands) mediate signaling pathways in gut epithelial cells and regulate the host immune function (73-75). Additionally, it has been reported that the Rgg proteins produced by the pathogenic *Streptococcus pyogenes* strain regulate the synthesis and metabolism of virulence factors in the bacteria and the effects are associated with the

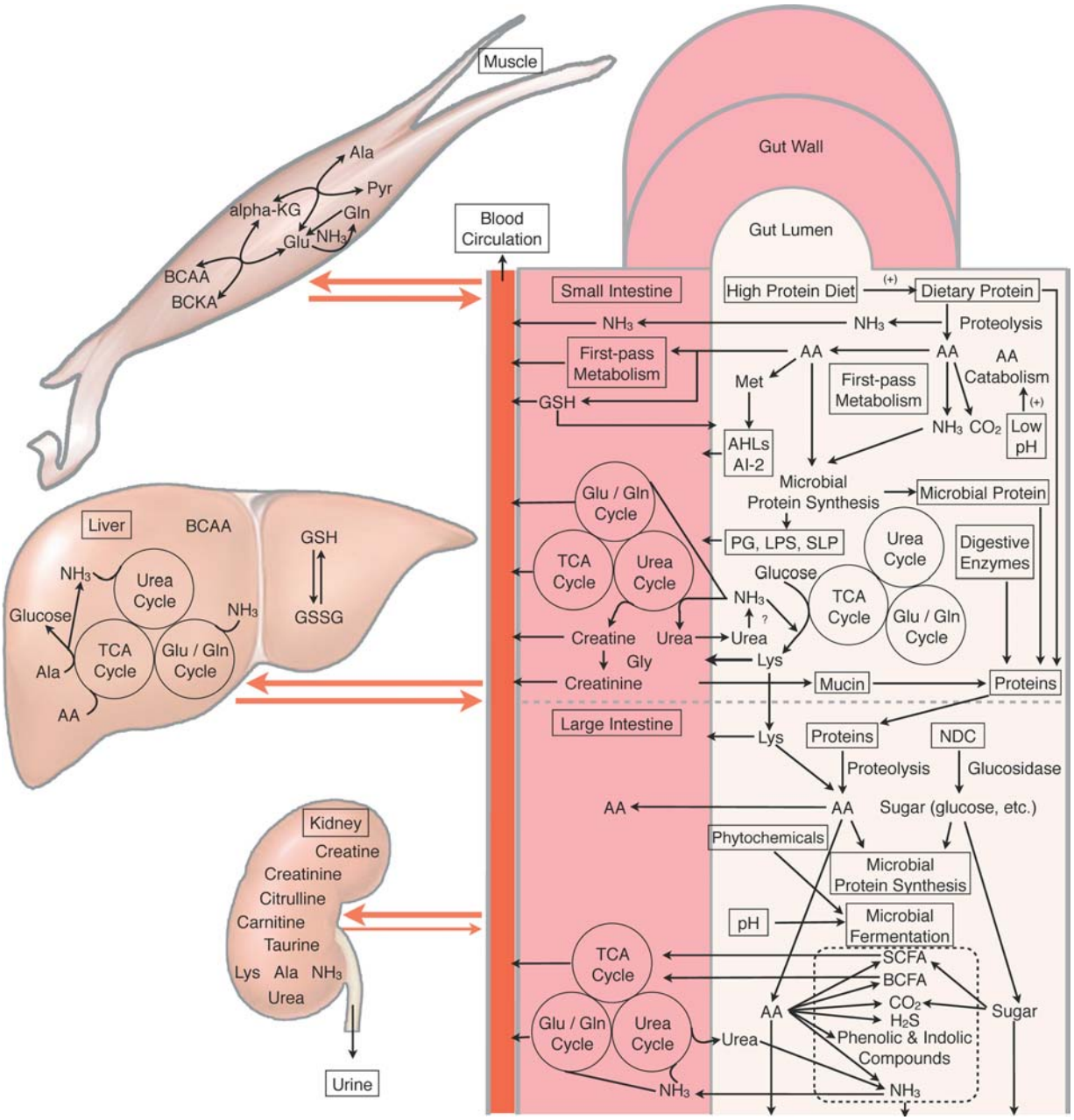

Figure 1. Schematic diagram of the multi-compartmental metabolism of amino acids in the gut and extraintestinal tissues of mono-gastric animals. AA, amino acids; AHLs, acyl homoserine lactones; AI-2, autoinducer-2; Ala, alanine; BCAA, branched chain amino acid; BCFA, branched-chain fatty acids; BCKA, branched-chain ketoacid; CO_2, carbon dioxide; GSH, glutathione; GSSG, glutathione disulfide; Glu, glutamate; Gln, glutamine; Glu / Gln Cycle, Glutamate / Glutamine Cycle; Gly, glycine; H_2S, hydrogen sulfide; alpha-KG, alpha-ketoglutarate; LPS, lipopolysacharrides; Lys, lysine; Met, methionine; NDC, non-digestible carbohydrates; NH_3, ammonia; PG, peptidoglycan; Pyr, pyrovate; SCFA, short chain fatty acids; SLP, S-layer protein; TCA Cycle, Tricarboxylic Acid Cycle.

catabolism of arginine and serine (76). In *Streptococcus bovis* HC5, the production of a broad-spectrum antibiotic bovicin HC5 inhibited the growth of pure cultures of hyper ammonia-producing bacteria as well as AA degradation and ammonia production by mixed ruminal bacteria (77).

Recent findings have indicated that some small molecules produced by gut bacteria play crucial role in quorum sensing and biological processes, such as biofilm formation and expression of virulence factors (78). Among these, the acyl homoserine lactones (AHLs) and autoinducer-2 (AI-2) are derived from S-adenosyl methionine, an important metabolite of methionine (79). It is now known that AI-2 is essential for the adherence to HeLa cells, mobility, and expression of virulence genes in enteropathogenic *E. coli* (80). In addition, formation of AI-

2 affects the attachment of *L. acidophilus* to Caco-2 cells *in vitro* (81). The production of AI-2 in *Streptococcus suis* is stimulated in the addition of 0.5% sodium chloride or glucose (82). Furthermore, *E. coli* interact with neighboring species by adjusting the production and consumption of AI-2 (83). Because AI-2 is produced and recognized both by gram-positive and gram-negative bacteria, pro- and anti-AI-2 interactions among gut bacteria and their association with the eukaryotic host within the digestive tract can affect the microbial community composition and host health (78).

Current data suggest that AHL molecules mediate the interactions between gut bacteria and the host. Also, N-3-oxododecanoyl homoserine lactone produced by *Pseudomonas aeruginosa* accelerates apoptosis in macrophages and neutrophils, while possessing an immunomodulatory effect on human peripheral blood mononuclear cells (84, 85). Conversely, as one of defense mechanisms, host cells can inactivate (via degradation) the AHL molecules produced by *P. aeruginosa in vitro* (86). Although it is not clear whether similar AHLs-mediated interactions occur within the digestive tract, the above findings raise important questions about the physiological significance of the microbial metabolites of methionine and related AA. Namely, can these metabolites serve as potential biomarkers for monitoring the microbe-microbe and microbe-host interactions at different niches along the digestive tract, as well as the health status and disease of the host. Identification of the multi-compartmental metabolic profiles of quorum-sensing signals and related molecules will aid in the development of diagnostic and therapeutic tools for the well-beings of both humans and animals (87).

