An Alternative Spermidine Biosynthetic Route is Critical for Growth of Campylobacter jejuni and is the Dominant Polyamine Pathway in the Human Gut Microbiota

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Running title: An alternative spermidine biosynthetic pathway

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Background: Many bacteria synthesize spermidine but lack orthologues of polyamine biosynthetic enzymes S-adenosylmethionine decarboxylase and spermidine synthase.

Results: An alternative spermidine biosynthetic pathway is essential in Campylobacter jejuni.

Conclusion: The alternative route via carboxyspermidine is the dominant pathway in the human gut microbiota and deep sea hydrothermal vents.

Significance: A multiplicity of polyamine biosynthetic pathways exist, providing novel targets for development of antimicrobial drugs.

The availability of fully sequenced bacterial genomes has revealed that many species known to synthesize the polyamine spermidine lack the spermidine biosynthetic enzymes S-adenosylmethionine decarboxylase (AdoMetDC) and spermidine synthase (SpdSyn). We found that such species possess orthologues of the sym-norspermidine biosynthetic enzymes carboxynorspermidine dehydrogenase (CANSDH) and carboxynorspermidine decarboxylase (CANSDC). By deleting these genes in the food-borne pathogen Campylobacter jejuni, we found that the CANSDC orthologue is responsible for synthesizing spermidine, not sym-norspermidine in vivo. In polyamine auxotrophic gene deletion strains of C. jejuni, growth is highly compromised but can be restored by exogenous sym-homospermidine and to a lesser extent by sym-norspermidine. The alternative spermidine biosynthetic pathway is present in many bacterial phyla and is the dominant spermidine route in the human gut, stomach and oral microbiomes, and appears to have supplanted the AdoMetDC/SpdSyn pathway in the gut microbiota. Approximately half of the gut Firmicutes species appear to be polyamine auxotrophs but all encode the potABCD spermidine/putrescine transporter. Orthologues encoding carboxyspermidine dehydrogenase and carboxyspermidine decarboxylase are found clustered with an array of diverse putrescine-biosynthetic genes in different bacterial genomes, consistent with a role in spermidine, rather than sym-norspermidine biosynthesis. Due to the pervasiveness of ε-Proteobacteria in deep sea hydrothermal vents, and to the ubiquity of the alternative spermidine biosynthetic pathway in that phylum, the carboxyspermidine route is also dominant in deep sea hydrothermal vents. The carboxyspermidine pathway for polyamine biosynthesis is found in diverse...
**human pathogens and this alternative spermidine biosynthetic route presents an attractive target for developing novel antimicrobial compounds.**

Polyamines are primordial polycationic cellular metabolites that are found in almost all cells. The most ubiquitous polyamine, the triamine spermidine (Fig. 1A), is synthesized from the diamine putrescine (1,4-diaminobutane) by addition of an aminopropyl group donated by decarboxylated S-adenosylmethionine (Fig. 1B). Two enzymes are known to be involved in spermidine biosynthesis from putrescine (Fig 1B): S-adenosylmethionine decarboxylase (AdoMetDC) (1) and the aminopropyltransferase spermidine synthase (SpdSyn) (2). Both AdoMetDC and SpdSyn are found in almost all eukaryotes, most archaea and in many bacterial phyla including the species *Escherichia coli* (3), *Bacillus subtilis* (4) and *Thermotoga maritima* (5). However, some bacterial species use the triamine *sym*-homospermidine (Fig. 1A) rather than spermidine, synthesized by homospermidine synthase or a deoxyhypusine synthase-like enzyme (6). It is surprising therefore that analysis of complete bacterial genomes reveals that there are many bacterial species that are known to synthesize spermidine but which do not possess AdoMetDC or SpdSyn orthologues eg., almost all ε-Proteobacteria (7), *Bacteroides*, *Prevotella* and *Porphyromonas* species within the Bacteroidetes phylum (8), species of *Clostridium* and *Eubacterium*, *Ruminococcus* and *Butyvibrio* in the Firmicutes phylum (9), and members of the *Deinococcus* phylum (10), amongst others. Recently we showed that an alternative *sym*-norspermidine (Fig. 1A) biosynthetic pathway synthesized *sym*-norspermidine from 1,3-diaminopropane in *Vibrio cholerae*, using aspartate β-semialdehyde, rather than decarboxylated S-adenosylmethionine as an aminopropyl group donor (11). The enzymes involved in synthesis of *sym*-norspermidine by the alternative pathway are carboxynorspermidine dehydrogenase (*CANS DH*) and carboxynorspermidine decarboxylase (*CANS DC*) (11). We speculated that in the majority of bacterial species that encode orthologues of *CANS DH* and *CANS DC*, the alternative pathway was used mainly for the synthesis of spermidine from putrescine, rather than the synthesis of *sym*-norspermidine from 1,3-diaminopropane. This assumption was made because most species possessing *CANS DH* and *CANS DC* orthologues do not possess orthologues of the enzymes required to synthesize 1,3-diaminopropane (diaminobutyrate aminotransferase (*DABA AT*) and diaminobutyrate decarboxylase (*DABA DC*)) nor orthologues of AdoMetDC and SpdSyn (11). Here we show that the food-borne pathogen *Campylobacter jejuni*, an ε-proteobacterium responsible for the majority of food poisoning cases in developed countries (12) synthesizes spermidine by the aspartate β-semialdehyde pathway. We also show that spermidine and carboxyspermidine decarboxylase (CASDC) are critical for growth of *C. jejuni* and that the function of spermidine in cell proliferation can be replaced by *sym*-homospermidine and to a lesser extent by *sym*-norspermidine. Orthologues genes potentially encoding carboxyspermidine dehydrogenase (CASDH) and CASDC are found in many bacterial phyla, including important human pathogens, and surprisingly CASDH/CASDC constitute the dominant polyamine biosynthetic pathway in deep sea hydrothermal vents and in the human gut microbiota.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains and Growth Conditions--**All strains were derived from the sequenced *C. jejuni* strain 81116 (NCTC 11828) (13). Bacteria were grown at 37 °C under microaerobic conditions (10% CO₂, 85% N₂, 5% O₂) on an orbital shaker (200 rpm) inside a MACS-MG-1000 controlled-atmosphere workstation (DW Scientific). When required, kanamycin or chloramphenicol was added to the growth medium at 50 µg/ml or 25 µg/ml, respectively. For growth assays, strains were grown overnight in Brucella broth (14) and washed twice in polyamine-deficient medium (Dulbecco’s modified Eagle’s medium, 3.7 g/l sodium bicarbonate, 500 mg/l aspartic acid, 500 mg/l serine, 200 mg/l sodium pyruvate, 100 mg/l cysteine, 500 mg/l glutamic acid, 100 mg/l proline, 5 mg/l ferric III chloride-6-hydrate, 10 mg/l adenine sulfate (all components supplied by Sigma-Aldrich)). Cells were diluted to an initial OD₆₀₀nm of 0.01 in polyamine-deficient medium ± polyamines or agmatine and incubated as described above.

