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Probiogenomic Analysis of Three Commonly Occuring Bifidobacterial Species

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ABSTRACT OF THE THESIS

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Bifidobacteria are gram-positive prokaryotes that contribute to a healthy gut microbiome and are known to play important roles in conferring health benefits to the host. Yet, very little is known about the mechanisms behind these probiotic effects. In this study, we identified transport systems and substrates within three distinct *Bifidobacterium* species: a probiotic strain found in the distal gut (*B. longum*), a probiotic strain found in breast milk and the gut (*B. animalis*), and an opportunistic pathogen found

in dental caries (*B. dentium*). Transport systems characteristic of each species were tabulated and then compared. We found that *B. longum* and *B. dentium* have carbohydrate and MDR transporters that reflect their diametric environmental locations in the gastrointestinal tract (GIT). *B. dentium* and *B. animalis* share acid resistance systems to survive in their low pH environments (the oral cavity for *B. dentium* and breast milk for *B. animalis*). In addition, we discovered an abundance of vitamin transporters in *B. longum* which may explain some of its proposed probiotic effects. Finally, the GadBC system for gamma-aminobutyrate production and secretion was found only in *B. dentium*, confirming several studies that suggest that this oral pathogen may have the potential to confer probiotic effects in the gut. The results indicate that the transportome of each species reflects adaptation to its ecological niche and reveal distinguishing features of each strain, which provide the basis for future industrial and medical applications of beneficial bacteria.

INTRODUCTION

The definition of probiotics, according to criteria set forth by the Food and Agriculture Organization (FAO) / World Health Organization (WHO), is live organisms which when administered orally have the capacity to successfully colonize the human gut and confer health benefits to the host [10]. These health benefits can include strengthening of the intestinal barrier, augmenting the immune response, secretion of antimicrobial molecules to antagonize pathogens, and competition for mucosal binding sites. Evidence for all of these benefits have been documented *in vivo*, but the molecular mechanisms of action are largely unknown or highly specific to one or two strains [11]. *Actinobacteria* diverged from other bacteria so long ago that it is impossible to identify the closest related bacterial group with certainty, making characterizations of their proteomes difficult. Exacerbating this problem is the fact that a subset of *Actinobacteria*, including *Bifidobacterium*, has been described as having attained novel genes by horizontal gene transfer (HGT) [13]. HGT allows for rapid environment-specific adaptation, which may lead to bacterial diversification and speciation [12].

Bifidobacteria are gram-positive *Actinobacteria* that are utilized and recognized for their probiotic effects on a wide range of ailments, including metabolic syndrome, obesity, inflammatory enteritis, bowel irritability, celiac disease, colorectal carcinogenesis, and even mental disorders like depression and anxiety [1-4]. Some of these probiotic effects seem to be a result of *Bifidobacterium*'s unusual ability to ferment oligosaccharides like fructooligosaccharides and galactooligosaccharides, while other effects may simply be due to their superior ability to outcompete other enteric bacteria and/or discourage the growth of some gram-negative pathogens [3, 6]. Because most

bifidobacteria are anaerobic, probiotic activities are mostly observed in oxygen-poor environments, such as the human gastrointestinal tract. However, some bifidobacteria are oxygen-tolerant and a few others are microaerophilic [7]. Due to the diverse characteristics of these bacteria, we sought to examine the transporter distributions of various species in the genus *Bifidobacterium* in order to elucidate any distinguishing features that may explain any established or supposed probiotic or pathogenic effects.

B. longum is one of the first species to colonize the sterile GIT's of newborns and tends to persist and dominate the gut microbiota throughout life, having been observed at high rates in the intestines of infants to centenarians [14, 18]. A progressive colonization of the gut by *B. longum* is thought to be an important step in the build up of immune system tolerance, and lack of such tolerance could possibly lead to food allergies and chronic inflammation [17]. Lack of aerobic or anaerobic respiratory components confirms that *B. longum* is a strict fermentative anaerobe, although the presence of homologues for enzymes that repair oxidative damage suggest it may be slightly aerotolerant [18]. Additionally, *B. longum* inhabits the lower GIT (a niche that is poor in mono- and disaccharides) and possesses extensive carbohydrate metabolism and transport proteins that specialize in breaking down plant-derived dietary fibers [18]. In a recent study, *B. longum* has been implicated in the amelioration of ulcerative colitis and liver injury in mice [20].

Previously, *B. animalis* and *B. lactis* were considered distinct species, but are now regarded as members of the same species with two subspecies: *animalis* and *lactis* [21]. As the name implies, *B. animalis* spp. *lactis* can utilize milk proteins and milk-derived peptides [22]. It is also resistant to acid and oxidative stress, allowing it to grow well in

milk-based media and making *B. animalis* a popular addition to fermented milk products like yogurt [23]. Similar to the effects of *B. longum*, *B. animalis* showed a preventative effect on acute colitis and colitis-associated colon cancer in mice via inhibition of NF-kappa-B activity [15, 24]. Anti-obesity properties were also observed in fatty rats given oral administration of *B. animalis* [25].

While most *Bifidobacterium* species are isolated from the human intestine and some are found in the vagina and breast milk, *B. dentium* is frequently isolated from human dental caries [16]. It is capable of acidogenesis in glucose-containing media, and can produce pH levels below 4.2 [27]. Therefore, while infiltration of tooth enamel by *B. dentium* may not be as life-threatening as conditions caused by other pathogenic bacteria, it is a significant contributor to the development of cavities, one of the most common chronic diseases that goes untreated in underdeveloped countries [28]. Interestingly, unlike other bifidobacterial genomes, not many genes seem to have been introduced into *B. dentium* via horizontal gene transfer [29]. Additionally, in contrast to *B. longum*, which is found in a very distal portion of the GIT, *B. dentium* inhabits the most proximal part, the oral cavity. As a result, it has a larger arsenal of genes to break down simple carbohydrates than most other bifidobacteria [26].

Probiogenomics involves the sequencing, identification and analysis of novel proteins and systems within probiotic gut bacteria in order to gain insight into the molecular basis for their health-promoting effects [11]. Here, we attempted such a study between two enteric probiotic strains, *B. longum JDM301* and *B. animalis* spp. *lactis BL3*, and one opportunistic pathogen found in the oral cavity, *B. dentium JCM1195*.

METHODS

Genome-BLAST (G-BLAST) was used to search for transport protein homologues

In order to generate the transportome for the three organisms, the proteomes of *B. longum* JDM301, *B. animalis* ssp. *lactis* BL3, and *B. dentium* JCM1195 were obtained from GenBank. These specific strains were chosen on the basis of published work confirming their probiotic or pathogenic effects, as well as the quality of the sequenced genomes. They were screened against the Transporter Classification Database (TCDB) for transport protein homologues using G-BLAST [30]. Using FASTA-formatted protein sequences as the queries, G-BLAST retrieves the top TC hits, along with relevant information including the TCID of the hit, the number of amino acyl residues in the hit, the e-value, the numbers of predicted transmembrane segments (TMSs) in both the query and hit, the degree of TMS overlap between query and hit, and the predicted substrate. A low e-value coupled with a high TMS overlap usually indicates a high degree of similarity between the query and hit [31]. TMS predictions were automatically generated by G-BLAST through the Web-based Hydropathy, Amphipathicity, and Topology (WHAT) program, which aligns plots of hydropathy and amphipathicity through the length of the protein [32]. Though TMS alignment is important for determining sufficient homology, proteins without TMSs were included since multicomponent systems often contain soluble components.