Polycationic nitrogenous substances (e.g., polyamines) are important products of AA metabolism in luminal microbes of the small intestine. Ornithine, which is derived from arginine, proline, glutamate, and glutamine, is decarboxylated by ornithine decarboxylase to produce putrescine. Putrescine is also formed from arginine sequentially via arginine decarboxylase (to yield agmatine) and decarboxylation of agmatine by agmatinase (to generate putrescine). Putrescine is converted into spermidine and spermine via spermidine synthase and spermine synthase, respectively. Polyamines have long been recognized to important roles in cell physiology. For example, polyamines act as antioxidants to protect cells from oxidative damage. As positively charged compounds, polyamines participate in many cellular processes through binding with RNA, DNA, nucleotide triphosphate, proteins, and other negatively charged molecules. Through these interactions, polyamines regulate gene expression, signal transduction, ion channel function, DNA and protein synthesis, and apoptosis. Thus, polyamines are essential for proliferation, differentiation, and function of bacteria and enterocytes. When cells are stimulated with growth factors, one of the first crucial events is the induction of polyamine synthesis, which precedes increases in DNA replication and protein synthesis. Thus, an increase in polyamine levels is associated with enhanced growth of intestinal mucosal cells and microorganisms. Conversely, depletion of cellular polyamines arrests cell growth.

6. DIETARY MODULATIONS OF AA METABOLISM IN GUT BACTERIA

Bacteria thriving in the digestive tract mainly rely on the utilization of substances of food origin. Therefore, diets have profound influence on bacterial metabolism, and specific dietary components may have selective effects on the population and metabolism of the microbiota. Over the last decades, there has been interest in effects of some dietary factors, such as protein, non-digestible carbohydrates, probiotics, synbiotics or phytochemicals on gut bacteria and host health (Figure 1). These results are highlighted in the following sections.

6.1. High protein diet

In the small intestine of mono-gastric animals, the daily requirement of AA for the first-pass metabolism and absorption of AA are constant under normal conditions. When AA are supplemented to the diet, the entry of AA into the portal vein increases (88). However, adaptive metabolism of the small intestine may occur in response to dietary protein intake. When high protein intake exceeds the capacity of digestive enzymes and AA transporters, some of protein and AA in the diet would enter the large intestine. To date, data on the regulation of AA metabolism in the small intestine and its luminal bacteria is limited. Also, little is known about high protein diet on the hindgut ecology (19).

Research showed that approximately 3-12 grams of dietary protein and peptides entered the human large intestine every day and served as nitrogen sources for the gut microbiota (14, 89). High levels of proteins and peptides in the large intestine could lead to an increased production of ammonia due to the actions of proteases, peptidases, deaminases, and deiminase produced by gut bacteria (23, 90, 91). When subjects are fed a high-protein diet, levels of sulfide and branched-chain fatty acids are elevated due to the bacterial fermentation of sulfur-containing AA and branched-chain AA (92, 93), but butyrate concentrations and numbers of butyrate-producing bacteria are decreased in the large intestine as well in the feces (94). It is widely regarded that butyrate is the main energy source for colonic epithelial cell, thus, a decrease in butyrate concentration and an increase in concentrations of ammonia and sulfide may explain the detrimental effect of high protein diet on the large intestine (e.g., increased incidence of colon cancer).

The structure and AA composition of proteins can affect their hydrolysis in the GI tract and nitrogen metabolism in gut bacteria. Results of *in vitro* study indicate that compared with casein, sulfate production from the bacterial fermentation of bovine serum albumin is higher and that dietary protein from the meat is an important substrate for sulfide generation (93). In addition, AA, such as tryptophan, proline, tyrosine, and isoleucine, strongly inhibit the protease activity of *Clostridium sporogenes* (90). In *Selenomonas ruminantium*, the urease activity is higher when serine or threonine is used as a substrate. However, urease activity decreases markedly when histidine is added to medium (95). These findings

suggest that proteins, peptides or AA in the large intestine not only affect the production of toxic substances, but also regulate the bacterial fermentation, AA degradation, and nitrogen recycling. Furthermore, the active metabolism of nitrogenous substances by bacteria in the digestive tract especially in the large intestine is also influenced by the availability and structure of carbohydrates (4, 89).

6.2. Non-digestible carbohydrates and availability of carbon source

Non-digestible carbohydrates (NDC) or dietary fibers are normal constituents of most foods derived from plants. They escape digestion in the small intestine as the host lacks the necessary degrading enzymes and pass into the large intestine. NDC include polysaccharides such as resistant starch, pectin, inulin, guar gum, wheat bran, cellulose, lignin, and oligosaccharides (89). According to the definition, prebiotics belong to the category of NDC and selectively stimulate the growth of potentially beneficial bacteria such as *Bifidobacterium* and *Lactobacillus* (89, 96). NDC affect the AA metabolism in gut bacteria either by regulating the bacterial composition and abundance or by providing the carbon source for the growth of microorganisms. In the three-stage continuous culture, the addition of inulin and galacto-oligosaccharides increased the numbers of lactobacilli in the proximal colon, together with a small increase in bifidobacteria, peptostreptococci, enterococci and *Clostridium perfringens* (97). The two carbohydrates stimulated the synthesis of nitroreductase and azoreductase (97). At neutral pH, the addition of starch to the media significantly decreased the bacterial production of amines, phenolic and indolic compounds, and branched-chain fatty acids both by pure and mixed bacteria cultures (14, 53, 69, 98). Similarly, the reduced production of phenolic and indolic compounds was reported for oligofructose added to the pure cultures of *Bifidobacterium* and *Bacteroides* (55). Recent studies on the effect of prebioctics on AA metabolism at the whole-body level revealed that galacto-oligosaccharides increased the urinary excretion of carnitine and taurine as well as fecal excretion of glycine in human infants, while the addition of prebiotics decreased the urinary excretion of lysine and alpha-keto-isocaproate (99). The findings indicate that NDC affect AA metabolism within the digestive tract and at the whole-body level. However, the underlying mechanism and effects on are not clear. Nonetheless, many gut bacteria such as bacteroides, bifidobacteria, ruminococci, eubacteria and clostridia can degrade polysaccharides and produce simple sugars (100). As one possible mechanism explaining the beneficial effect of NDC, the simple sugars produced could be used by the bacteria or shared with other bacteria species and affect their AA metabolism. Interestingly, in the presence of glucose, significant reduction in protease production by *Clostridium sporogenes* was observed (90). The production of phenolic and indolic compounds from aromatic AA also decreased in mixed bacteria in response to the addition of glucose (101).

The highly stimulated fermentation and growth of bacteria in the proximal colon by NDC resulted in the formation of short-chain fatty acids and thus a decrease in luminal pH (89, 97, 102). As one of the important parameters of the gut environment, the decreased luminal pH can affect microbial metabolism and composition in the large intestine (10, 25). Consistent with data on carbohydrate availability, the microbial production of amines, branched-chain fatty acids, phenolic and indolic compounds from AA decreased at pH 5.5 compared with neutral pH (14, 53, 101). However, the above conclusions are based on *in vitro* studies. In the large intestine, apart from the short-chain fatty acids, the shifts of individual fatty acids also affect the luminal pH. Therefore, it seems essential to determine the role of individual fatty acids on the growth, community composition and AA metabolism of gut bacteria both *in vitro* and *in vivo* (25). Meanwhile, little is known about the substrate preferences of the majority of gut bacteria (10). Therefore, cautions must be taken when prebiotics are introduced into the gut, because they may stimulate one or more element of the microbiota in addition to the target groups whose activation may trigger a series of unfavorable biochemical reactions and serious health problems (10). Furthermore, factors such as nature of the substrate, dietary components, gut environment, microbial composition and metabolic cross-feedings might be important when evaluating and assessing the mechanism of the beneficial effect of NDC or dietary component (10, 103).

6.3 Probiotics and synbiotics

Probiotics are microbial food supplements that beneficially affect the host by improving its intestinal microbial balance, while synbiotics are the combination of prebiotics and prebiotics (96). Meanwhile, many bacteriocin-producing species belong to the category of probiotics. Increasing evidence shows that probiotics and synbiotics are beneficial to the health of humans and animals by improving the nitrogen metabolism within the gut and at the whole-body level (7). Here, we provide an update on the progress of research regarding the regulatory role of probiotics and symbiotics on host AA metabolism.