**HPLC Analysis of Cellular Polyamines--**Cells were harvested by centrifugation (2880 x g, 10 min), washed twice in polyamine-deficient medium, and then twice in phosphate-buffered saline. MOPS lysis buffer (100 mM MOPS, 50 mM NaCl, 20 mM MgCl₂) at pH 8.0 was added.
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at 5 µl per mg cell fresh weight, and cells were subjected to three cycles of freeze/thawing. Trichloroacetic acid was added to a final concentration of 10%, and cells were incubated on ice for 5 min. After centrifugation (18,000 g, 5 min, 4 ºC), 5 µl of supernatant were derivatized using the AccQ-Fluor reagent kit for labelling amino acids (Waters). For normalisation, 1,7-diaminoheptane was included as an internal standard. Labelled polyamines were separated by HPLC using a Luna 5µm C18 (2) 100A column (250 x 4.6 mm; Phenomenex) with fluorescence detection (excitation 248 nm, emission 398 nm).

Solvent A was 70 mM acetic acid, 25 mM triethylamine, pH 4.82; solvent B was 80% acetonitrile, 20% H2O (v/v); solvent C was methanol, and the gradient was run for 65 min at a flow rate of 1.2 ml/min with the following concentrations: t = 0 min, 100 % A; t = 1 min, 78 % A, 22 % B; t = 27 min, 55 % A, 39 % B, 6 % C; t = 27.5 min, 53 % A, 33 % B, 14 % C; t = 34 min, 20 % A, 10 % B, 70 % C; t = 37 min, 100 % B; t = 58 min, 100 % A.

Construction of gene deletion mutants of C. jejuni 81116-Suicide plasmids were constructed for each gene knockout. Each plasmid consisted of the DNA flanking the gene sequence to be knocked out, with an internal BamHI site into which a selectable marker kanamycin resistance cassette was located. The rest of the vector, pGemTeasy (Promega), does not replicate in C. jejuni. The two regions flanking the insertion site were amplified using genomic DNA from 81116 as a template, and the ends of these were designed to overlap and incorporate a BamHI site. Primer “gene” disruption 1 was used with “gene” disruption 2 in PCR (each gene and the primer sequences are shown in Supplemental Table S1). Primer “gene” disruption 3 was used with “gene” disruption 4. The PCR was performed with Hotstar Taq polymerase (Qiagen) according to the manufacturer’s instructions. Cycle parameters: 95 ºC 15 min; 95 ºC 30 s, 50 ºC 30 s, 72 ºC 2 min for 30 cycles, followed by 72 ºC for 15 min. Each product was purified using Qiaquick PCR purification kit (Qiagen) according to the manufacturer’s instructions. Products were then combined, diluted 1/20 and 1 µl used as template for a further PCR reaction using primer “gene” disruption 1 and primer “gene” disruption 4. This PCR used the same cycling parameters. The product was first purified using Qiaquick PCR purification kit (Qiagen) according to the manufacturer’s instructions, before being ligated into pGemTeasy and transformed into E.coli TOP10 competent cells (Invitrogen). Clones (white on X-Gal LB plates) were checked by sequencing using M13 universal forward and reverse primers and correct inserts retained. The resultant vectors have a unique BamHI site flanked by targeting DNA in the pGemTeasy backbone.

To insert the selection marker, a BamHI flanked kanamycin resistance cassette was ligated into each vector digested with BamHI and transformed into E.coli TOP10 competent cells (Invitrogen). Selection was performed on kanamycin–LB plates and then plasmids were checked by sequencing using M13 universal forward and reverse primers and correct inserts retained. As these pGemTeasy-based plasmids are unable to replicate in C. jejuni they act as suicide vectors introducing the selectable marker into each gene locus by homologous recombination. The suicide plasmids were transformed into C. jejuni 81116 by electroporation and selected on Brucella solid medium containing kanamycin. Gene deletions were verified by retaining three independent clones, which were checked by isolating genomic DNA from each and then using PCR to assess the presence of the kanamycin resistance gene in the target locus using primers outside the disruption cassette, and inside kanamycin resistance gene.

Complementation of the CASDC gene deletion mutant with the cognate CASDC gene.-The CASDC gene was cloned under a constitutive promoter, the low level metK promoter, in a suicide vector that contained the flanking regions corresponding to the pseudogene cj0046 of C. jejuni 11168 CASDC gene deletion strain (ΔC8J_0715). The promoter-gene-antibiotic selectable marker was inserted into the chromosome by homologous recombination to give a stable single copy of the gene (c_ΔC8J_0715).

Synthesis of sym-Homospermidine.-sym-Homospermidine 1 was prepared as outlined in Scheme 1. 1,7-(bis)cyano-N-benzyl-4-azaheptane, 3. Compound 3 was synthesized using a modification of the procedure of Covassin et al. (15). A 100 ml round bottom flask was charged with 17.97 g (130.2 mmol) of anhydrous potassium carbonate and 1.80 g (10.85 mmol) of potassium iodide in 40 ml of
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Acetonitrile. A 4.69 g portion of N-benzylamine 2 (4.79 ml, 43.34 mmol) was then added, followed by the slow addition of 9.87 g (9.11 ml, 95.55 mmol) of 4-chlorobutyronitrile in 20 ml of acetonitrile. The reaction was refluxed for 16 hours, and was then cooled to room temperature, filtered and the solvent was removed in vacuo. The yellow, oily residue was chromatographed on silica gel (hexane:EtOAc 4:1) to afford 8.1 g of pure 3 as a pale yellow oil. 1H NMR (400 MHz, CDCl3) δ 7.38–7.22 (m, 5H), 3.54 (s, 2H), 2.55 (t, J = 6.8 Hz, 4H), 2.36 (t, J = 6.8 Hz, 4H), 1.79 (quintet, J = 6.8 Hz, 4H).