Manual examination of homologues to determine false positives

An arbitrary e-value cutoff of 0.00001 was used subsequent to running G-BLAST. Proteins exhibiting e-values greater than this cutoff were examined using

topological data to determine if the queries and hits were true homologues. A false-positive G-BLAST result can occur if two proteins share similarity in the hydrophilic regions, but not in the transmembrane segments. Manually studying the hydropathy profiles for many hits helped determine if the program had missed a TMS or predicted a TMS in an incorrect region.

Identification of substrates and complete systems

Well-characterized protein homologues were assigned a general substrate category and a predicted specific substrate designation. For systems with missing components, the genome context provided by NCBI allowed identification of adjacent proteins that may have been missed by G-BLAST.

RESULTS

Overview of transporter proteins and systems by TC subclass

TCDB organizes membrane transporter proteins using five levels of hierarchical criteria. The criteria are as follows: (i) mode of transport, (ii) energy-coupling mechanism, (iii) phylogenetic coupling within a family (or superfamily), (iv) phylogenetic coupling within a subfamily (or family within a superfamily), and (v) substrate specificity. These criteria then correspond to class, subclass, family (or superfamily), subfamily (or family within a superfamily), and substrate-specific system, respectively [33, 34]. Utilizing this classification system, membrane transport proteins are organized into one of five well-characterized categories (channels, secondary carriers, primary active transporters, group translocators, transmembrane electron flow carriers), or into one of two less well-defined categories (auxiliary transport proteins, putative transporters of unknown function or mechanism of function).

The proteomes of *B. longum* JDM301, *B. dentium* JCM1195T, and *B. animalis* subspecies, *lactis* BL3 were screened against TCDB using G-BLAST. Table 1 shows the distribution of transport proteins by subclass found in the three species of Bifidobacteria examined. Only transmembrane segment-containing permease proteins, and soluble transport proteins that appear to be part of a complete system were included in these results. However, many distinct query transport proteins can be homologues of the same component within a system, meaning that the number of proteins found within a subclass of substrate category doesn't necessarily correspond to the number of unique substrates

transported. Table 2 shows the distribution of transport systems by subclass, which elucidates the number of unique systems within each subclass.

A major difference is in the smaller size of the proteome of *B. animalis* (1532 proteins) compared to those of *B. longum* (1959) and *B. dentium* (2141). Reflecting this trend, *B. animalis* has the fewest well-characterized transporter proteins and systems (238, 156), followed by those of *B. longum* (339, 191) and *B. dentium* (364, 216). The vast majority of proteins and systems are found in either the 2.A subclass, porters, or the 3.A subclass, pyrophosphate-bond hydrolysis-driven transporters.

TC class 1 consists of channels and pores, which usually catalyze energy-independent transport without a carrier-mediated mechanism [33]. TC subclass 1.A represents alpha-type channels that catalyze facilitated diffusion of a class of molecules. All three organisms possess a similar number of these systems, with *B. longum* having the fewest (10). TC subclass 1.B beta-barrel, which are primarily found in the outer membranes of gram-negative bacteria, is absent in all three organisms [35]. Actinobacteria, despite being gram-positive, are known to have outer membrane pore-forming proteins, but these are not necessarily beta-barrels and few have been characterized in these organisms [38]. Additionally, there are few differences in the distributions of transport systems in subclass 1.C, pore-forming toxins, and subclass 1.E, holins, between the three organisms.

TC Subclass 2.A, which is composed of uniporters, symporters and antiporters, is the largest group of transport systems in the three organisms representing between 34% and 42% of all systems within each organism. Within this subclass, there is quite a significant difference in the distribution of proteins and systems with *B. dentium* having

the most (107, 91), followed by *B. longum* (75, 64) and *B. animalis* (58, 53). Subclass 2.C systems, ion gradient-driven energizers, are unsurprisingly lacking because these transporters work in concert with 1.B systems, which are primarily found in gram-negative bacteria [34].

The second largest group of transport systems in the three organisms is the 3.A phosphate-bond-hydrolysis-driven transporters, which comprise between 24% to 40% of all systems within these organisms. Though the number of 3.A transport proteins is much larger than that of 2.A transport proteins, the number of systems in 3.A is much lower because 3.A systems typically have more components than 2.A systems. Therefore, it is especially significant that *B. longum* has 12 more 3.A systems than 2.A systems (76 to 64). This is in contrast to *B. dentium*, which has 32 more 2.A systems than 3.A systems (91 to 59), and *B. animalis*, which has 16 more 2.A systems than 3.A systems (53 to 37). The remaining class 3 systems are found in the 3.D subclass with each organism having between two to four such systems.

Subclass 4.A consists of phosphotransfer-driven rPTS-type group translocators; *B. longum* and *B. dentium* each have one such system present. Subclass 4.B is the nicotinamide ribonucleoside uptake transporters, which are absent in all three organisms. There are four proteins of subclass 4.C, the acyl CoA Ligase-coupled transporters, and between one and two 4.C systems are present in each organism. Subclass 4.D includes polysaccharide synthase/exporters (glycosyl transferases), which are sometimes implicated in a two step process of exopolysaccharide production and secretion. *B. longum* has two such systems, while *B. dentium* and *B. animalis* each have one. Subclass

4.F systems, which facilitate phospholipid transport to the lumenal surface of the ER in eukaryotes, are absent in *B. longum*, but *B. animalis* and *B. dentium* each possess two.

Subclasses 5.A and 5.B are the transmembrane two-electron transfer carriers and transmembrane one-electron transfer carriers, respectively. These systems are largely lacking in the organisms examined, except for one 5.B system found in *B. animalis*.

Subclass 8.A includes auxiliary transport proteins, which do not directly transport substrates across membranes but aid in the function of one or more established transport systems. There are between 11-15 such systems in each organism with an unremarkable pattern.

The remaining proteins in each organism are organized under subclass 9.A, known transporters that function by an unknown mechanism of action, or 9.B, putative transporters of uncertain function and/or mechanism. Each of the species has 2-3 systems of subclass 9.A., but between 21 to 26 systems of subclass 9.B. However, *B. animalis*' 26 9.B systems makes up 16.7% of all known systems in its transportome, much higher than the percentages of 9.B systems in *B. longum* and *B. dentium*, 11% and 12%, respectively.

Comparison of transporter systems by substrate

Like the comparisons of the TC subclasses, it is important to take into account both the raw number of systems and the percentages of substrate systems because of the differing sizes of the three transportomes. Table 3 shows the unique systems found in each organism for a given substrate category. The most obvious trend is that out of 155 transport systems in *B. animalis*, 48 (31%) are transport systems of unknown function, compared to 23% in *B. dentium* and 19% in *B. longum*. Because of this high percentage

of unidentified systems, *B. animalis* expectedly contains the least number of systems in virtually every other substrate category.

The number of transport systems within an organism is a reflection of the variety of substrates that can be transported. Probiotic organisms are known for their ability to withstand antibiotic treatment and ability to ferment carbohydrates indigestible to humans [13, 39]. As such, a known probiotic organism like *B. animalis* should possess a variety of drug and carbohydrate transporters. However, Table 4 reveals that *B. animalis* has the lowest percent of systems specific for drugs (5% compared to 10% and 12%), sugars (2.6% compared to 3.5% and 6%), and polysaccharides (3.2% compared to 7% and 7.4%). The low percentages of transport systems within *B. animalis* suggests either that (i) many of the systems in *B. animalis* have yet to characterized as transporters of known function or (ii) *B. animalis* confers its probiotic effects with a narrower range of substrates than other probiotics in this genus (see discussion).