Recent findings indicate probiotics or synbiotics modulate the AA metabolism in animal models and humans in a multi-compartmental mode. Compared to germ-free mice, the introduction of *Lactobacillus paracasei* to the intestine of germ free mice reduced concentrations of serine and glutamine in the duodenum and concentrations of glutamate, glutamine, glutathione, methionine, alanine, glycine, cycteine and creatine in the jejunum and ileum (104). Also, in response to the supplementation with *L. paracasei* or *Lactobacillus rhamnosus*, hepatic concentrations of leucine and isoleucine, as well as concentrations of citrulline, lysine and creatine in urine were decreased (105). On contrary, concentrations of glutamate, glutamine, lysine and glucose increased in plasma and the fecal excretion of lysine and glucose increased (105). In another study, the authors found that supplementing *L. rhamnosus* or *L. rhamnosus* plus galacto-oligosaccharides to human baby microbiota mice resulted in increased concentrations of alpha-keto-isovalerate and creatine in urine (99). Both probiotics and synbiotics enhanced fecal excretion of glutamate, glutamine, branched-chain AA, alanine, and glycine. Compared with

the probiotic *L. rhamnosus*, dietary supplementation with galacto-oligosaccharides reduced the fecal excretion of aspartate, asparagine, lysine, methionine and aromatic AA (99). The supplementation with both probiotics and synbiotics reduced hepatic concentrations of alanine in the liver (99). The dietary supplementation of a synbiotic food containing *Lactobacillus acidophilus*, *Bifidobacterium longum* and fructooligosaccharides reduced the fecal excretion of tyrosine, phenylalanine, alanine, glutamate, lysine, glycine, valine and isoleucine in humans (106). These differential results may result from variations in the metabolic characteristics of humans and mice, probiotics and prebiotics used, etc. However, all the findings point to a role for probiotics and synbiotics in regulating AA metabolism in intestinal microbes. Collectively, the results provide us with a broader view of effects of probiotics and synbiotics on health. Further studies are essential to uncover the underlying mechanisms and the potential biological significance.

6.4. Phytochemicals

Phytochemicals are bioactive non-nutrient plant compounds present in fruits, vegetables, grains, and other plant foods. Ingestion of these substances by humans and animals has been linked to reductions in risk of major chronic diseases (107). A number of studies over the last decades showed that phytochemicals might affect the growth and metabolism of proteolytic and AA-fermenting rumen bacteria. For example, work with pure cultures of ruminal bacteria revealed that saponin from *Yucca schidigera* inhibited the growth of *Ruminobacter amylophilus*, *Butyrivibrio fibrisolvens*, *Prevotella bryantii*, and *Streptococcus bovis* and with a modest decrease in ammonia production (108, 109). It was demonstrated that condensed tannin from *Lotus corniculatus* inhibited the growth of *Clostridium proteoclasticum*, *B. fibrisolvens*, *Eubacterium* spp. *Ruminococcus albus*, *Fibrobacter succinogenes* and *S. bovis*, and rates of proteolysis *in vitro* (110). In addition, the growth of proteolytic bacteria such as *B. fibrisolvens*, *R. amylophilus* and *S. bovis* was reduced by sainfoin (*Onobrychris viciifolia*) condensed tannin (111). The inhibitory effect of tannin to bacteria was due to the ability of tannins to form complexes with the cell envelop of bacteria, leading to morphological changes of the cell wall and the extracellular enzymes secreted (111, 112). Also, essential oil inhibited the growth of most pure bacteria tested, with *S. bovis* being the most resistant species, and *P. ruminicola*, *Clostridium sticklandii*, and *Peptostreptococcus anaerobius* the most sensitive species (113). The mode of action of essential oil on the formation of ammonia involves reduction in deamination of AA by hyper-ammonia-producing bacteria (114).

Plant phenolic compounds from tea and berries have also been reported to have antimicrobial properties and modulate gut microbiota in humans. Studies with pure bacteria cultures isolated from human fecal samples demonstrated that the growth of certain pathogenic AA-fermenting bacteria such as *Clostridium perfringens*, *Clostridium difficile*, *Streptococcus pneumoniae*, *Bacteroides fragilis*, *Staphylococcus aureus*, *Salmonella typhimurium* and *Escherichia coli* was significantly repressed by tea phenolics and their derivatives, while commensal anaerobes like *Lactobacillus rhamnosus*, *Bifidobacterium* spp. and *Clostridium* sp. were less severely affected (115). It was also found that caffeic acid generally exerted a more significant inhibitory effect on the growth of pathogens (e.g., *B. fragilis* and *C. difficile*) than epicatechin, catechin, gallic acid and 3-O-methylgallic acid (115). These results suggest that the chemical structure of a phytochemical might be important for its antimicrobial effect (115). The anthocyanins from berries were also effective in inhibiting the growth of pathogenic *Staphylococcus* spp., *Salmonella* spp. *Bacillus cereus* and *Helicobacter pylori* (116, 117). The anti-growth effect of berry phenolics on *Helicobacter pylori* may be mediated through urease inhibition and disruption of ATP production from proline due to an inhibition of proline dehydrogenase (118). Thus, modulation of the gut microbial ecology may be one of the mechanisms for phytochemicals such as polyphenol, tannin, saponin and essential oil to promote the health status of humans and animals (119). However, further *in vitro* and *in vivo* studies are warranted to define the effects of these phytochemicals on microbial nitrogen metabolism and microbial community structure in relation to differences in diets and gut environments. Other issues to be considered should include chemical structure, antimicrobial properties of phytochemicals, long-term effects, the interplay between phytochemicals and gut microbiome, and the consequence to host health (114, 119, 120).

7. CONCLUSIONS AND PERSPECTIVES

During the evolution of animals and their gut microbiota, dietary AA may serve as one of the selective forces that shape the diversity of the AA-fermenting bacteria and their metabolic pathways for AA utilization in the digestive tract. Through the metabolism of AA, gut bacteria survive, thrive and compete with each other in the complex dietary context and gut environment. The AA-derived molecules produced by gut bacteria create new niches for the well-beings of the bacteria but also present new pressure for the surrounding prokaryotic species and eukaryotic host. Ultimately, gut bacteria affect host health by modulating host nutritional status and metabolic processes in response to consumption of different diets. To date, little is known about the genetics and physiology of the gut microorganisms, which limits our understanding of the functional aspects of the microbiome (the whole microbial community) (9). At present, little is known about utilization of nitrogenous substances (e.g., plant proteins, animal proteins, peptides, free amino acids, and ammonia) by small-intestinal bacteria in nonruminants (e.g., pigs and chicken). Fortunately, with the development of new sequencing techniques and new metabolite analysis platforms, our appreciation of the holistic biological processes in the gut and the whole body is expanding rapidly. Finally, the integration of metagenomics and metabolomics (11), as well as the development of systems biology, will surely help us better understand the mechanism of the *in vivo* interplays among dietary AA, gut microbiome and host under different nutritional and health conditions (8). Collectively, this work will lead to

development of new concepts and methods for the formulation and management of foods and drugs to improve the well beings of humans, animals, and environment.