1,7-diamino-5-azaheptane (homospermidine), 4. A 3.0 g portion (12.39 mmol) of 3, 5.0 ml of concentrated ammonium hydroxide and 5.0 mL of Raney nickel (50% in water) were placed in a hydrogenation bottle, followed by the cautious addition of 100 ml of methanol, and the mixture was hydrogenated (70 psi) (16-17) with shaking for 64 hours. The resulting mixture was filtered through a Celite plug to remove the catalyst, and the solvent was removed in vacuo to afford 4 as a pale yellow oil (3.10 g, quantitative yield). The product was of sufficient purity to be used in the next reaction without further purification.

1H NMR (400 MHz, CDCl3) δ 7.35–7.15 (m, 5H), 3.53 (s, 2H), 2.65 (bs, 4H), 2.41 (s, 4H), 1.59 (bs, 4H), 1.47 (s, 8H).

1,7-diamino-N5-benzyl-5-azaheptane, 1. A 2.95 g (11.82 mmol) of 4, 0.15 g of 20% Pd/C and 0.15 g of 20% Pd(18)2/C (19) were placed in a hydrogenation bottle, followed by the cautious addition of 100 ml of methanol, and the mixture was hydrogenated (70 psi) with shaking for 24 hours. The resulting mixture was filtered through a Celite plug to remove the catalyst, and the solvent was removed in vacuo to afford 1 as a clear oil. The oil was taken up in 3 ml of 12.1 M HCl, reconstituted at the rotary evaporator and then recrystallized (water/methanol) to yield 2.69 g (65.0%) of homospermidine hydrochloride 1 as a white crystalline solid. 1H NMR (400 MHz, CDCl3) δ 2.66 (t, J = 6.8 Hz, 4H), 2.57 (t, J = 6.8 Hz, 4H), 1.58–1.38 (m, 13H).

RESULTS

Campylobacter jejuni synthesizes spermidine by an S-adenosylmethionine decarboxylase and spermidine synthase-independent pathway-The ε-proteobacterium C. jejuni strain 81116 was grown in polyamine-deficient, defined minimal medium, and cells from the exponential and stationary phases were analysed for polyamine content by HPLC (Fig. 2A). Spermidine was the only polyamine detected (0.87 ± 0.19 and 0.30 ±0.11 nmol/mg fresh weight at exponential (OD600nm 0.2-0.3) and stationary (OD600nm 0.9-1.0) phase, respectively). There are no discernable orthologues of AdoMetDC and SpdSyn in any Campylobacter genome but we noticed that orthologues of CANSDH (universally annotated as saccharopine dehydrogenase) and CANSDC were present in all of these genomes. No orthologues of DABA AT and DABA DC, which together are able to synthesize 1,3-diaminopropane, the precursor of sym-norspermidine, are present in Campylobacter genomes. It therefore seemed likely that the CANSDH- and CANSDC-encoding genes of Campylobacter species actually encoded carboxyspermidine dehydrogenase (CASDH) and carboxyspermidine decarboxylase (CASDC) enzymes. Orthologues encoding arginine decarboxylase (ADC), agmatine deiminase/amidohydrolase (AIH) and N-carbamoylputrescine amidohydrolase (NCPAH) are present in all Campylobacter genomes and so we hypothesized that C. jejuni 81116 synthesizes spermidine by the pathway shown in Fig. 2B.

An alternative spermidine biosynthetic pathway is present in C. jejuni-We constructed gene deletions in C. jejuni 81116 genes (Fig. 2B) putatively encoding ADC (ΔC8J_0715), AIH (ΔC8J_0892), NCPAH (ΔC8J_0890), CASDH (ΔC8J_0166) and CASDC (ΔC8J_1418). With each gene deletion, spermidine accumulation was abolished after growth in polyamine-deficient medium (Fig. 3) except for deletion of CASDH, which surprisingly resulted in a very large increase in the amount of spermidine. We were unable to separate a carboxyspermidine synthetic standard from putrescine by HPLC (Fig. 3). This is reminiscent of the difficulty of detecting carboxynorspermidine in the gene deletion of carboxynorspermidine decarboxylase in Vibrio cholerae (11). However, we showed recently that the C. jejuni recombinant CASDC enzyme is active with both carboxynorspermidine and carboxyspermidine as substrates in vitro,
producing \textit{sym}-norspermidine and spermidine, respectively (20).

\textit{Spermidine is critical for growth of \textit{C. jejuni} 81116-The ADC (\textit{C8J}_0715), CASDH (\textit{C8J}_0166) and CASDC (\textit{C8J}_1418) gene deletion strains were grown in polyamine-deficient liquid medium (Fig. 4A). Gene deletion strains for ADC and CASDC were severely compromised for growth but the CASDH gene deletion strain grew normally. After 25 hr of growth the ADC gene deletion strain consistently grew slightly better than the CASDC knockout mutant. When the ADC gene deletion strain was grown with 500 \( \mu \text{M} \) exogenous agmatine (the product of ADC), growth was restored more efficiently than with 500 \( \mu \text{M} \) spermidine in the medium. Spermidine at 500 \( \mu \text{M} \) restored the growth of the ADC and CASDC gene deletion strains to the same extent. Expression of a chromosomally-integrated recombinant CASDC ORF expressed from a \textit{metK} promoter in the CASDC gene deletion strain (\textit{C8J}_1418) completely restored growth of the CASDC gene deletion strain (Fig. 4A) and restored spermidine biosynthesis (Fig. 4B). The AIH (\textit{C8J}_0892) and NCPAH (\textit{C8J}_0890) gene deletion strains were severely compromised for growth in polyamine-deficient medium but growth was mostly restored by addition of 500 \( \mu \text{M} \) spermidine (Fig. 4C).

\textit{Sym-norspermidine, spermidine and \textit{sym}-homospermidine support growth of \textit{C. jejuni}—We assessed the relative ability of different triamines (Fig. 1A) at 500 \( \mu \text{M} \) to restore growth of the CASDC gene deletion strain (\textit{C8J}_1418). Spermidine and \textit{sym}-homospermidine restored growth with similar efficiencies, however, \textit{sym}-norspermidine was less effective (Fig. 4D). When the accumulation of each exogenously supplied triamine in the CASDC gene deletion strain was assessed by HPLC, \textit{sym}-norspermidine and \textit{sym}-homospermidine were found to have accumulated to similar levels but spermidine was much less abundant in the cells (Fig. S1), suggesting that \textit{sym}-norspermidine and \textit{sym}-homospermidine need to be present at higher levels to replace the function of spermidine. We investigated whether \textit{sym}-norspermidine would outcompete spermidine for uptake as detected by growth restoration. However, equal amounts of \textit{sym}-norspermidine and spermidine in the growth medium restored growth of the CASDC gene deletion strain to the level of the spermidine-only rescue (Fig. S2). In this growth experiment, spermidine and \textit{sym}-norspermidine accumulated in the CASDC gene deletion strain cells to similar levels when present in the growth medium individually or when mixed (Fig. S3A). The growth restoration by \textit{sym}-norspermidine was surprising to us so we assessed whether the \textit{sym}-norspermidine stock solutions might have contained small amounts of spermidine. If any spermidine was present in the \textit{sym}-norspermidine stock solution, it was at levels less than 0.1\% of the \textit{sym}-norspermidine content (Fig. S3B).