Investigation of unique and shared transport systems

To better elucidate differences and similarities between the species, Table 5 organizes all systems found in *B. longum*, *B. dentium*, and *B. animalis* into a single table, and notes in which species a certain system is found. In total, there were 347 transport systems, and, of these, 64 were present in all three species. Within these 64 common systems, the most abundant transporter types were cation transport systems with 11, followed by proteins/peptides, amino acids and their derivatives, and polysaccharides, each organism with 5 systems of each category. *B. animalis* shares 120 systems (77%)

with either one or both of the other organisms, making it the least specialized *Bifidobacterium* of the species studied.

As for differences, *B. longum* has 81 unique systems and *B. dentium* has 77 unique systems, which correspond to 42% and 36% of the totals, respectively. An interesting observation is that the unique systems in *B. dentium* are skewed toward the 2.A subclass (35, 45.5%), while the unique systems in *B. longum* are skewed toward the 3.A subclass (43, 53%). This may imply that *B. dentium* has adapted to an aerobic environment (oral cavity), since many 2.A systems require the proton motive force (pmf) for function.

Distribution of carbohydrate transport systems reflects each organism's ecological niche

The composition and flow of nutrients through the distal portion of the bowel, where *B. longum* largely resides, is mostly made up of molecules that are not able to be metabolized and are passed over by the microbiota farther up the gut [37]. Those microbes in the oral cavity, like *B. dentium*, however, have access to the full contents of ingested foods and would be expected to have an extensive arsenal of genes capable of taking advantage of the opportunities [37]. This is reflected well in the number of total carbohydrate transport systems in *B. dentium* (28) compared to the lower bowel-dwelling *B. longum* (21) and *B. animalis* (10). *B. dentium* also has nearly double the number of unique carbohydrate transport systems as *B. longum* (7), which possibly reflects the higher variety of sugars available in the oral cavity relative to the distal portions of the gastrointestinal tract.

B. longum and *B. dentium* have distinct resistomes

It is known that many gut microbes including probiotics species, in general, possess many multi-drug resistance transporters and other proteins to help render antibiotics less effective [42]. Out of 31 such drug transport systems, *B. longum* and *B. dentium* have 23 and 22, respectively. *B. animalis*, on the other hand, has 10 drug transport systems. Out of these, only one is unique to it, reflecting the trend of *B. animalis* having the least specialized transportome of the three organisms and, thus, a more generic resistome. In contrast, *B. longum* and *B. dentium* have distinct resistome profiles, with B. longum possessing 14 unique systems and *B. dentium* possessing 8. Between the two organisms, *B. longum* appears to be able to export more antibiotics than *B. dentium*.

Pore-forming toxins are shared between the organisms

TC subclass 1.C, pore-forming toxins, are a hallmark of many pathogenic bacteria [40]. In many of those organisms, the soluble pore-forming toxins bind to and lyse the membranes of prokaryotic and host cells [41]. There were only four such systems found in the Bifidobacteria studied, and, two out of the four pore-forming toxin systems are shared between all three organisms. One system is shared between *B. longum* and *B. dentium*, while one is unique to *B. animalis*. Notably, the pathogenic species, *B. dentium*, did not have a single unique pore-forming toxin system. Because of the presence of these systems in both probiotic and pathogenic strains, it seems unlikely that *B. dentium* uses

this method to infect host cells. These systems may exist to attack other prokaryotes in each Bifidobacterium's niche conferring a competitive advantage to these organisms.

All three species contain an exopolysaccharide (EPS) secretion system necessary for adhesion to epithelial cells

Previous studies of a strain of *B. animalis* have revealed that a gene, Balat_1410, is necessary for adherence to human intestinal epithelium [43]. Balat_1410 was screened against TCDB using TC-BLAST and was found to be the 8.A.3.1.2 system, which is implicated in exopolysaccharide (EPS) synthesis and export. This system was found not only in the strain of *B. animalis*, but also in *B. longum* and *B. dentium*. Another EPS secretion system, CpsU (2.A.66.2.16) was found to be shared between *B. animalis* and *B. dentium*. There is evidence that CpsU is a membrane translocator for EPS [44]. In addition, *B. longum* has a unique EPS system, 3.A.1.132.3. It appears to be a fusion protein homologous to both GldF and GldG, which are components of an EPS transporter.

Systems shared by *B. dentium* and *B. animalis* reflect their ability to withstand low pH environments

More so than the gut-dwelling *B. longum* and *B. animalis*, *B. dentium* is subject to a wide range of pH's and must withstand pH levels below 5.5, the critical point at which tooth enamel begins to break down [37]. *B. animalis* is less acid resistant than *B. dentium*, but more so than *B. longum* because it is found in a more acidic environment than the gut,

breast milk [23]. Expectedly, *B. dentium* has the most proton-transporting systems (8), as well as the most proton-export proteins (17).

Previous studies had indicated that *B. dentium*'s high acid tolerance may be related to the presence of a H+-translocating F1-F0-type ATPase, a glutamate decarboxylase (GadB), and a GABA antiporter (GadC). The F1-F0-ATPase is considered crucial to maintaining the intracellular pH at 7.5, while GadB and GadC have been reported in other bacteria to form a glutamate-dependent acid resistance system 2 (AR2) [37]. Ventura, et al. showed that GadB and GadC, among other genes, were upregulated 90 and 51 fold, respectively, in response to acid stress [37]. All three proteins are found in *B. dentium*, while *B. animalis* possesses the F1-F0 ATPase and GadC.

Vitamin transporters in *B. longum* shows it is a significant producer and supplier of vitamins in the gut microbiome

Lactic acid bacteria, including bifidobacteria have been reported as being capable of *de novo* synthesis of essential vitamins including riboflavin, B12, and niacin, and then supply them to the host [45]. A total of seven vitamin transport systems were found in the three organisms, and six of them were discovered in *B. longum*. The other two species each had four vitamin systems. The specific vitamins that were transported were niacin, biotin, and riboflavin. A transporter for thiamine precursors, 3.A.1.30.1, was found to be shared by all three organisms, which suggests that they can all take part in thiamin biosynthesis.

A possible virulence secretion system is present in *B. longum*

For all other systems discussed in the results, only those with many component proteins crucial to transport (like large TMS-containing permeases) were counted. Only one component of the 3.A.7.17.1 system, EssC, is found in *B. longum*. However, it is the only Virulence- or DNA transfer-related secretory pathway family member in any of the three species. EssC is important in that it shows considerable sequence identity with both the Type IV Secretory Pathway Family (3.A.7) and the mycobacterial protein secretion systems, also referred to as Type VII Secretion Systems (3.A.24). Because EssC is the so-called "missing link" between Type IV and Type VII secretion systems, it's presence suggests the possibility that either secretion system may be present in *B. longum*, and that the other constituents are insufficiently well characterized or are too small to be recognized by BLAST. There is potential here for further study.

DISCUSSION

In this report we utilized probiogenomics, the process of sequencing and analyzing probiotic and non-probiotic gut bacteria, to elucidate similarities and differences in the transporter profiles of different species of Bifidobacteria [11]. The proteomes of two Bifidobacteria with well-documented probiotic effects residing in the GIT, *B. longum* JDM301 and *B. animalis* subspecies *lactis* BL3, and one implicated in opportunistic pathogenicity in the oral cavity, *B. dentium* JCM 1195, were screened against TCDB using G-BLAST. The G-BLAST results showed Bifidobacterial homologues of established transport proteins, generating the "transportome" [46]. Comparing and contrasting the presence or absence of certain systems using these transportomes revealed some key similarities and differences between the three *Bifidobacterium* species.