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9. REFERENCES

1. Dwayne C Savage: Microbial ecology of the gastrointestinal tract. *Ann Rev Microbial* 31, 107-133 (1977)

2. Guoyao Wu: Amino acids: metabolism, functions, and nutrition. *Amino Acids* 37, 1-17 (2009)

3. Guoyao Wu, Fuller W Bazer, Teresa A Davis, Sung Woo Kim, Peng Li, J Marc Rhoads, M Carey Satterfield, Stephen B Smith, Thomas E Spencer and Yulong Yin: Arginine metabolism and nutrition in growth, health and disease. *Amino Acids* 37(1), 153-168 (2009)

4. John H Cummings and George T Macfarlane: Colonic microflora: Nutrition and health. *Nutrition* 13(5), 476-478 (1997)

5. Malcolm F Fuller and Peter J Reeds: Nitrogen cycling in the gut. *Annu Rev Nutr* 18, 385-411 (1998)

6. Cornelia C Metges: Contribution of microbial amino acids to amino acid homeostasis of the host. *J Nutr* 130, 1857S-1864S (2000)

7. Werner G Bergen and Guoyao Wu: Intestinal nitrogen recycling and utilization in health and disease. *J Nutr* 139(5), 821-825 (2009)

8. Jeremy K Nicholson, Elaine Holmes and Ian D Wilson: Gut microorganisms, mammalian metabolism and personalized health care. *Nat Rev Microbiol* 3(5), 431-438 (2005)

9. Ruth E Ley, Daniel A Peterson and Jeffrey I Gordon: Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell* 124, 837-848 (2006)

10. Harry J Flint, Sylvia H Duncan, Karen P Scott and Petra Louis: Interactions and competition within the microbial community of the human colon: links between diet and health. *Environ Microbiol* 9(5), 1101-1111 (2007)

11. Peter J Turnbaugh and Jeffrey I Gordon: An invitation to the marriage of metagenomics and metabolomics. *Cell* 134, 708-713 (2008)

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

13. Margaret J Duncan: Genomics of oral bacteria. *Crit Rev Oral Biol Med* 14, 175-187 (2003)

14. Elizabeth A Smith and George T Macfarlane: 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 (1998)

15. X Wang, S P Heazlewood, D O Krause and T H J Florin: Molecular characterization of the microbial species that colonize human ileal and colonic mucosa by using 16S rDNA sequence analysis. *J Appl Microbiol* 95, 508-520 (2003)

16. Hidenori Hayashi, Rei Takahashi, Takahiro Nishi, Mitsuo Sakamoto and Yoshimi Benno: Molecular analysis of jejunal, ileal, caecal and recto-sigmoidal human colonic microbiota using 16S rRNA gene libraries and terminal restriction fragment length polymorphism. *J Med Microbiol* 54, 1093-1101 (2005)

17. Mei Wang, Siv Ahrné, Bengt Jeppsson and Göran Molin: Comparison of bacterial diversity along the human intestinal tract by direct cloning and sequencing of 16S rRNA genes. *FEMS Microbiol Ecol* 54, 219-231 (2005)

18. Sergey R Konstantinov, Ajay A Awati, Barbara A Williams, Bevis G Miller, Philip Jones, Christopher R Stokes, Antoon D L Akkermans, Hauke Smidt and Willem M de Vos: Post-natal development of the porcine microbiota composition and activities. *Environ Microbiol* 8(7), 1191-1199 (2006)

19. Carien C G M Booijink, Erwin G Zoetendal, Michiel Kleerebezem and Willem M de Vos: Microbial communities in the human small intestine: coupling diversity to metagenomics. *Future Microbiol* 2(3), 285-295 (2007)

20. Carien C G M Booijink: Analysis of diversity and function of the human small intestinal microbiota (Thesis). *Wageningen University Press*, The Netherlands (2009)

21. Zhao-lai Dai, Jing Zhang, Guoyao Wu and Wei-Yun Zhu: Utilization of amino acids by bacteria from the pig small intestine. *Amino Acids* DOI 10.1007/s00726-010-0556-9 (2010)

22. Tugrul T Kararli: Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. *Biopharm Drug Dispos* 16, 351-380 (1995)

23. R John Wallace: Ruminal microbial metabolism of peptides and amino acids. *J Nutr* 126, 1326S-1334S (1996)

24. Sylvia H Duncan, G E Lobley, G Holtrop, J Ince, A M Johnstone, P Louis and H J Flint: Human colonic microbiota associated with diet, obesity and weight loss. *Int J Obes (Lond)* 32, 1720-1724 (2008)

25. Sylvia H Duncan, Petra Louis, John M Thomson and Harry J Flint: The role of pH in determining the species composition of the human colonic microbiota. *Environ Microbiol* 11(8), 2112-2122 (2009)

26. Aileen Joy O Libao-Mercado, Cuilan L Zhu, John P Cant, Hélène Lapierre, Jean-Noël Thibault, Bernard Sève, Malcolm F Fuller and Cornelis F M de Lange: 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 (2009)

27. J R Ling and I P Armstead: The *in vitro* uptake and metabolism of peptides and amino acids by five species of rumen bacteria. *J Appl Bacteriol* 78, 116-124 (1995)

28. James B Russell, Herbert J Strobel, Arnold J M Driessen and Wil N Konings: Sodium-dependent transport of neutral amino acids by whole cells and membrane vesicles of *Streptococcus bovis*, a ruminal bacterium. *J Bacteriol* 170(8), 3531-3536 (1988)

29. Guangjiong Chen and James B Russell: Transport of glutamine by *Streptococcus bovis* and conversion of glutamine to pyroglutamic acid and ammonia. *J Bacteriol* 171(6), 2981-2985 (1989)

30. Guangjiong Chen and James B Russell: Sodium-dependent transport of branched-chain amino acids by a monensin-sensitive ruminal peptostreptococcus. *Appl Environ Microbiol* 55(10), 2658-2663 (1989)

31. Guangjiong Chen and James B Russell: Transport and deamination of amino acids by a gram-positive, monensin-sensitive ruminal bacterium. *Appl Environ Microbiol* 56(7), 2186-2192 (1990)

32. Scott A Martin: Nutrient transport by ruminal bacteria: a review. *J Anim Sci* 72, 3019-3031 (1994)

33. J S Van Kessel and J B Russell: Energetics of arginine and lysine transport by whole cells and membrane vesicles of strain SR, a monensin-sensitive ruminal bacterium. *Appl Environ Microbiol* 58(3), 969-972 (1992)

34. Bruce J Paster, James B. Russell, C M J Yang, J M Chow, Carl R Woese and R Tanner: Phylogeny of the ammonia-producing ruminal bacteria *Peptostreptococcus anaerobius*, *Clostridium sticklandii*, and *Clostridium aminophilum* sp. nov. *Int J Syst Bacteriol* 43(1), 107-110 (1993)

35. Cengiz Atasoglu, Carmen Valdes, Nicola D Walker, C James Newbold and R John Wallace: De novo synthesis of amino acids by the ruminal bacteria *Prevotella bryantii* B14, *Selenomonas ruminantium* HD4, and *Streptococcus bovis* ES1. *Appl Environ Microbiol* 64(8), 2836-2843 (1998)

36. A Bach, S Calsamiglia and M D Stern: Nitrogen Metabolism in the Rumen. *J Dairy Sci* 88(E Suppl), E9-E21 (2005)

37. David Torrallardona, C Ian Harris and Malcolm F Fuller: Lysine synthesized by the gastrointestinal microflora of pigs is absorbed, mostly in the small intestine. *Am J Physiol Endocrinol Metab* 284, E1177-E1180 (2003)

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

39. Larry Reitzer: Nitrogen assimilation and global regulation in *Escherichia coli*. *Annu Rev Microbiol* 57,155-176 (2003)

40. James B Russell, J D O'Connor, D G Fox, P J Van Soest and C J Sniffen: A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. *J Anim Sci* 70(11), 3551-3561 (1992)

41. James B Russell, C J Sniffen and P J Van Soest. Effect of carbohydrate limitation on degradation and utilization of casein by mixed rumen bacteria. *J Dairy Sci* 66(4), 763-775 (1983)

42. Cornelia C Metges and Klaus J Petzke: Utilization of essential amino acids synthesized in the intestinal microbiota of monogastric mammals. *Br J Nutr* 94, 621-622 (2005)