The effect of spermidine and \textit{sym}-norspermidine dosage (500, 750 and 1000 \( \mu \text{M} \)) on growth restoration of the CASDC gene deletion strain was examined. Growth restoration was concentration-dependent although 750 \( \mu \text{M} \) and 1000 \( \mu \text{M} \) spermidine were equally effective at almost completely restoring growth. This effect was not observed with the less effective \textit{sym}-norspermidine, and 1000 \( \mu \text{M} \) \textit{sym}-norspermidine was more effective than 750 \( \mu \text{M} \) \textit{sym}-norspermidine, although 1000 \( \mu \text{M} \) \textit{sym}-norspermidine was markedly less effective than 500 \( \mu \text{M} \) spermidine at growth restoration (Fig. 5A). The relative efficiencies of different diamines for growth restoration of the \textit{C. jejuni} 81116 ADC gene deletion strain (\textit{C8J}_0715) were determined. Putrescine, 1,3-diaminopropane and cadaverine were equally ineffective at restoring growth (Fig. 5B) and HPLC analysis indicated a complete lack of accumulation of these diamines in the cells (results not shown).

\textit{CASDH and CASDC are found in diverse bacterial phyla—Orthologues of both CASDH and CASDC are pervasive in the Firmicutes and Proteobacteria, although infrequent in the \textit{\varepsilon}-Proteobacteria (Table 1). The alternative pathway is also found in the lesser known (and lesser sequenced) phyla including Deinococcus-Thermi, Verrucomicrobia, Spirochaetes, Deferrribacteria, Fusobacteria, Lentisphaeriae, Elusimicrobia, Chrysiogenetes and Fibrobacteres. However, it is rare in the Cyanobacteria and Archaea, and although some distant homologues of CASDH are found in many Actinobacteria, there are no CASDC orthologues and thus the pathway appears to be absent from the Actinobacteria. Only two \textit{\varepsilon}-proteobacterial genomes possess an AdoMetDC orthologue, \textit{Nitratiruptor} sp. SB155-}
2 (YP_00156990) and *Caminibacter medialanticus* TB-2 (ZP_01872533) and so the CASDH/CASDC pathway is almost the only pathway for spermidine biosynthesis in this phylum. A number of important human pathogens possess the CASDH/CASDC pathway including *Streptococcus pneumoniae*, *Bordetella pertussis*, *Pasteurella multocida*, *Bartonella* species, *Brucella* species, *Moraxella catarrhalis*, *Vibrio cholerae*, *Helicobacter pylori*, *Bacteroides fragilis*, *Porphyromonas gingivalis*, the food-borne pathogen *C. jejuni* and the botulism bacterium *Clostridium botulinum*.

CASDH and CASDC are often found clustered with putrescine biosynthetic genes—Analysis of bacterial genomes indicates that CASDH- and CASDC-encoding ORFs are frequently localised to clusters of putrescine biosynthetic genes including alanine racemase- and aspartate aminotransferase-fold arginine decarboxylases together with agmatine ureohydrolase, or agmatine deiminase and N-carbamoylputrescine amidohydrolase (Fig. 6). Less frequently, alanine racemase- and aspartate aminotransferase-fold ornithine decarboxylase orthologues are found clustered with CASDH and CASDC. Usually CASDH is positioned immediately upstream of CASDC, however, the situation is reversed in the β-proteobacterium *Bordetella pertussis* Tohoma 1 and uncultured Termite Groups 1 Rs-D17. Uniquely in *Magnetococcus* sp. MC-1, the CASDH and CASDC pair are arranged divergently and must be transcribed from an intervening divergent promoter. In some Firmicutes species, both CASDH/CASDC and AdoMetDC/SpdSyn pairs can be found in the same cluster. In the gut firmicute species *Ruminococcus gnavus* and *Clostridium leptum*, an identical gene cluster is present containing an aspartate aminotransferase-fold arginine decarboxylase, spermidine synthase, agmatine ureohydrolase, CASDH and CASDC (Fig. 6). However, *C. leptum* has retained an AdoMetDC immediately upstream of the cluster, whereas *R. gnavus* has lost AdoMetDC from its genome (Fig. 6). Other gut firmicutes such as the opportunistic pathogen *Clostridium difficile* appear to possess only the AdoMetDC/SpdSyn pathway for spermidine biosynthesis, with no orthologues of CASDH and CASDC detectable in the genome. The pattern of presence and absence of the AdoMetDC/SpdSyn and CASDH/CASDC pathways in the Firmicutes as a whole suggests that the CASDH/CASDC pathway is enriched in the gut microbiota and is in the process of supplanting the AdoMetDC/SpdSyn pathway.

*CASDH/CASDC is the dominant polyamine biosynthetic pathway in the human gut microbiota*—As CASDH and CASDC are pervasive in the Bacteroidetes and Firmicutes phyla, we analysed the polyamine biosynthetic pathways in the 55 most ubiquitous and abundant species in the human gut microbiota described by Qin *et al.*, (21). In Fig. 7 it can be seen that not a single species possesses AdoMetDC and SpdSyn only, and the AdoMetDC/SpdSyn pathway is found in only five species, all of which contain the CASDH/CASDC pathway. There are 15 Firmicutes species and 22 Bacteroidetes species that possess the CASDH/CASDC pathway, and 1 Bacteroidetes species and 15 Firmicutes species are putatively auxotrophic for polyamine biosynthesis (Fig. 7). Each of the gut polyamine putative auxotrophic species nevertheless possesses the spermidine-preferential ABC cassette uptake transporter (22) encoded by the *potABCD* genes (Table 2) except for the actinobacterial species *Collinsella aerofaciens*. In this transport system, PotA is an energy-generating ATPase, PotB and PotC each have six transmembrane-spanning segments and form a channel for spermidine and putrescine, and PotD is a periplasmic spermidine-binding protein. In each of the polyamine auxotrophic species the *potABCD* genes are clustered into an operon, and for five species, the *potC* and *potD* genes are fused (Table 2). This is an unusual fusion since *potC* encodes one of the two transmembrane channel proteins and *potD* encodes the substrate binding protein. The *potC-potD* fusion is also found in some of the gut firmicute polyamine prototrophs but this fusion appears to be limited to the Firmicutes.