An interesting observation from our analysis was that *B. animalis* has the least specialized transportome of the three species studied, meaning that it possesses the fewest unique transport systems. For example, *B. animalis* only has two carbohydrate transport systems and one drug transport system that were not shared with either of the other Bifidobacteria. Unexpectedly, *B. animalis* shares more transport systems with the oral cavity-dwelling *B. dentium* (44), than with its fellow GIT resident, *B. longum* (13). In a way, *B. animalis* possesses hybrid characteristics because it is a probiotic species that resides in an anaerobic environment (the GIT) like *B. longum*, but its transportome is more similar to that of the opportunistic pathogen that thrives in dental carries, *B. dentium* [16]. Because *B. animalis* has well-documented probiotic effects and shares so many systems with *B. dentium*, it seems possible that *B. dentium* has similar probiotic

effects in the gut. An instance of a strain of bacteria that is pathogenic in one tissue but probiotic in another was observed in several strains of *Bacteroides* [Zafar and Saier, unpublished results]. This makes *B. dentium* an interesting target of future study.

Some important similarities between the three organisms are the pore-forming toxin systems and a common exopolysaccharide (EPS) synthesis and secretion system. The majority of the pore-forming toxins were shared between two or more of the Bifidobacterial species. Because of the presence of these systems in both the probiotic strains and the pathogenic strain, it seems unlikely that *B. dentium* uses such toxins as a means of antagonizing host cells as do other pathogenic bacteria (40, 41). These systems possibly exist to attack other prokaryotes in each Bifidobacterium's nichem, conferring a competitive advantage. This substantiates the idea that the pathogenicity of *B. dentium* is largely due to its secretion of acid, which is a byproduct of carbohydrate fermentation [27]. The EPS secretion system that is shared between all three organisms, 8.A.3.1.2, showed strong similarity to an EPS secretion system that was shown to be necessary for adherence to the intestinal epithelium [43]. This suggests that, though *B. dentium* is primarily found in the oral cavity, it uses the same system as the gut probiotic species to adhere to the mucosa in the human gastrointestinal tract. There are other EPS secretion systems, CpsU and GldFG, that were found in the Bifidobacteria, but further investigation is needed to confirm if they function in epithelial cell adhesion as does 8.A.3.1.2.

Analysis of the multidrug resistance (MDR) transport systems showed that while *B. longum* and *B. dentium* have similar total numbers of these systems, *B. longum* has 14 unique drug transport systems and *B. dentium* has 8. *B. dentium* is regularly subject to a

number of antimicrobials that *B. longum* may not be, such as food preservatives, various mouthwashes, and oral biocides such as chlorhexidine [37]. It has been demonstrated that over 100 genes are upregulated in *B. dentium* in response to diluted mouth-wash, and several of these genes encode members of the MFS. The data concerning specific genes that were upregulated were not presented in the publication presenting these results, so it was not possible to determine which transporter systems they were [37]. However, despite presumably having more antimicrobials to resist, *B. dentium* still has fewer unique drug transport systems compared to *B. longum*, and despite both species being present in breast milk, *B. longum*'s slightly more varied resistome may explain why it seems to persist as the dominant colonizer of the infant gut over *B. dentium* [19].

We also found evidence that *B. longum* and *B. dentium* possess several features in their transportomes that reflect their adaptation to their respective environments. In particular, the distribution of carbohydrate and H^+-translocating systems, as well as the ratio of TC subclass 2.A to 3.A systems, were quite distinct. In fact, the abundance of carbohydrate transporters and high acid tolerance in B. dentium appear to be linked. The fermentation of dietary carbohydrates by plaque bacteria may lead to pH levels as low as 4, requiring the presence of H^+ pumps in cariogenic oral bacteria to maintain a neutral intracellular pH [47]. Adaptation to the oral cavity is reflected in the fact that the F0-F1-ATPase, as well as the GadB-GadC system, necessary for acid resistance, are present in *B. dentium*, partially present in *B. animalis*, but absent in *B. longum*. As *B. animalis* is also found in breast milk and known to able to withstand acidic environments, this observation could have been anticipated [22, 23]. Furthermore, previous studies have noted that *B. dentium* is an extremely efficient producer of GABA due to the GadB-GadC

system, and that *B. dentium* modulates visceral sensitivity due to the analgesic effects of its secreted GABA [48-50]. These studies combined with our results strongly suggest that *B. dentium* could be exploited as an enteric probiotic species.

TABLES

Table 1. Overview of transport proteins by TC subclass. The number of proteins in each TC subclass is listed along with the percentage of proteins in each TC subclass. The total number of transport proteins found in each organism is listed at the bottom. *B. longum* JDM301 is abbreviated as "*B. long*," *B. dentium* JCM1195 is abbreviated as "*B. dent*," and *B. animalis* spp. *lactis* BL3 is abbreviated as "*B. anim.*"

	B. long	B. dent	B. anim	B. long %	B. dent %	B. anim %
1.A alpha-type channels	10	14	14	2.9%	3.8%	5.88%
1.B beta-barrel porins	0	0	0	0.0%	0.0%	0.00%
1.C Pore-forming toxins	4	3	4	1.2%	0.8%	1.68%
1.E Holins	1	2	3	0.3%	0.5%	1.26%
2.A Porters (uniporters, symporters, antiporters)	75	107	58	22.1%	29.4%	24.37%
2.C Ion-gradient-driven energizers	0	0	0	0.0%	0.0%	0.00%
3.A P-P bond hydrolysis-driven transporters	197	176	101	58.1%	48.4%	42.44%
3.B Decarboxylation-driven transporters	0	0	0	0.0%	0.0%	0.00%
3.D Oxidoreduction-driven transporters	7	5	5	2.1%	1.4%	2.10%
4.A Phosphotransfer-driven group translocators	1	1	0	0.3%	0.3%	0.00%
4.B Nicotinamide ribonucleoside uptake transporters	0	0	0	0.0%	0.0%	0.00%
4.C Acyl-CoA ligase-coupled transporters	4	4	4	1.2%	1.1%	1.68%
4.D Polysaccharide synthase exporters	2	1	1	0.6%	0.3%	0.42%
4.F The Choline/EthanolaminePhosphotransferase 1 (CEPT1) Family	0	3	3	0.0%	0.8%	1.26%
5.A Transmembrane two-electron transfer carriers	0	0	0	0.0%	0.0%	0.00%
5.B Transmembrane one-electron transfer carriers	0	0	1	0.0%	0.0%	0.42%
8.A Auxilary transport proteins	11	15	13	3.2%	4.1%	5.46%
9.A Recognized transporters of unknown biochemical mechanism	2	2	3	0.6%	0.5%	1.26%
9.B Putative transport proteins	25	31	28	7.4%	8.5%	11.76%
Total	339	364	238	100.0%	100.0%	100.00%

Table 2. Overview of transport systems by TC subclass. The number of systems in each TC subclass is listed along with the percentage of systems in each TC subclass. The total number of transport systems found in each organism is listed at the bottom. *B. longum* JDM301 is abbreviated as "*B. long*," *B. dentium* JCM1195 is abbreviated as "*B. dent*," and *B. animalis* spp. *lactis* BL3 is abbreviated as "*B. anim.*"