43. David Torrallardona, C Ian Harris and Malcolm F Fuller: Pigs' gastrointestinal microflora provide them with essential amino acids. *J Nutr* 133, 1127-1131 (2003)

44. Cornelia C Metges, Antoine E El-Khoury, Lidewij Henneman, Klaus J Petzke, Ian Grant, Shahinaze Bedri, Paulo P Pereira, Alfred M Ajami, Malcolm F Fuller and Vernon R Young: Availability of intestinal microbial lysine for whole body lysine homeostasis in human subjects. *Am J Physiol Endocrinol Metab* 277, 597-607 (1999)

45. Cornelia C Metges, Klaus J Petzke, Shahinaze Bedri, Malcolm F Fuller and Vernon R Young: The quantitative contribution of microbial lysine to lysine flux in healthy male subjects. *FASEB J* 11, A149 (1997)

46. Guoyao Wu: Urea synthesis in enterocytes of developing pigs. *Biochem J* 312, 717-23 (1995)

47. Barbara Stoll, Joseph Henry, Peter J Reeds, Hung Yu, Farook Jahoor and Douglas G Burrin: Catabolism dominates the first-pass intestinal metabolism of dietary essential amino acids in milk protein-fed piglets. *J Nutr* 128, 606–614 (1998)

48. Guoyao Wu: Intestinal mucosal amino acid catabolism. *J Nutr* 128, 1249-1252 (1998)

49. Lixiang Chen, Yu-Long Yin, Wenjuan S Jobgen, Scott C Jobgen, Darrell A Knabe, Wei-Xin Hu and Guoyao Wu: In vitro oxidation of essential amino acids by jejunal mucosal cells of growing pig. *Livest Sci* 109, 19-23 (2007)

50. Lixiang Chen, Peng Li, Junjun Wang, Xilong Li, Haijun Gao, Yulong Yin, Yongqing Hou and Guoyao Wu: Catabolism of nutritionally essential amino acids in developingmporcine enterocytes. *Amino Acids* 37, 143-152 (2009)

51. George T Macfarlane and John H Cummings: The colonic flora, fermentation and large bowel digestive function In: The large intestine: physiology, pathophysiology and disease. Eds: S F Philipps, J H Pemberton, R G Shorter, *Raven Press*, New York (1991)

52. H A Barker: Amino acid degradation by anaerobic bacteria. *Annu Rev Biochem* 50, 23-40 (1981)

53. Elizabeth A Smith and George T Macfarlane: Studies on amine production in the human colon: Enumeration of amine forming bacteria and physiological effects of carbohydrate and pH. *Anaerobe* 2, 285-297 (1996)

54. Dixie D Whitt and R D Demoss: Effect of microflora on the free amino acid distribution in various regions of the mouse gastrointestinal tract. *Appl Microbiol* 30(4), 609-615 (1975)

55. Roel Van der Meulen, Nicholas Camu, Timo Van Vooren, Christophe Heymans and Luc De Vuyst: In vitro kinetic analysis of carbohydrate and aromatic amino acid metabolism of different members of the human colon. *Int J Food Microbiol* 124, 27–33 (2008)

56. Michael A Mahowald, Federico E Rey, Henning Seedorf, Peter J Turnbaugh, Robert S Fulton, Aye Wollam, Neha Shah, Chunyan Wang, Vincent Magrini, Richard K Wilson, Brandi L Cantarel, Pedro M Coutinho, Bernard Henrissat, Lara W Crock, Alison Russell, Nathan C Verberkmoes, Robert L Hettich and Jeffrey I Gordon: Characterizing a model human gut microbiota composed of members of its two dominant bacterial phyla. *Proc Natl Acad Sci U S A* 106(14), 5859-5864 (2009)

57. Peter A Bron, Corinne Grangette, Annick Mercenier, Willem M de Vos and Michiel Kleerebezem: Identification of *Lactobacillus plantarum* genes that are induced in the gastrointestinal tract of mice. *J Bacteriol* 186(17), 5721-5729 (2004)

58. Maria L Marco, Roger S Bongers, Willem M de Vos and Michiel Kleerebezem: Spatial and temporal expression of *Lactobacillus plantarum* genes in the gastrointestinal tracts of mice. *Appl Environ Microbiol* 73(1), 124-132 (2007)

59. Yang Fang, Junjun Wang, Xiaojie Li, Tianyi Ying, Shiyan Qiao, Defa Li and Guoyao Wu: 2-DE and MS analysis of interactions between *Lactobacillus fermentum* I5007 and intestinal epithelial cells. *Electrophoresis* 28, 4330-4339 (2007)

60. John D Hayes, Jack U Flanagan and Ian R Jowsey: Glutathione transferases. *Annu Rev Pharmacol Toxicol* 45, 51-88 (2005)

61. E Scharmach, S Hessel, B Niemann and A Lampen: Glutathione S-transferase expression and isoenzyme composition during cell differentiation of Caco-2 cells. *Toxicology* 265, 122-126 (2009)

62. Sandrine P Claus, Tsz M Tsang, Yulan Wang, Olivier Cloarec, Eleni Skordi, François-Pierre Martin, Serge Rezzi, Alastair Ross, Sunil Kochhar, Elaine Holmes and Jeremy K Nicholson: Systemic multicompartmental effects of the gut microbiome on mouse metabolic phenotypes. *Mol Syst Biol* 4, 219 (2008)

63. Steven B Heymsfield, Carlos Arteaga, Clifford McManus, Janet Smith and Steven Moffitt: Measurement of muscle mass in humans: validity of the 24-hour urinary creatinine method. *Am J Clin Nutr* 37, 478-494 (1983)

64. D E Metzler: The metabolism of nitrogen and amino acids. In: Biochemistry: the Chemical Reactions of Living Cells, Eds: J Hayhurst, 1358-1419, *Elsevier Science*, USA (2003)

65. Guoyao Wu, Fuller W Bazer, Teresa A Davis, Laurie A Jaeger, Greg A Johnson, Sung Woo Kim, Darrell A Knabe, Cynthia J Meininger, Thomas E Spencer and Yu-Long Yin: Important roles for the arginine family of amino acids in swine nutrition and production. *Livest Sci* 112, 8-22 (2007)

66. Paul D Cotter and Colin Hill: Surviving the acid test: Responses of gram-positive bacteria to low pH. *Microbiol Mol Biol Rev* 67(3), 429-453 (2003)

67. Nobuhiro Takahashi. Acid-neutralizing activity during amino acid fermentation by *Porphyromonas gingivalis*, *Prevotella intermedia* and *Fusobacterium nucleatum*. *Oral Microbiol Immunol* 18(2), 109-113 (2003)

68. Yaling Liu, Yiqian Dong, Yi-Ywan M Chen, and Robert A Burne: Environmental and growth phase regulation of the *Streptococcus gordonii* arginine deiminase gene. *Appli Enviro Microbiol* 74 (16), 5023-5030 (2008)

69. Clive Allison and George T Macfarlane: Influence of pH, nutrient availability, and growth rate on amine production by *Bacteroides fragilis* and *Clostridium perfringens*. *Appl and Environ Microbiol* 55(11), 2894-2898 (1989)

70. Hope Richard and John W Foster: *Escherichia coli* glutamate- and arginine-dependent acid resistance systems increase internal pH and reverse transmembrane potential. *J Bacteriol* 186(18), 6032-6041 (2004)

71. Edward Guccione, Maria del Rocio Leon-Kempis, Bruce M Pearson, Edward Hitchin, Francis Mulholland, Pauline M van Diemen, Mark P Stevens and David J Kelly: Amino acid-dependent growth of *Campylobacter jejuni*: key roles for aspartase (AspA) under microaerobic and

oxygen-limited conditions and identification of AspB (Cj0762), essential for growth on glutamate. *Mol Microbiol* 69(1), 77-93 (2008)