Intriguingly, of the 15 Firmicutes species that possess the CASDH/CASDC pathway, 11 possess SpdSyn but not AdoMetDC. Analysis of putrescine biosynthetic pathways indicate that within the list of 55 gut species (23), Firmicutes species use the aspartate aminotransferase-fold ADC and Bacteroidetes use the alanine racemase-fold ADC. When the polyamine biosynthetic pathway as a whole is assessed in these 55 species, seven different biosynthetic pathway configurations can be discerned (Fig. 8). The first part of the pathway consists of two separate submodules: one of three forms of ADC
to form agmatine from arginine, and either AUH or AIH/NCPAH to form putrescine from agmatine. All pathways have a second module of CASDH/CASDC and may have an additional module of AdoMetDC and SpdSyn or only SpdSyn.

**DISCUSSION**

An alternative pathway for spermidine biosynthesis, i.e. one that does not use AdoMetDC and SpdSyn was first proposed by Tait (24) thirty five years ago. He proposed aspartate β-semialdehyde as the source of the aminopropyl group for spermidine biosynthesis from putrescine and suggested a carboxyspermidine intermediate in the α-proteobacterial species *Paracoccus denitrificans* and *Rhodobacter sphaeroides*. Previously, we demonstrated that the genes encoding CANSDH and CANSDC were essential for sym-norspermidine biosynthesis in *Vibrio cholerae* (11). The 1,3-diaminopropane precursor of sym-norspermidine was synthesized by a fusion protein comprised of DABA AT and DABA DC. However, because there is relatively little overlap between genomes possessing DABA AT and DABA DC orthologues with those containing CANSDH and CANSDC orthologues (except for the Vibrionales and a limited number of other species), we tentatively suggested that the carboxypolyamine pathway was used primarily for spermidine biosynthesis in other species (11). We obtained an X-ray crystal structure of the *C. jejuni* CANSDC orthologue and determined that it has a relatively equal preference for carboxynorspermidine and carboxyspermidine, in contrast to the *V. cholerae* CANSDC, which has a marked preference for carboxynorspermidine (20). Here we have shown that the *C. jejuni* enzyme is a CASDC and not a CANSDC in vivo, and is essential for spermidine biosynthesis. Only spermidine is present in *C. jejuni* and there is no pathway for 1,3-diaminopropane biosynthesis and none is detectable by HPLC. Consequently, sym-norspermidine is not synthesized in the *C. jejuni* cells.

In contrast to *V. cholerae*, where deletion of the gene encoding CANSDH leads to complete elimination of sym-norspermidine biosynthesis (11), deletion of the *C. jejuni* CASDH does not abolish spermidine accumulation. Instead, it paradoxically produces a very large increase in spermidine accumulation. This unexpected result of deletion of the CASDH-encoding gene suggests that a metabolic bypass is activated by the removal of CASDH, and that CASDH is a rate-limiting enzyme regulating spermidine levels. Over-expression of CANSDH in *V. cholerae* caused a large accumulation of spermidine, which is normally undetectable in these cells, suggesting that the excess CANSDH had depleted its main substrate 1,3-diaminopropane, and as a result then converted putrescine to spermidine. (11). The diversity of mechanisms for bypassing metabolic blocks has been revealed by a recent study of an *E. coli* mutant lacking 4-phosphorylthreonate dehydrogenase, which is required for biosynthesis of the key enzyme cofactor pyridoxal-5'-phosphate. A multicopy suppressor strategy identified seven different genes in three serendipitous pathways that could restore pyridoxal-5'-phosphate synthesis (25). Even in polyamine biosynthesis there is a precedent for bypassing metabolic blocks. The *Pseudomonas aeruginosa* PAO1 N-carbamoylputrescine amidohydrolase gene deletion produced a leaky biosynthetic phenotype and it was consequently found that acetylputrescine amidohydrolase, which has a very similar substrate to N-carbamoylputrescine (26) is induced in the gene deletion mutant and allows bypass of the metabolic block. The CANSDH/CASDH enzyme is related to homospermidine synthase, lysine-6-dehydrogenase, saccharopine dehydrogenase and aspartate dehydrogenase (6). There may be a dehydrogenase in *C. jejuni* that can serendipitously substitute the activity of the missing CASDH. Aspartate β-semialdehyde is able to non-enzymatically form a Schiff base with carboxynorspermidine (27), which may facilitate a biosynthetic bypass. The evidence that supports a role for CASDH homologues in the carboxypermidine pathway is: deletion of the CASDH-encoding gene causes a very large increase in spermidine, which at least suggests involvement in polyamine metabolism; CASDH is found adjacent to CASDC in several α-proteobacterial lineages; CASDH is found adjacent to putrescine biosynthetic genes; CASDH is closely related to CANSDH of *V. cholerae*, which is able to synthesize carboxyspermidine as well as carboxynorspermidine.

A gene described as a CANSDC was deleted in the gastric pathogen *Helicobacter pylori* 26695 as part of a systematic screen for genes essential
An alternative spermidine biosynthetic pathway

for growth in this strain (28). The CANSDC orthologue was essential for growth as determined by a colony growth assay. However, H. pylori contains spermidine as its only polyamine (29), and as no orthologue of AdoMetDC is present, the H. pylori CANSDC must be considered a CASDC. There is no AdoMetDC orthologue in H. pylori, however, a divergent SpdSyn-like gene is present, which was shown by Chalker et al., (28) to be inessential for colony growth.

Spermidine can be replaced by sym-homospermidine for growth restoration of C. jejuni spermidine auxotrophs-Depletion of spermidine auxotrophs, indicates that a higher concentration of spermidine than spermidine, they are flexible linear chains and can bend, whereas sym-norspermidine, which is one carbon shorter than spermidine is unlikely to stretch.