	B. long	*B. denti*	*B. anim*	*B. long %*	*B. dent %*	*B. anim%*
1.A alpha-type channels	10	14	14	5.2%	6.5%	9.0%
1.B beta-barrel porins	0	0	0	0.0%	0.0%	0.0%
1.C Pore-forming toxins	3	3	3	1.6%	1.4%	1.9%
1.E Holins	1	2	2	0.5%	0.9%	1.3%
2.A Porters (uniporters, symporters, antiporters)	64	91	53	33.5%	42.1%	34.0%
2.C Ion-gradient-driven energizers	0	0	0	0.0%	0.0%	0.0%
3.A P-P bond hydrolysis-driven transporters	76	59	37	39.8%	27.3%	23.7%
3.B Decarboxylation-driven transporters	0	0	0	0.0%	0.0%	0.0%
3.D Oxidoreduction-driven transporters	4	2	2	2.1%	0.9%	1.3%
4.A Phosphotransfer-driven group translocators	1	1	0	0.5%	0.5%	0.0%
4.B Nicotinamide ribonucleoside uptake transporters	0	0	0	0.0%	0.0%	0.0%
4.C Acyl-CoA ligase-coupled transporters	1	1	2	0.5%	0.5%	1.3%
4.D Polysaccharide synthase exporters	2	1	1	1.0%	0.5%	0.6%
4.F The Choline/EthanolaminePhosphotransferase 1 (CEPT1) Family	0	2	2	0.0%	0.9%	1.3%
5.A Transmembrane two-electron transfer carriers	0	0	0	0.0%	0.0%	0.0%
5.B Transmembrane one-electron transfer carriers	0	0	1	0.0%	0.0%	0.6%
8.A Auxilary transport proteins	6	12	10	3.1%	5.6%	6.4%
9.A Recognized transporters of unknown biochemical mechanism	2	2	3	1.0%	0.9%	1.9%
9.B Putative transport proteins	21	26	26	11.0%	12.0%	16.7%
Total	191	216	156	100.0%	100.0%	100.0%

Table 3. Overview of transport proteins by substrate category. The number of proteins that transport a given substrate category is listed along with the percentage of such proteins. The total number of transport proteins found in each organism is listed at the bottom. *B. longum* JDM301 is abbreviated as "*B. long*," *B. dentium* JCM1195 is abbreviated as "*B. dent*," and *B. animalis* spp. *lactis* BL3 is abbreviated as "*B. anim.*"

	B. long	*B. dent*	*B. anim*	*B. long %*	*B. Dent %*	*B. anim %*
Anions	9	17	15	2.65%	4.67%	6.30%
Cations	40	36	34	11.80%	9.89%	14.29%
Electrons	0	0	1	0.00%	0.00%	0.42%
Water	1	1	1	0.29%	0.27%	0.42%
Amines	1	5	2	0.29%	1.37%	0.84%
Amino acids and derivatives	42	54	32	12.39%	14.84%	13.45%
Carboxylates	9	9	4	2.65%	2.47%	1.68%
Drugs	35	27	12	10.32%	7.42%	5.04%
Nonselective	10	7	6	2.95%	1.92%	2.52%
Nucleobases and nucleosides	4	16	5	1.18%	4.40%	2.10%
Siderophores	1	3	1	0.29%	0.82%	0.42%
Sugars	20	30	4	5.90%	8.24%	1.68%
Sugar alcohols	1	2	1	0.29%	0.55%	0.42%
Sugar derivatives	26	14	11	7.67%	3.85%	4.62%
Vitamins	12	4	8	3.54%	1.10%	3.36%
DNA	1	1	1	0.29%	0.27%	0.42%
Lipids	6	6	6	1.77%	1.65%	2.52%
Polysaccharides	38	41	13	11.21%	11.26%	5.46%
Proteins and peptides	36	22	17	10.62%	6.04%	7.14%
Unknown	47	69	64	13.86%	18.96%	26.89%
Total	339	364	238	100.00%	100.00%	100.00%

Table 4. Overview of transport systems by substrate category. The number of systems that transport a given substrate category is listed along with the percentage of such systems. The total number of transport systems found in each organism is listed at the bottom. *B. longum* JDM301 is abbreviated as "*B. long*," *B. dentium* JCM1195 is abbreviated as "*B. dent*," and *B. animalis* spp. *lactis* BL3 is abbreviated as "*B. anim.*"

	B. long	*B. denti*	*B. anim*	*B. long %*	*B. dent %*	*B. anim %*
Anions	6	14	9	3.14%	6.48%	5.77%
Cations	27	25	22	14.14%	11.57%	14.10%
Electrons	0	0	1	0.00%	0.00%	0.64%
Water	1	1	1	0.52%	0.46%	0.64%
Amines	1	4	2	0.52%	1.85%	1.28%
Amino acids and derivatives	20	26	15	10.47%	12.04%	9.62%
Carboxylates	8	8	4	4.19%	3.70%	2.56%
Drugs	20	18	8	10.47%	8.33%	5.13%
Nonselective	8	6	6	4.19%	2.78%	3.85%
Nucleobases and nucleosides	4	10	5	2.09%	4.63%	3.21%
Siderophores	1	2	1	0.52%	0.93%	0.64%
Sugars	7	13	4	3.66%	6.02%	2.56%
Sugar alcohols	1	2	1	0.52%	0.93%	0.64%
Sugar derivatives	11	6	7	5.76%	2.78%	4.49%
Vitamins	6	3	4	3.14%	1.39%	2.56%
DNA	1	1	1	0.52%	0.46%	0.64%
Lipids	3	3	4	1.57%	1.39%	2.56%
Polysaccharides	14	16	5	7.33%	7.41%	3.21%
Proteins and peptides	16	8	8	8.38%	3.70%	5.13%
Unknown	36	50	48	18.85%	23.15%	30.77%
Total	191	216	156	100.00%	100.00%	100.00%

Table 5. Occurrence of transport systems in *B. longum*, *B. animalis*, and *B. dentium*. The systems highlighted in cyan are unique to *B. longum*, those highlighted in green are unique to *B. animalis*, and those highlighted in red are unique to *B. dentium*. *B. longum* JDM301 is abbreviated as "*B. long*," *B. dentium* JCM1195 is abbreviated as "*B. dent*," and *B. animalis* spp. *lactis* BL3 is abbreviated as "*B. anim.*"

	B. long	*B. anim*	*B. dent*	General substrate category	Specific substrate
1.A.11.1.3	X	X	X	Amines	ammonia
2.A.3.1.3	X	X	X	Amino acids and derivatives	tryptophan, tyrosine, phenylalanine
2.A.3.3.22	X	X	X	Amino acids and derivatives	Amino acids
3.A.1.24.4	X	X	X	Amino acids and derivatives	L-methionine
3.A.1.3.24	X	X	X	Amino acids and derivatives	Amino acids
3.A.1.3.9	X	X	X	Amino acids and derivatives	glutamate
3.A.1.7.2	X	X	X	Anions	phosphate
9.B.27.2.3	X	X	X	Anions	Selenite
1.A.14.2.2	X	X	X	Carboxylates	Acetate
2.A.69.3.1	X	X	X	Carboxylates	Auxin
2.A.33.1.5	X	X	X	Cations	Na+, H+
2.A.36.3.2	X	X	X	Cations	Na+, K+, Rb+, Li+, H+
2.A.38.4.3	X	X	X	Cations	K+
2.A.4.1.1	X	X	X	Cations	Cd2+, Zn2+, Co2+, Cu2+, Ni2+
2.A.45.2.6	X	X	X	Cations	Silicon
2.A.72.1.3	X	X	X	Cations	K+
3.A.3.2.21	X	X	X	Cations	Ca2+

Table 5. (ctd.)