72. Patrice L Moreau: The lysine decarboxylase CadA protects *Escherichia coli* starved of phosphate against fermentation acids. *J Bacteriol* 189(6), 2249-2261 (2007)

73. Ulrich E Schaible and Stefan H E Kaufmann: A nutritive view on the host–pathogen interplay. *Trends Microbiol* 13(8), 373-380 (2005)

74. Sergey R Konstantinov, Hauke Smidt, Willem M de Vos, Sven C M Bruijns, Satwinder Kaur Singh, Florence Valence, Daniel Molle, Sylvie Lortal, Eric Altermann, Todd R Klaenhammer and Yvette van Kooyk: S layer protein A of *Lactobacillus acidophilus* NCFM regulates immature dendritic cell and T cell functions. *Proc Natl Acad Sci U S A* 105 (49), 19473-19478 (2008)

75. Sarah Lebeer, Jos Vanderleyden and Sigrid C J De Keersmaecker: Host interactions of probiotic bacterial surface molecules: comparison with commensals and pathogens. *Nat Rev Microbiol* 8, 171-184 (2010)

76. Michael S Chaussee, Greg A Somerville, Larry Reitzer and James M Musser: Rgg coordinates virulence factor synthesis and metabolism in *Streptococcus pyogenes*. J Bacteriol 185(20), 6016-6024 (2003)

77. Janaína R Lima, Andréa de O Barros Ribon, James B Russell and Hilário C Mantovani: Bovicin HC5 inhibits wasteful amino acid degradation by mixed ruminal bacteria *in vitro*. *FEMS Microbiol Lett* 292, 78-84 (2009)

78. Andrew Camilli and Bonnie L Bassler: Bacterial small-molecule signaling pathways. *Science* 311, 1113-1116 (2006)

79. Laurent Keller and Michael G Surette: Communication in bacteria: an ecological and evolutionary perspective. *Nat Rev Microbiol* 4, 249-258 (2006)

80. Marcelo P Sircili, Matthew Walters, Luis R Trabulsi and Vanessa Sperandio: Modulation of enteropathogenic *Escherichia coli* virulence by quorum sensing. *Infect Immun* 72(4), 2329-2337 (2004)

81. B L Buck, M A Azcarate-Peril and T R Klaenhammer: Role of autoinducer-2 on the adhesion ability of *Lactobacillus acidophilus*. *J Appl Microbiol* 107, 269-279 (2009)

82. X G Han and C P Lu: Detection of autoinducer-2 and analysis of the profile of luxS and pfs transcription in *Streptococcus suis* serotype 2. *Curr Microbiol* 58, 146-152 (2009)

83. Karina B Xavier and Bonnie L Bassler: Interference with AI-2-mediated bacterial cell–cell communication. *Nature* 437, 750-753 (2005)

84. Kazuhiro Tateda, Yoshikazu Ishii, Manabu Horikawa, Tetsuya Matsumoto, Shinichi Miyairi, Jean Claude Pechere, Theodore J Standiford, Masaji Ishiguro and Keizo Yamaguchi: The *Pseudomonas aeruginosa* autoinducer N-3-oxododecanoyl homoserine lactone accelerates apoptosis in macrophages and neutrophils. *Infect Immun* 71 (10), 5785-5793 (2003)

85. Doreen S W Hooi, Barrie W Bycroft, Siri Ram Chhabra, Paul Williams and David I Pritchard: Differential immune modulatory activity of *Pseudomonas aeruginosa* quorum-sensing signal molecules. *Infect Immun* 72 (11), 6463-6470 (2004)

86. Carlene K Chun, Egon A Ozer, Michael J Welsh, Joseph Zabner and E P Greenberg: Inactivation of a *Pseudomonas aeruginosa* quorum-sensing signal by human airway epithelia. *Proc Natl Acad Sci U S A* 101(10), 3587-3590 (2004)

87. F Boyen, V Eeckhaut, F Van Immerseel, F Pasmans, R Ducatelle and F Haesebrouck: Quorum sensing in veterinary pathogens: Mechanisms, clinical importance and future perspectives. *Vet Microbiol* 135, 187-195 (2009)

88. J B van Goudoever, B Stoll, J F Henry, D G Burrin and P J Reeds: Adaptive regulation of intestinal lysine metabolism. *Proc Natl Acad Sci USA* 97 (21), 11620-11625 (2000)

89. Michael Blaut: Relationship of prebiotics and food to intestinal microflora. *Eur J Nutr* 41 (Suppl 1), I/11–I/16 (2002)

90. Clive Allison and George T Macfarlane: Regulation of protease production in *Clostridium sporogenes*. *Appl Environ Microbiol* 56(11), 3485-3490 (1990)

91. Alan W Walker, Sylvia H Duncan, E Carol McWilliam Leitch, Matthew W Child and Harry J Flint: pH and peptide supply can radically alter bacterial populations and short-chain fatty acid ratios within microbial communities from the human colon. *Appl Environ Microbiol* 71(7), 3692-3700 (2005)

92. G T Macfarlane, G R Gibson, E Beatty and J H Cummings: Estimation of short chain fatty acid production from protein by human intestinal bacteria, based on branched chain acid measurements. *FEMS Microbiol Ecol* 101, 81-86 (1992)

93. Elizabeth A Magee, Caroline J Richardson, Róisín Hughes and John H Cummings: Contribution of dietary protein to sulfide production in the large intestine: an *in vitro* and a controlled feeding study in humans. *Am J Clin Nutr* 72, 1488-1494 (2000)

94. Sylvia H Duncan, Alvaro Belenguer, Grietje Holtrop, Alexandra M Johnstone, Harry J Flint and Gerald E Lobley: Reduced dietary intake of carbohydrates by obese subjects results in decreased concentrations of butyrate and

butyrate-producing bacteria in feces. *Appl Environ Microbiol* 73(4), 1073-1078 (2007)

95. Charles J Smith and Marvin P Bryant: Introduction to metabolic activities of intestinal bacteria. *Am J Clin Nutr* 32, 149-157 (1979)

96. Glenn R Gibson and Marcel B Roberfroid: Dietary modulation of the human colony microbiota: Introducing the concept of prebiotics. *J Nutr* 125, 1401-1412 (1995)

97. A J McBain and G T Macfarlane: Modulation of genotoxic enzyme activities by non-digestible oligosaccharide metabolism in in-vitro human gut bacterial ecosystems. *J Med Microbiol* 50, 833-842 (2001)

98. Elizabeth A Smith and George T Macfarlane: Formation of phenolic and indolic compounds by anaerobic bacteria in the human large intestine. *Microb Ecol* 33, 180-188 (1997)

99. Francois-Pierre J Martin, Norbert Sprenger, Ivan K S Yap, Yulan Wang, Rodrigo Bibiloni, Florence Rochat, Serge Rezzi, Christine Cherbut, Sunil Kochhar, John C Lindon, Elaine Holmes and Jeremy K Nicholson: Panorganismal gut microbiome-host metabolic crosstalk. *J Proteome Res* 8, 2090-2105 (2009)

100. G T Macfarlane and S Macfarlane: Factors affecting fermentation reactions in the large bowel. *Proc Nutr Soc* 52, 361-313 (1993)

101. Elizabeth A Smith and George T Macfarlane: Enumeration of human colonic bacteria producing phenolic and indolic compounds: effects of pH, carbohydrate availability and retention time on dissimilatory aromatic amino acid metabolism. *J Appl Bacteriol* 81, 288-302 (1996)

102. Petra Louis and Harry J Flint: Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. *FEMS Microbiol Lett* 294(1), 1-8 (2009)

103. Petra Louis, Karen P Scott, Sylvia H Duncan and Harry J Flint: Understanding the effects of diet on bacterial metabolism in the large intestine. *J Appl Microbiol* 102, 1197-1208 (2007)