Deep sea hydrothermal vent communities and the human gastrointestinal tract are enriched for the carboxyspermidine pathway-The CASDH/CASDC pathway is present in many bacterial phyla, but it is notably absent in the Chloroflexi, Chlorobi, almost all of the Cyanobacteria, in Acidobacteria, Planctomycetes and most prominently in the Actinobacteria. These observations are based entirely on sequence similarity analyses and not gene annotations. Almost all CASDH orthologues are annotated erroneously as saccharopine dehydrogenase. In phyla such as the Chloroflexi, Chlorobi and the filamentous Cyanobacteria, sym-homospermidine is the main polyamine. The ubiquity of the CASDH/CASDC pathway in the ε-Proteobacteria means that it is the dominant spermidine biosynthetic pathway in deep sea hydrothermal vent communities, as a consequence of the prominence of the ε-Proteobacteria in that environment (31). Other phyla in deep sea hydrothermal vents possess CASDH/CASDC orthologues including the Deferribacteres species Deferribacter desulfuricans SSM1 (32), which also possess the AdoMetDC/SpdSyn pathway and accumulates spermidine but not sym-norspermidine (18), and in δ-Proteobacteria such as Geobacter sulfurreducens and Desulfovibrio vulgaris. The alternative carboxyspermidine biosynthetic pathway is therefore pervasive in deep sea hydrothermal vent communities.

Another ecological niche that has assimilated the CASDH/CASDC pathway is the human gastrointestinal tract. Of the two dominant bacterial phyla within the human large intestine, the Bacteroidetes species universally possess the CASDH/CASDC route as their only polyamine biosynthetic pathway, although Parabacteroides johnsonii has become a polyamine auxotroph due to degradation of its CASDH-/CASDC-encoding genes. Horizontal transfer almost certainly explains the presence of AdoMetDC/SpdSyn genes in Bacteroides capillosus, so that two physically clustered spermidine biosynthetic pathways are present in the genome of this Bacteroidetes species. Half of the Firmicutes species amongst the 55 most abundant bacterial species in the human gut (21) appear to be polyamine auxotrophs but all possess orthologues of the potABCD spermidine transporter genes. This is a common occurrence in bacteria with reduced genomes, such as in intracellular pathogens, where biosynthetic pathways are lost.
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and so the bacterium becomes dependent on uptake. In the intracellular pathogen *Mycoplasma genitalium*, the polyamine biosynthetic pathway has been lost but all components of the potABC (no potD orthologue) spermidine transporter are essential (33). The other half possess the CASDH/CASDC pathway but also have a SpdSyn orthologue, however, there are no Firmicutes species that possess an AdoMetDC orthologue but not SpdSyn. The gut firmicute *Butyvibrio crossotus* possesses CASDH, CASDC and SpdSyn orthologues and has been shown previously to accumulate spermidine as its only polyamine (18). An active AdoMetDC in the absence of an aminopropyltransferase like SpdSyn would lead to an accumulation of decarboxylated S-adenosylmethionine, a potent inhibitor of methyltransferases (34). The CASDH/CASDC pathway appears to have replaced the AdoMetDC/SpdSyn pathway in many gut Firmicutes species, with the SpdSyn remaining as a remnant, although it is possible that the SpdSyn orthologue has acquired a new function. There is only one actinobacterial species amongst the 55 most ubiquitous, abundant human gut bacterial species but *Collinsella aerofaciens* does not possess any recognisable polyamine biosynthetic genes and has been shown previously to lack polyamine biosynthesis (18), i.e. it is a polyamine auxotroph. It also lacks orthologues of the potABCD genes encoding the spermidine-preferential transporter, and therefore it is formally possible that the SpdSyn orthologue has acquired a new function. There is only one actinobacterial species amongst the 55 most ubiquitous, abundant human gut bacterial species but *Collinsella aerofaciens* does not possess any recognisable polyamine biosynthetic genes and has been shown previously to lack polyamine biosynthesis (18), i.e. it is a polyamine auxotroph. It also lacks orthologues of the potABCD genes encoding the spermidine-preferential transporter, and therefore it is formally possible that the SpdSyn orthologue has acquired a new function.

The bacterial AdoMetDC/SpdSyn pathway was first described in *E. coli* (3) and this is the only polyamine biosynthetic pathway in the Enterobacteria, which entirely lack the CASDH/CASDC route. It is surprising therefore that the AdoMetDC/SpdSyn pathway is only a minor polyamine biosynthetic route in the human gastrointestinal tract. However, it is known now that the proteobacteria, which includes *E. coli*, make up less than 3% of the gut microbiota (35). Not only is the CASDH/CASDC pathway dominant in the gut (Bacteroidetes and Firmicutes) and stomach (*Helicobacter*), the oral microbiome is composed of a large contingent of Bacteroidetes species which also contain the CASDH/CASDC route as the only polyamine biosynthetic pathway, eg. *Prevotella*, *Bacteroides* and *Porphyromonas* species, which have been shown to accumulate spermidine as the sole polyamine (8). Taking into account the number of bacterial cells in the human gastrointestinal tract, the CASDH/CASDC pathway is likely to be present in more cells within the human supra-organism than the AdoMetDC/SpdSyn pathway.

The AdoMetDC/SpdSyn route for spermidine biosynthesis is widely distributed in bacteria, archaea and eukaryotes. In contrast, the CASDH/CASDC route is absent from the archaea, except for a clear case of horizontal gene transfer in the euryarchaeote *Methanoplanus petrolearius* DSM 11571, and is absent in eukaryotes. Probably the CASDH/CASDC route evolved after the AdoMetDC/SpdSyn pathway. It is clear that CASDC has evolved from other polyamine-related basic amino acid decarboxylases (36) and CASDH has evolved from an enzyme family that includes homospermidine synthase, lysine 6-dehydrogenase, saccharopine dehydrogenase, aspartate dehydrogenase and homoserine dehydrogenase (6). The intriguing question is why the CASDH/CASDC pathway is supplanting AdoMetDC/SpdSyn in the gut Firmicutes species. The co-substrate of CASDH is aspartate β-semialdehyde and the cofactor is NADPH (27), and CASDC requires the cofactor pyridoxal-5'-phosphate (37) (20). The AdoMetDC/SpdSyn pathway does not required exogenous cofactors but does require S-adenosylmethionine as a co-substrate. Not only is S-adenosylmethionine a relatively energetically expensive molecule being made from ATP and methionine, but the co-product of SpdSyn (methylthioadenosine) must be salvaged to recover methionine. The necessity for salvage may confer a fitness cost in a fast growing environment like the human gut, which is one of the most densely populated biomes known. It is notable that species using the CASDH/CASDC pathway are frequently found in anaerobic environments.