3.A.3.23.1	X	X	X	Cations	cations
3.A.3.25.3	X	X	X	Cations	cations
3.D.10.1.6	X	X	X	Cations	H+
3.D.2.2.2	X	X	X	Cations	H+
2.A.1.3.30	X	X	X	Drugs	Lincomycin
3.A.1.106.3	X	X	X	Drugs	Nisin, Polymyxin
3.A.1.135.5	X	X	X	Drugs	Daunomycin, 2,7-bis(carboxyethyl)-5(6)-carboxyfluorescein-acetoxymethylester
3.A.1.135.6	X	X	X	Drugs	erythromycin, tetracycline, Hoechst 33342
2.A.103.1.7	X	X	X	Lipids	Lipid II
4.C.1.1.14	X	X	X	Lipids	fatty acids
1.C.113.1.6	X	X	X	Nonselective	small molecules
1.C.82.1.1	X	X	X	Nonselective	small molecules
2.A.1.6.10	X	X	X	Nonselective	Metabolites
2.A.40.1.3	X	X	X	Nucleobases and nucleosides	Pyrimidines
2.A.40.2.1	X	X	X	Nucleobases and nucleosides	Purines
2.A.2.2.3	X	X	X	Polysaccharides	Raffinose
3.A.1.1.27	X	X	X	Polysaccharides	Maltose, Maltotriose, Maltotetraose, Trehalose
3.A.1.1.28	X	X	X	Polysaccharides	Raffinose, Stachyose
3.A.1.1.45	X	X	X	Polysaccharides	Maltose
9.B.28.1.3	X	X	X	Polysaccharides	Maltose

Table 5. (ctd.)

1.A.33.1.4	X	X	X	Proteins and peptides	Prefolded proteins
1.A.62.3.2	X	X	X	Proteins and peptides	Oligopeptides
3.A.1.103.3	X	X	X	Proteins and peptides	O-antigen precursors
3.A.1.5.35	X	X	X	Proteins and peptides	Peptides
3.A.5.2.2	X	X	X	Proteins and peptides	proteins
1.A.8.2.7	X	X	X	Sugar alcohols	water, Dihydroxyacetone, glycerol
3.A.1.1.48	X	X	X	Sugar derivatives	Lacto-N-biose
8.A.3.1.2	X	X	X	Sugar derivatives	Exopolysaccharides
2.A.1.2.14	X	X	X	Sugars	L-arabinose, Arabinose
2.A.1.3.41	X	X	X	Unknown	Unknown
2.A.1.67.1	X	X	X	Unknown	Unknown
2.A.98.1.3	X	X	X	Unknown	Unknown
3.A.1.125.3	X	X	X	Unknown	Unknown
8.A.5.1.3	X	X	X	Unknown	Unknown
8.A.9.1.1	X	X	X	Unknown	Unknown
8.A.9.1.2	X	X	X	Unknown	Unknown
9.B.1.1.5	X	X	X	Unknown	Unknown
9.B.105.1.5	X	X	X	Unknown	Unknown
9.B.146.1.1	X	X	X	Unknown	Unknown
9.B.146.1.6	X	X	X	Unknown	Unknown
9.B.36.1.3	X	X	X	Unknown	Unknown
9.B.76.1.1	X	X	X	Unknown	Unknown

Table 5. (ctd.)

9.B.97.5.1	X	X	X	Unknown	Unknown
2.A.1.82.4	X	X	X	Vitamins	Niacin
2.A.88.1.8	X	X	X	Vitamins	Biotin
3.A.1.30.1	X	X	X	Vitamins	Thiamin precursors
1.A.8.3.1	X	X	X	Water	Water
2.A.3.1.20	X	X		Amino acids and derivatives	L-alanine, D-alanine, L-Serine, D-serine, Glycine
3.A.1.5.11	X	X		Amino acids and derivatives	Glutathione
1.A.35.3.1	X	X		Cations	Mg2+, Ca2+, Ni2+
2.A.55.3.3	X	X		Cations	Mn2+
2.A.9.3.6	X	X		Proteins and peptides	proteins
2.A.86.1.7	X	X		Siderophores	Heme
2.A.66.4.4	X	X		Sugar derivatives	Lipid II peptidoglycan precursors
4.D.1.1.1	X	X		Sugar derivatives	Lipopolysaccharides
3.A.1.31.1	X	X		Unknown	Unknown
8.A.5.1.4	X	X		Unknown	Unknown
9.B.27.2.5	X	X		Unknown	Unknown
9.B.74.1.1	X	X		Unknown	Unknown
3.A.1.17.13	X	X		Vitamins	Riboflavin
2.A.3.7.1		X	X	Amino acids and derivatives	Gamma-aminobutyrate, glutamate
3.A.1.24.5		X	X	Amino acids and derivatives	L-histidine
3.A.1.3.16		X	X	Amino acids and derivatives	aspartate, glutamate

Table 5. (ctd.)

3.A.1.3.2		X	X	Amino acids and derivatives	Glutamine
3.A.1.3.27		X	X	Amino acids and derivatives	Glutamine, glutamate, Asparagine
1.A.43.1.13		X	X	Anions	Camphor, Fl-
2.A.49.5.4		X	X	Anions	Cl-
4.F.1.1.7		X	X	Anions	phosphate
4.F.1.3.1		X	X	Anions	phosphate
2.A.69.4.6		X	X	Carboxylates	Auxin
3.A.1.120.8		X	X	Carboxylates	Acetate
2.A.4.6.1		X	X	Cations	zinc ions
3.A.1.139.2		X	X	Cations	Fe2+
3.A.1.15.3		X	X	Cations	Zn2+
3.A.2.1.10		X	X	Cations	protons, H+
3.A.3.5.4		X	X	Cations	Ag+
3.A.12.1.4		X	X	DNA	DNA
2.A.1.3.22		X	X	Drugs	tetracycline
2.A.1.46.5		X	X	Drugs	Quinolone
3.A.1.120.3		X	X	Drugs	Oleandomycin
9.A.40.2.3		X	X	Nonselective	Small molecules
2.A.1.3.37		X	X	Nucleobases and nucleosides	Uridine, Deoxyuridine, 5-fluorouridine
2.A.66.2.16		X	X	Polysaccharides	Exopolysaccharides
2.A.2.3.3		X	X	Sugar derivatives	Isoprimeverose
9.B.18.1.2		X	X	Sugar derivatives	Exopolysaccharides
2.A.1.5.3		X	X	Sugars	Sucrose, Maltose, H+
1.A.1.5.25		X	X	Unknown	Unknown

Table 5. (ctd.)

2.A.1.2.93		X	X	Unknown	Unknown
2.A.1.7.13		X	X	Unknown	Unknown
2.A.1.85.1		X	X	Unknown	Unknown
2.A.114.1.7		X	X	Unknown	Unknown
2.A.128.1.5		X	X	Unknown	Unknown
2.A.69.3.5		X	X	Unknown	Unknown
2.A.7.3.31		X	X	Unknown	Unknown
3.A.1.140.4		X	X	Unknown	Unknown
8.A.11.1.2		X	X	Unknown	Unknown
8.A.21.2.2		X	X	Unknown	Unknown
8.A.49.1.1		X	X	Unknown	Unknown
8.A.5.1.6		X	X	Unknown	Unknown
9.B.142.5.1		X	X	Unknown	Unknown
9.B.148.3.3		X	X	Unknown	Unknown
9.B.226.1.8		X	X	Unknown	Unknown
9.B.265.1.1		X	X	Unknown	Unknown
9.B.273.1.1		X	X	Unknown	Unknown
2.A.3.1.13		X		Amines	Putrescine
2.A.26.1.9		X		Amino acids and derivatives	Branched amino acids
2.A.3.2.7		X		Amino acids and derivatives	Arginine
9.B.104.1.4		X		Amino acids and derivatives	Serine
1.A.43.1.2		X		Anions	Camphor, Fl-
1.A.43.3.4		X		Anions	Fl-
2.A.49.9.2		X		Anions	F-, H+

Table 5. (ctd.)