104. Francois-Pierre J Martin, Yulan Wang, Norbert Sprenger, Elaine Holmes, John C Lindon, Sunil Kochhar, and Jeremy K Nicholson: Effects of probiotic *Lactobacillus paracasei* treatment on the host gut tissue metabolic profiles probed via Magic-Angle-Spinning NMR spectroscopy. *J Proteome Res* 6, 1471-1481 (2007)

105. Francois-Pierre J Martin, Yulan Wang, Norbert Sprenger, Ivan K S Yap, Torbjörn Lundstedt, Per Lek, Serge Rezz, Ziad Ramadan, Peter van Bladeren, Laurent B Fay, Sunil Kochhar, John C Lindon, Elaine Holmes and Jeremy K Nicholson: Probiotic modulation of symbiotic gut microbial–host metabolic interactions in a humanized microbiome mouse model. *Mol Syst Biol* 4, 157 (2008)

106. Maurice Ndagijimana, Luca Laghi, Beatrice Vitali, Giuseppe Placucci, Patrizia Brigidi and M Elisabetta Guerzoni: Effect of a synbiotic food consumption on human gut metabolic profiles evaluated by ^{1}H Nuclear Magnetic Resonance spectroscopy. *Int J Food Microbiol* 134, 147-153 (2009)

107. Rui Hai Liu: Potential synergy of phytochemicals in cancer prevention: mechanism of action. *J Nutr* 134, 3479S-3485S (2004)

108. R J Wallace, L Arthaud and C J Newbold: Influence of *Yucca schidigera* extract on ruminal ammonia concentration and ruminal micro-organisms. *Appl Environ Microbiol* 60, 1762-1767 (1994)

109. Y Wang, T A McAllister, L J Yanke and P R Cheeke: Effect of steroidal saponin from *Yucca schidigera* extract on ruminal microbes. *J Appl Microbiol* 88, 887-896 (2000)

110. B R Min, G T Attwood, W C McNabb, A L Molan and T N Barry: The effect of condensed tannins from *Lotus corniculatus* on the proteolytic activities and growth of rumen bacteria. *Anim Feed Sci Technol* 121, 45-58 (2005)

111. G A Jones, T A McAllister, A D Muir and K J Cheng: Effects of sainfoin (*Onobrychis viciifolia* Scop.) condensed tannins on growth and proteolysis by four strains of ruminal bacterium. *Appl Environ Microbiol* 60, 1374-1378 (1994)

112. Alexandra H Smith, Erwin Zoetendal and Roderick I Mackie: Bacterial mechanisms to overcome inhibitory effects of dietary tannins. *Microb Ecol* 50, 197-205 (2005)

113. F M McIntosh, P Williams, R Losa, R J Wallace, D A Beever and C J Newbold: Effects of essential oils on ruminal microorganisms and their protein metabolism. *Appl Environ Microbiol* 69, 5011-5014 (2003)

114. R J Wallace: Antimicrobial properties of plant secondary metabolites. *Proc Nutr Soc* 63, 621-629 (2004)

115. Hui Cheng Lee, Andrew M Jenner, Chin Seng Low and Yuan Kun Lee: Effect of tea phenolics and their aromatic fecal bacterial metabolites on intestinal microbiota. *Res Microbiol* 157, 876-884 (2006)

116. R Puupponen-Pimiä, L Nohynek, S Hartmann-Schmidlin, M Kähkönen, M Heinonen, K Määttä-Riihinen and K-M Oksman-Caldentey: Berry phenolics selectively inhibit the growth of intestinal pathogens. *J Appl Microbiol* 98, 991-1000 (2005)

117. Liisa J Nohynek, Hanna-Leena Alakomi, Marja P Kähkönen, Marina Heinonen, Ilkka M Helander, Kirsi-Marja Oksman-Caldentey and Riitta H Puupponen-Pimiä: Berry phenolics: antimicrobial properties and mechanisms

of action against severe human pathogens. *Nutr Cancer* 54(1), 18-32 (2006)

118. Y T Lin, Y I Kwon, R G Labbe and K Shetty: Inhibition of *Helicobacter pylori* and associated urease by oregano and cranberry phytochemical synergies. *Appl Environ Microbiol* 71(12), 8558-8564 (2005)

119. J M Laparra and Y Sanz: Interactions of gut microbiota with functional food components and nutraceuticals. *Pharmacol Res* 61, 219-225 (2010)

120. Amlan K Patra and Jyotisna Saxena: Dietary phytochemicals as rumen modifiers: a review of the effects on microbial populations. *Antonie Van Leeuwenhoek* 96, 363-375 (2009)

121. W J Loesche and R J Gibbons: Amino acid fermentation by *Fusobacterium nucleatum*. *Arch Oral Biol* 13, 191-201 (1968)

122. Yoram Bouhnik, Sophie Alain, Alain Attar, Bernard Flourié, Laurent Raskine, Marie José Sanson-Le Pors and Jean-Claude Rambaud: Bacterial populations contaminating the upper gut in patients with small intestinal bacterial overgrowth syndrome. *Am J Gastroenterol*, 94, 1327-1331 (1999)

123. C D Boiangiu, E Jayamani, D Brügel, G Herrmann, J Kim, L Forzi, R Hedderich, I Vgenopoulou, A J Pierik, J Steuber and W Buckel: Sodium ion pumps and hydrogen production in glutamate fermenting anaerobic bacteria. *J Mol Microbiol Biotechnol* 10(2-4), 105-119 (2005)

124. W L George, B D Kirby, V L Sutter, D M Citron and S M Finegold. Gram-negative anaerobic bacilli: Their role in infection and patterns of susceptibility to antimicrobial agents. II. Little-known *Fusobacterium* species and miscellaneous genera. *Rev Infect Dis* 3(3), 599-626 (1981)

125. J Potrykus, R L White and S L Bearne: Proteomic investigation of amino acid catabolism in the indigenous gut anaerobe *Fusobacterium varium*. *Proteomics* 8, 2691-2703 (2008)

126. H A Campbell, L T Mashburn, E A Boyse and L J Old: Two L-asparaginases from *Escherichia coli* B. Their separation, purification, and antitumor activity. *Biochemistry* 6, 721-730 (1967)

127. J S Heller, R Rostomily, D A Kyriakidis and E S Canellakis: Regulation of polyamine biosynthesis in Escherichia coli by basic proteins. *Proc Natl Acad Sci U S A* 80(17), 5181-5184 (1983)

128. T T Kararli: Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. *Biopharm Drug Dispos* 16, 351-380 (1995)

129. I H Gewolb, R S Schwalbe, V L Taciak, T S Harrison and P Panigrahi: Stool microflora in extremely low birthweight infants. *Arch Dis Child Fetal Neonatal Ed* 80(3), F167-173 (1999)

130. H Richard and J W Foster: *Escherichia coli* Glutamate- and arginine-dependent acid resistance systems increase internal pH and reverse transmembrane potential. *J Bacteriol* 186(18), 6032-6041 (2004)

131. P Schierack, N Walk, K Reiter, K D Weyrauch and L H Wieler: Composition of intestinal Enterobacteriaceae populations of healthy domestic pigs. *Microbiology* 153(Pt 11), 3830-3837 (2007)

132. Guangjiong Chen and James B Russell: Transport of glutamine by *Streptococcus bovis* and conversion of glutamine to pyroglutamic acid and ammonia. *J Bacteriol* 171(6), 2981-2985 (1989)

133. A A Salyers, S E West, J R Vercellotti and T D Wilkins: Fermentation of mucins and plant polysaccharides by anaerobic bacteria from the human colon. *Appl Environ Microbiol* 34(5), 529-533 (1977)