Because prominent human pathogens such as *H. pylori*, *P. gingivalis*, *S. pneumoniae*, *Brucella* species, *Bordetella pertussis*, *Pasteurella multocida*, *Bartonella* species, *Moraxella catarrhalis*, *Vibrio cholerae*, *Bacteroides fragilis* and food-associated bacteria such as the opportunistic pathogen *Campylobacter jejuni* and the deadly *Clostridium botulinum* possess the
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CASDH/CASDC pathway, a new potential target for the development of antimicrobial compounds has been revealed by our findings. Most of these pathogens also use the arginine route, rather than the ornithine route for putrescine biosynthesis, so the entire polyamine biosynthetic pathway in these bacterial species differs from the human host, and therefore presents a novel target for exploration of antimicrobial drug development.

References

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Acknowledgements

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Abbreviations
- ADC, arginine decarboxylase; AdoMetDC, S-adenosylmethionine decarboxylase; AIH, agmatine deiminase/iminohydrolase; CANSDC, carboxynorspermidine decarboxylase; CANSDH, carboxynorspermidine dehydrogenase; CASDC, carboxyspermidine decarboxylase; CASDH, carboxyspermidine dehydrogenase; DABA AT, diaminobutyrate aminotransferase; DABA DC, diaminobutyrate decarboxylase; NCPAH, N-carbamoylputrescine amidohydrolase; ODC, ornithine decarboxylase; SpdSyn, spermidine synthase
**FIGURE LEGENDS**

**SCHEME 1. Synthetic strategy for sym-homospermidine.**

**FIGURE 1. Bacterial polyamines and the decarboxylated S-adenosylmethionine pathway for spermidine biosynthesis.** A, aminopropyl groups are shown in red; aminobutyl groups in blue. B, Biosynthesis of spermidine from putrescine by transfer of an aminopropyl group from decarboxylated S-adenosylmethionine.

**FIGURE 2. Spermidine biosynthesis in *C. jejuni*.** A, HPLC chromatogram of polyamines from *C. jejuni* wild-type (81116) cells grown in polyamine-deficient medium. Polyamines were extracted from log phase (OD$_{600}$, 0.2 to 0.3) and stationary phase (OD$_{600}$, 0.9 to 1.0) cells. R, fluorescent labelling dye; Spd, spermidine; IS, internal standard (1,7-diaminoheptane). B, putative pathway for spermidine biosynthesis in *C. jejuni*. Candidate *C. jejuni* open reading frames encoding pathway enzymes are indicated. ADC, arginine decarboxylase; AIH, agmatine deiminase/ iminohydrolase; NCPAH, N-carbamoylputrescine amidohydrolase; CASDH, carboxyspermidine dehydrogenase; CASDC, carboxyspermidine decarboxylase. The aminopropyl group transfer is shown in blue.

**FIGURE 3. HPLC chromatograms of polyamines from *C. jejuni* gene deletion mutants grown in polyamine-deficient medium.** Cells were grown as described in experimental procedures. R, fluorescent labelling dye; Spd, spermidine; IS, internal standard (1,7-diaminoheptane).

**FIGURE 4. Cell growth in *C. jejuni* deletion mutants.** Cells were grown in polyamine-deficient medium ± polyamines as described in experimental procedures. Data represent the means of triplicate cultures ± standard deviation. A, cell growth for wild-type (81116), ΔC8J_0715 (arginine decarboxylase; ADC), ΔC8J_0166 (carboxyspermidine dehydrogenase; CASDH) and ΔC8J_1418 (carboxyspermidine decarboxylase; CASDC) deletion strains, and the genetically complemented strain c ΔC8J_1418. a, wildtype parental strain; b, ΔC8J_1418 (CASDC); c, ΔC8J_1418 (CASDC) plus Spd; d, ΔC8J_0715 (ADC); e, ΔC8J_0715 (ADC) plus Agm; f, ΔC8J_0715 (ADC) plus Spd; g, ΔC8J_0166 (CASDH); h, genetically complemented ΔC8J_1418 (CASDC), i.e. (c ΔC8J_1418). Where added to the medium, spermidine and agmatine were at 500 µM final concentration. Spd, spermidine; Agm, agmatine. B, HPLC chromatograms of *C. jejuni* gene deletion strain ΔC8J_1418 (CASDC) and the same strain genetically complemented by expressing a chromosomally located copy of the CASDC-encoding ORF (c ΔC8J_1418). C, a, *C. jejuni* 81116 wildtype parental strain; b, ΔC8J_0890 (NCPAH); c, ΔC8J_0890 plus 500 µM Spd; d, ΔC8J_0892 (AIH); e, ΔC8J_0892 plus 500 µM Spd. D, a, *C. jejuni* 81116 wildtype parental strain; b, ΔC8J_1418 (CASDC); c, ΔC8J_1418 plus 500 µM spermidine; d, ΔC8J_1418 plus 500 µM sym-norspermidine; e, ΔC8J_1418 plus 500 µM sym-homospermidine.

**FIGURE 5. Growth restoration of *C. jejuni* polyamine auxotrophic strains by exogenous polyamines.** Cells were grown in polyamine-deficient medium ± polyamines as described in experimental procedures. A, Dose response of cell growth in ΔC8J_1418 (carboxyspermidine decarboxylase; CASDC) gene deletion mutant in polyamine-deficient medium supplemented with spermidine or sym-norspermidine. a, *C. jejuni* wild-type parental strain (81116); b, ΔC8J_1418 (CASDC); c, ΔC8J_1418 plus 500 µM spermidine; d, ΔC8J_1418 plus 750 µM spermidine; e, ΔC8J_1418 1000 µM spermidine; f, ΔC8J_1418 plus 500 µM sym-norspermidine; g, ΔC8J_1418 plus 750 µM sym-norspermidine; h, ΔC8J_1418 plus 1000 µM sym-norspermidine. B, Dose response of cell growth in ΔC8J_0715 (arginine decarboxylase;
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ADC) gene deletion mutant in polyamine-deficient medium supplemented with polyamines. 

a, C. jejuni wild-type parental strain (81116); b, ΔC8J_0715; c, ΔC8J_0715 plus 500 μM agmatine; d, ΔC8J_0715 plus 500 μM putrescine; e, ΔC8J_0715 plus 500 μM cadaverine; f, ΔC8J_0715 plus 500 μM 1,3-diaminopropane; g, ΔC8J_0715 plus 500 μM spermidine; h, ΔC8J_0715 plus 500 μM sym-norspermidine.