3.A.1.17.2		X		Anions	Aromatic sulfonate
1.A.22.1.9		X		Cations	ions
2.A.38.4.6		X		Cations	K+
9.A.8.1.1		X		Cations	Fe2+
9.B.100.1.1		X		Cations	H+
2.A.1.3.61		X		Drugs	Hoechst 3342, doxorubicin, daunorubicin, tetraphenylphosphonium, ethidium bromide, Rhodamine 6G
5.B.1.2.2		X		Electrons	Electrons
2.A.103.1.4		X		Lipids	Lipid II
4.C.1.1.15		X		Lipids	Lauric Acid
1.C.109.1.4		X		Nonselective	small molecules
1.A.23.5.1		X		Nucleobases and nucleosides	Cyclic nucleotides
2.A.40.7.2		X		Nucleobases and nucleosides	Hypoxanthine, Guanosine
1.E.40.3.1		X		Proteins and peptides	endolysin
1.E.43.1.9		X		Proteins and peptides	endolysin
2.A.7.5.4		X		Sugars	D-glucose
9.B.28.1.8		X		Sugars	Maltose
1.A.78.2.6		X		Unknown	Unknown
2.A.109.1.7		X		Unknown	Unknown
3.A.1.106.4		X		Unknown	Unknown
3.A.29.1.2		X		Unknown	Unknown
8.A.24.1.8		X		Unknown	Unknown

Table 5. (ctd.)

9.A.24.4.3		X		Unknown	Unknown
9.B.105.2.4		X		Unknown	Unknown
9.B.115.1.2		X		Unknown	Unknown
9.B.183.1.6		X		Unknown	Unknown
9.B.196.2.2		X		Unknown	Unknown
9.B.261.1.2		X		Unknown	Unknown
9.B.55.1.2		X		Unknown	Unknown
2.A.21.2.5	X		X	Amino acids and derivatives	Proline
2.A.3.1.10	X		X	Amino acids and derivatives	S-Methylmethionine
2.A.7.3.6	X		X	Amino acids and derivatives	Threonine/Homoserine
2.A.78.1.1	X		X	Amino acids and derivatives	BCAAs
3.A.1.3.14	X		X	Amino acids and derivatives	L-cystine
3.A.1.3.25	X		X	Amino acids and derivatives	glutamate, Asparagine, Glutamine
2.A.109.1.2	X		X	Anions	Te ions
2.A.49.5.5	X		X	Anions	Cl-
2.A.108.2.10	X		X	Cations	Fe2+
2.A.37.1.2	X		X	Cations	K+
2.A.1.3.39	X		X	Drugs	Linezold, Tetraphenylphosphonium chloride, SDS, Trimethoprim, Chloramphenicol
2.A.28.2.6	X		X	Drugs	Macrolides
2.A.66.1.32	X		X	Drugs	Drugs
3.A.1.122.16	X		X	Drugs	Macrolides

Table 5. (ctd.)

2.A.103.1.9	X		X	Lipids	Lipids
1.C.109.1.2	X		X	Nonselective	small molecules
2.A.1.2.25	X		X	Nucleobases and nucleosides	nucleosides: inosine, adenosine and guanosine; bases: hypoxanthine, adenine, guanine, 2-fluoroadenine
2.A.40.7.4	X		X	Nucleobases and nucleosides	Hypoxanthine, Guanosine
2.A.2.5.1	X		X	Polysaccharides	Oligogalacturonide
3.A.1.1.10	X		X	Polysaccharides	Alginate
3.A.1.1.20	X		X	Polysaccharides	fructooligosaccharide
3.A.1.1.43	X		X	Polysaccharides	Melibiose, Trehalose
9.B.28.1.10	X		X	Polysaccharides	Maltose
4.A.1.2.14	X		X	Sugar derivatives	Beta-Glucoside
2.A.1.1.42	X		X	Sugars	Glucose
3.A.1.2.1	X		X	Sugars	Ribose
3.A.1.2.22	X		X	Sugars	Sugars
2.A.1.24.4	X		X	Unknown	Unknown
2.A.7.3.47	X		X	Unknown	Unknown
3.A.1.122.8	X		X	Unknown	Unknown
3.A.1.136.1	X		X	Unknown	Unknown
9.B.104.1.2	X		X	Unknown	Unknown
9.B.74.1.2	X		X	Unknown	Unknown
2.A.1.6.4	X			Amino acids and derivatives	Proline/glycine-betaine
2.A.26.1.2	X			Amino acids and derivatives	Isoluecine/valine

Table 5. (ctd.)

2.A.35.1.3	X			Amino acids and derivatives	Tyrosine
2.A.7.17.2	X			Amino acids and derivatives	Aromatic amino acids
2.A.79.1.3	X			Amino acids and derivatives	Threonine
3.A.1.17.9	X			Amino acids and derivatives	Taurine
3.A.1.4.2	X			Amino acids and derivatives	Hydrophobic amino acids
3.A.1.4.8	X			Amino acids and derivatives	Leucine, isoleucine, valine, threonine, alanine
2.A.20.1.7	X			Anions	Phosphate
3.A.1.16.3	X			Anions	Nitrate, Nitrite, Cyanate
8.A.7.1.1	X			Anions	Phosphate
1.A.16.1.3	X			Carboxylates	Formate
2.A.1.11.3	X			Carboxylates	Oxalate/Formate
2.A.16.2.2	X			Carboxylates	Malate
2.A.66.1.24	X			Carboxylates	Citrate
3.A.1.120.6	X			Carboxylates	Acetate
3.D.10.1.4	X			Carboxylates	Succinate, Quinone
1.A.1.17.2	X			Cations	K+
2.A.107.1.1	X			Cations	Mn2+
3.A.1.1.50	X			Cations	glycerophosphocholine
3.A.1.122.15	X			Cations	Heavy metals
3.A.1.15.11	X			Cations	Zn2+
3.A.1.23.2	X			Cations	Co2+
3.A.2.1.6	X			Cations	Na+, H+
3.A.3.5.18	X			Cations	Cu+

Table 5. (ctd.)

3.D.1.8.1	X			Cations	H+
9.A.40.1.2	X			Cations	Co2+
9.B.10.1.1	X			Cations	Zn2+
2.A.1.21.22	X			Drugs	Macrolides like erythromycin; oleando-mycin; azithromycin
2.A.1.21.3	X			Drugs	Tetracycline
2.A.1.3.25	X			Drugs	Actinorhodin
2.A.1.3.5	X			Drugs	Pristinamycin I, Pristinamycin II, Rifamycin
3.A.1.121.3	X			Drugs	virginiamycin, lincomycin
3.A.1.121.4	X			Drugs	Ethidium/Fluoroquinolones
3.A.1.122.1	X			Drugs	Macrolides
3.A.1.122.2	X			Drugs	Antimicrobial peptides
3.A.1.122.7	X			Drugs	Macrolide
3.A.1.124.1	X			Drugs	Nisin
3.A.1.124.3	X			Drugs	Nukacin
3.A.1.124.5	X			Drugs	Salivaricin
1.A.22.1.2	X			Nonselective	Nonspecific ions
1.E.24.1.5	X			Nonselective	small molecules
2.A.1.1.92	X			Nonselective	Metabolites
9.A.40.2.2	X			Nonselective	Small molecules
3.A.1.1.17	X			Polysaccharides	Trehalose, maltose, sucrose
3.A.1.1.18	X			Polysaccharides	Trehalose
3.A.1.1.44	X			Polysaccharides	Maltose, maltodextrin

Table 5. (ctd.)