134. I M Robinson, M J Allison and J A Bucklin: Characterization of the cecal bacteria of normal pigs. *Appl Environ Microbiol* 41(4), 950-955 (1981)

135. Guangjiong Chen and James B Russell: Fermentation of peptides and amino acids by a monensin-sensitive ruminal peptostreptococcus. *Appl Environ Microbiol* 54(11), 2742-2749 (1988)

136. S R Elsden and M G Hilton: Volatile acid production from threonine, valine, leucine and isoleucine by clostridia. *Arch Microbiol* 117(2), 165-172 (1978)

137. M L Britz and R G Wilkinson: Leucine dissimilation to isovaleric and isocaproic acids by cell suspensions of amino acid fermenting anaerobes: the Stickland reaction revisited. *Can J Microbiol* 28(3), 291-300 (1982)

138. Leonard Hubert Stickland: Studies in the metabolism of the strict anaerobes (genus Clostridium): The chemical reactions by which *Cl. sporogenes* obtains its energy. *Biochem J* 28(5), 1746-1759 (1934)

139. J M Poston: Leucine 2,3-aminomutase, an enzyme of leucine catabolism. *J Biol Chem* 251(7), 1859-1863 (1976)

140. G Attwood, D Li, D Pacheco and M Tavendale: Production of indolic compounds by rumen bacteria isolated from grazing ruminants. *J Appl Microbiol* 100(6), 1261-1271 (2006)

141. J Kenklies, R Ziehn, K Fritsche, A Pich and J R Andreesen: Proline biosynthesis from L-ornithine in *Clostridium sticklandii*: purification of delta1-pyrroline-5-carboxylate reductase, and sequence and expression of the encoding gene, proC. *Microbiology* 145 (Pt 4), 819-826 (1999)

142. S E Perkins, J G Fox, N S Taylor, D L Green and N S Lipman: Detection of *Clostridium difficile* toxins from the

small intestine and cecum of rabbits with naturally acquired enterotoxemia. *Lab Anim Sci* 45, 379-384 (1995)

143. L G Arroyo, S A Kruth, B M Willey, H R Staempfli, D E Low and J S Weese: PCR ribotyping of *Clostridium difficile* isolates originating from human and animal sources. *J Med Microbiol* 54(Pt 2), 163-166 (2005)

144. V Zidaric, M Zemljic, S Janezic, A Kocuvan and M Rupnik: High diversity of *Clostridium difficile* genotypes isolated from a single poultry farm producing replacement laying hens. *Anaerobe* 14(6), 325-327 (2008)

145. J Avbersek, S Janezic, M Pate, M Rupnik, V Zidaric, K Logar, M Vengust, M Zemljic, T Pirs and M Ocepek: Diversity of *Clostridium difficile* in pigs and other animals in Slovenia. *Anaerobe* 15(6), 252-255 (2009)

146. R J Wallace: Catabolism of amino acids by *Megasphaera elsdenii* LC1. *Appl Environ Micribiol* 51(5): 1141-1143 (1986)

147. H Werner: *Megasphaera elsdenii*-a normal inhabitant of human large intestine. *Zentralbl Bakteriol Orig A* 223(2), 343-347 (1973)

148. P T Sugihara, V L Sutter, H R Attebery, K S Bricknell and S M Finegold: Isolation of *Acidaminococcus fermentans* and *Megasphaera elsdenii* from normal human feces. *Appl Microbiol* 27(1), 274-275 (1974)

149. De Man Rogosa: *Acidaminococcus* gen. n., *Acidaminococcus fermentans* sp. n., anaerobic gram-negative diplococci using amino acids as the sole energy source for growth. *J Bacteriol* 98(2), 756-766 (1969)

150. Y Kamio and Y Terawaki: Purification and properties of *Selenomonas ruminantium* lysine decarboxylase. *J Bacteriol* 153(2), 658-664 (1983)

151. S Liao, P Poonpairoj, K C Ko, Y Takatuska, Y Yamaguchi, N Abe, J Kaneko and Y Kamio: Occurrence of agmatine pathway for putrescine synthesis in *Selenomonas ruminatium*. Biosci Biotechnol *Biochem* 72(2), 445-455 (2008)

152. J N Lopes and F S Cruz: Chemically defined media for growing anaerobic bacteria of the genus Veillonella. *Antonie Van Leeuwenhoek* 42(4), 411-420 (1976)

153. Mareike Kraatz and David Taras: *Veillonella magna* sp. nov., isolated from the jejunal mucosa of a healthy pig, and emended description of *Veillonella ratti*. *Int J Syst Evol Microbiol* 58(Pt 12), 2755-2761 (2008)

154. L L Myers and D S Shoop: Association of enterotoxigenic *Bacteroides fragilis* with diarrheal disease in young pigs. *Am J Vet Res* 48(5), 774-775 (1987)

155. J M Howe, W R Featherston, W J Stadelman and G J Banwart: Amino acid composition of certain bacterial cell-wall proteins. *Appl Microbiol* 13(5), 650-652 (1965)

156. D B Purser and SM Buechler: Amino acid composition of rumen organisms. *J Dairy Sci* 49(1), 81-84 (1966)

157. Milton J Allison and J L Peel: The biosynthesis of valine from isobutyrate by *Peptostreptococcus elsdenii* and *Bacteroides ruminicola*. *Biochem J* 121, 431-437 (1971)

158. Milton J Allison, Albert L Baetz and Juergen Wiegel: Alternative pathways for biosynthesis of leucine and other amino acids in *Bacteroides ruminicola* and *Bacteroides fragilis*. Appl Environ Microbiol 48(6), 1111-1117 (1984)

159. W A Hullah and T H Blackburn: Uptake and incorporation of amino acids and peptides by *Bacteroides amylophilus*. *Appl Microbiol* 21(2), 187-191 (1971)

160. G C Mead: The amino acid-fermenting Clostridia. *J Gen Microbiol* 67, 47-56 (1971)

161. N J Hoogenraad and F J R Hird: The chemical composition of rumen bacteria and cell walls from rumen bacteria. *Br J Nutr* 24, 119-127 (1970)

162. Frank D Sauer, James D Erfle and Subramaniam Mahadevan: Amino acid biosynthesis in mixed rumen cultures. *Biochem J* 150, 357-372 (1975)

163. J S Van Kessel and J B Russell: Energetics of Arginine and Lysine Transport by whole cells and membrane vesicles of strain SR, a monensin-sensitive ruminal bacterium. *Appl Environ Microbiol* 58(3), 969-972 (1992)

164. Graeme T Attwood, Athol V Klieve, Diane Ouwerkerk and Bharat KC Patel: Ammonia-hyperproducing bacteria from New Zealand ruminants. *Appl Environ Microbiol* 64(5), 1796-1804 (1998)

165. Jennifer L Rychlik and James B Russell: The adaptation and resistance of *Clostridium aminophilum* F to the butyrivibriocin-like substance of *Butyrivibrio fibrisolvens* JL5 and monensin. *FEMS Microbiol Lett* 209(1), 93-98 (2002)

166. Terence R Whitehead and Michael A Cotta: Isolation and identification of hyper-ammonia producing bacteria from swine manure storage pits. *Curr Microbiol* 48(1), 20-26 (2004)

167. R C Anderson, M D Flythe, N A Krueger, T R Callaway, T S Edrington, R B Harvey and D J Nisbet: Decreased competiveness of the foodborne pathogen *Campylobacter jejuni* during co-culture with the hyper-ammonia producing anaerobe *Clostridium aminophilum*. *Folia Microbiol (Praha)* 55(4), 309-311 (2010)

Abbreviations: AA, amino acids; AHL, acyl-homoserine lactones; AI-2, autoinducer-2; AR2 and AR3, acid resistance system 2 and 3; GABA, gamma-aminobutyric acid; GI tract, gastrointestinal tract; NDC, non-digestible carbohydrates

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