3.A.1.1.7	X			Polysaccharides	Maltose, trehalose
3.A.1.2.8	X			Proteins and peptides	Autoinducer-2
3.A.1.5.26	X			Proteins and peptides	Glutathione
3.A.1.5.39	X			Proteins and peptides	Peptides
3.A.16.1.1	X			Proteins and peptides	Misfolded proteins
3.A.16.1.2	X			Proteins and peptides	Misfolded proteins
3.A.16.1.3	X			Proteins and peptides	Misfolded proteins
3.A.25.2.1	X			Proteins and peptides	Pre-proteins
3.A.6.1.1	X			Proteins and peptides	Proteins
3.A.7.17.1	X			Proteins and peptides	EssA and EssB
3.A.9.1.2	X			Proteins and peptides	Proteins
2.A.1.7.1	X			Sugar derivatives	Fucose
2.A.66.6.2	X			Sugar derivatives	Exopolysaccharides
3.A.1.1.33	X			Sugar derivatives	N,N'-diacetylchitobiose
3.A.1.132.3	X			Sugar derivatives	Exopolysaccharides
4.D.2.1.6	X			Sugar derivatives	Glycosyl
3.A.1.1.34	X			Sugars	Arabinose
3.A.1.139.1	X			Sugars	UDP-glucose
3.A.1.2.23	X			Sugars	Sugars
2.A.1.67.2	X			Unknown	Unknown
2.A.7.3.52	X			Unknown	Unknown
3.A.1.105.9	X			Unknown	Unknown

Table 5. (ctd.)

3.A.1.132.6	X			Unknown	Unknown
3.A.1.140.1	X			Unknown	Unknown
3.A.1.147.9	X			Unknown	Unknown
9.B.106.2.2	X			Unknown	Unknown
9.B.106.3.1	X			Unknown	Unknown
9.B.111.1.3	X			Unknown	Unknown
9.B.124.1.1	X			Unknown	Unknown
9.B.126.1.2	X			Unknown	Unknown
9.B.143.4.2	X			Unknown	Unknown
2.A.1.81.5	X			Vitamins	Riboflavin
3.A.1.25.5	X			Vitamins	Biotin, Riboflavin
3.A.12.1.1	X				DNA
2.A.3.5.1			X	Amines	Ethanolamine
3.A.1.11.9			X	Amines	Spermidine, Putrescine
3.A.1.17.4			X	Amines	Taurine
2.A.23.4.1			X	Amino acids and derivatives	Serine, Threonine, Na+
2.A.3.1.21			X	Amino acids and derivatives	L-Serine, L-threonine, L-cysteine
2.A.3.1.8			X	Amino acids and derivatives	Asparagine
2.A.3.13.3			X	Amino acids and derivatives	L-Leucine
2.A.7.17.1			X	Amino acids and derivatives	phenylalanine, tryptophan, tyrosine
2.A.75.1.2			X	Amino acids and derivatives	lysine, Histidine, Arginine

Table 5. (ctd.)

2.A.76.1.10			X	Amino acids and derivatives	Serine, Threonine, Homoserine, Homoserine lactone
3.A.1.122.19			X	Amino acids and derivatives	Acetoin
3.A.1.24.2			X	Amino acids and derivatives	Methionine sulfoxide, Methionine
3.A.1.3.17			X	Amino acids and derivatives	Histidine, lysine, Arginine
3.A.1.4.10			X	Amino acids and derivatives	Alanine, Serine, Threonine, Valine, Isoleucine, Leucine
1.A.43.1.7			X	Anions	Fl-
1.A.43.3.1			X	Anions	Camphor, Fl-
1.A.43.3.3			X	Anions	Fl-
2.A.1.17.3			X	Anions	Cyanate
2.A.102.4.5			X	Anions	Sulfite
8.A.7.1.2			X	Anions	Phosphate
2.A.1.23.2			X	Carboxylates	Cholate, taurocholate
2.A.7.21.4			X	Carboxylates	Orotate
2.A.8.1.1			X	Carboxylates	Gluconate
2.A.8.1.10			X	Carboxylates	D-glycerate
1.A.35.3.4			X	Cations	Mg2+
2.A.107.1.2			X	Cations	manganese
2.A.35.1.1			X	Cations	Na+, H+
2.A.37.2.1			X	Cations	Na+, H+
2.A.38.4.4			X	Cations	K+
2.A.55.3.7			X	Cations	H+, Metal ions

Table 5. (ctd.)

2.A.66.1.43			X	Cations	Al3+
2.A.1.21.11			X	Drugs	Drugs
2.A.115.2.8			X	Drugs	Novobiocin
2.A.6.5.11			X	Drugs	drugs
2.A.66.1.13			X	Drugs	Fluoroquinolones, Tigecycline
2.A.66.1.23			X	Drugs	dipeptides, FMN, FAD
2.A.66.1.25			X	Drugs	drugs
3.A.1.137.2			X	Drugs	Antimicrobial peptides
4.D.1.1.14			X	Lipids	fatty acids
1.A.22.1.10			X	Nonselective	ions
1.A.23.2.2			X	Nonselective	ions
2.A.1.1.27			X	Nucleobases and nucleosides	Myoinositol, H+
2.A.1.7.5			X	Nucleobases and nucleosides	2-deoxy-D-ribose, 2-deoxyribose
3.A.1.33.1			X	Nucleobases and nucleosides	Methylthioadenosine
2.A.66.2.20			X	Polysaccharides	Polysaccharides
2.A.66.2.23			X	Polysaccharides	Xanthan precursors
3.A.1.1.21			X	Polysaccharides	Xylobiose
3.A.1.1.23			X	Polysaccharides	Cellobiose, Celltriose
9.B.28.1.2			X	Polysaccharides	Maltose
1.E.21.2.2			X	Proteins and peptides	endolysin
1.E.40.3.3			X	Proteins and peptides	endolysin

Table 5. (ctd.)

9.A.47.2.1			X	Proteins and peptides	proteins
3.A.1.106.7			X	Siderophores	Salmochelin, Enterochelin
3.A.1.21.2			X	Siderophores	Fe3+-carboxymycobactin
2.A.1.18.2			X	Sugar alcohols	Ribitiol, H+
3.A.1.1.29			X	Sugar derivatives	Aldouronate
2.A.1.1.81			X	Sugars	Glucose
2.A.1.68.1			X	Sugars	Glucose
3.A.1.1.30			X	Sugars	Glucose
3.A.1.1.36			X	Sugars	sucrose, maltose, glucose, fructose
3.A.1.1.52			X	Sugars	sucrose, maltose, glucose, fructose, esculin
3.A.1.2.20			X	Sugars	Xylose, glucose
8.A.8.1.7			X	Sugars	Fructose
2.A.11.2.1			X	Unknown	Unknown
2.A.86.1.5			X	Unknown	Unknown
8.A.49.1.3			X	Unknown	Unknown
8.A.5.1.7			X	Unknown	Unknown
9.B.14.1.17			X	Unknown	Unknown
9.B.142.3.8			X	Unknown	Unknown
9.B.2.1.23			X	Unknown	Unknown
9.B.223.1.3			X	Unknown	Unknown
9.B.250.1.4			X	Unknown	Unknown

Table 5. (ctd.)

9.B.250.1.4			X	Unknown	Unknown
9.B.257.1.3			X	Unknown	Unknown
9.B.67.8.4			X	Unknown	Unknown
9.B.74.1.3			X	Unknown	Unknown
9.B.97.1.9			X	Unknown	Unknown
3.A.1.17.11			X	Vitamins	Riboflavin

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