FIGURE 6. Polyamine-related gene clusters containing carboxyspermidine dehydrogenase (CASDH) and carboxyspermidine decarboxylase (CASDC) orthologues in bacterial genomes. Protein accession numbers are shown below the first and last ORFs. Bacterial phyla are listed in brackets. AUH, agmatine ureohydrolase; AAT, aspartate aminotransferase fold; AR, alanine racemase fold; hypo, hypothetical protein.

FIGURE 7. Spermidine biosynthetic pathways in the human gut microbiota. Protein accession numbers are given for carboxyspermidine dehydrogenase (CASDH), carboxyspermidine decarboxylase (CASDC), AdoMetDC and SpdSyn ORFs in the 55 most abundant, ubiquitous bacterial species in the human gut (21). Firmicutes species (F) are shown in pink and Bacteroidetes species in green (B). Absent ORFs are not coloured.

FIGURE 8. Polyamine biosynthetic pathway configurations in human gut microbiota species. aAR-ADC, ancestral alanine racemase-fold biosynthetic arginine decarboxylase (23); AR-ADC, alanine racemase-fold biosynthetic arginine decarboxylase (23); biosynthetic aspartate aminotransferase-fold arginine decarboxylase (23); AUH, agmatine ureohydrolase.
Table 1

Number of genomes encoding both carboxyspermidine dehydrogenase (CASDH) and carboxyspermidine decarboxylase (CASDC) in bacterial phyla

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<tr>
<td>Firmicutes</td>
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<tr>
<td>δ-Proteobacteria</td>
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</tr>
<tr>
<td>Deinococcus-Thermi</td>
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</tr>
<tr>
<td>Thermi</td>
<td>4</td>
</tr>
<tr>
<td>β-Proteobacteria</td>
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<td>4</td>
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<td>Elusimicrobia</td>
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<td>Fibrobacteres</td>
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<tr>
<td>Archaea</td>
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</table>
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Table 2

Prominent human gut bacterial putative polyamine auxotrophs possessing the spermidine-specific PotABCD transporter

<table>
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<tr>
<th>Species</th>
<th>PotA Accession</th>
<th>PotB Accession</th>
<th>PotC Accession</th>
<th>PotD Accession</th>
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Protein accession numbers are followed by the amino acid size in brackets.
Scheme 1

4-chlorobutyronitrile acetonitrile → 77% yield

20% Pd/C
20% Pd(OH)₂/C
MeOH 65% yield

Raney Ni
H₂ 50 psi
MeOH, NH₄OH
100% yield

1

77% yield

2
Fig 1

A

sym-norspermidine

\[
\begin{align*}
\text{H}_2\text{N} & - \text{N} - \text{H} - \text{N} - \text{NH}_2 \\
\text{H}_2\text{N} & - \text{N} - \text{H} - \text{N} - \text{NH}_2
\end{align*}
\]

spermidine

\[
\begin{align*}
\text{H}_2\text{N} & - \text{N} - \text{H} - \text{N} - \text{NH}_2 \\
\text{H}_2\text{N} & - \text{N} - \text{H} - \text{N} - \text{NH}_2
\end{align*}
\]

sym-homospermidine

\[
\begin{align*}
\text{H}_2\text{N} & - \text{N} - \text{H} - \text{N} - \text{NH}_2 \\
\text{H}_2\text{N} & - \text{N} - \text{H} - \text{N} - \text{NH}_2
\end{align*}
\]

B

S-adenosylmethionine

\[
\begin{align*}
\text{H}_2\text{N} & - \text{N} - \text{H} - \text{N} - \text{NH}_2 \\
\text{H}_2\text{N} & - \text{N} - \text{H} - \text{N} - \text{NH}_2
\end{align*}
\]

S-adenosylmethionine decarboxylase (AdoMetDC)

putrescine

spermidine synthase (SpdSyn)

spermidine

decarboxylated S-adenosylmethionine
Fig 2

A  
Retention time (min)

B  
arginine  
arginine  
NH2

C8J_0715  
(ADC)  
agmatine  
H2N

C8J_0892  
(AIH)  
N-carbamoyl putrescine  
H2N

C8J_0890  
(NCPAH)  
putrescine  
H2N

spermidine  
H2N

C8J_1418  
(CASDC)  
carboxyspermidine  
H2N

aspartate  
β-semialdehyde  
H3N+
Fig 4
Fig 5

A

B

OD \text{nm} \times 600

hours

OD \text{nm} \times 600

hours
**Fig 6**

- Ruminococcus gnavus ATCC 29149 (Firmicutes)
- Bacteroides capillosus ATCC 29799 (Bacteroidetes)
- Clostridium leptum DSM 753 (Firmicutes)
- Eubacterium rectale ATCC 33656 (Firmicutes)
- Streptococcus pneumoniae TIGR4 (Firmicutes)
- Clostridium butyricum SS521 (Firmicutes)
- Ethanoligenes harbinense YUAN-3 (Firmicutes)
- Pelobacter propionicus DSM 2379 (δ-Proteobacteria)
- Desulfovibrio vulgaris str.
- Hildenborough (δ-Proteobacteria)
- Pelobacter propionicus DSM 2379 (δ-Proteobacteria)
- Desulfovibrio vulgaris str.
- Miyazaki F (δ-Proteobacteria)
- Deferrribacteres desulfuricans SSM1 (Deferrribacteres)
- Geobacter lovleyi SZ (δ-Proteobacteria)
- Saccharophagus degradans 2-40 (ε-Proteobacteria)
- Geobacter bendifiensis Bem (ε-Proteobacteria)
- Sphingobium japonicum UT26S (α-Proteobacteria)
- Novosphingobium aromaticivorans DSM 12444 (α-Proteobacteria)
- Erythrobacter litoralis
- HTCC 2594 (α-Proteobacteria)
- Sphingopyxis alaskensis RB2256 (α-Proteobacteria)
- Allochromatium vinosum DSM180 (γ-Proteobacteria)
- Spirosoma linguale DSM74 (Bacteroidetes)
- Nitriruptor sp. SB155-2 (γ-Proteobacteria)
- Moraxella catarrhalis RH4 (γ-Proteobacteria)
- Sulfospirillum deleyianum DSM 6946 (γ-Proteobacteria)
- Sulfurimonas autotrophica DSM 16294 (γ-Proteobacteria)
- Nautilia profundicola AmH (γ-Proteobacteria)
- Paludibacter propionicigenes WB4 (Bacteroidetes)
- Deinococcus maricopensis DSM 21211(Deinococcus-Thermi)
- Methanoplanus petrolearius DSM 11571
- (Archaea, Euryarchaeota, Methanomicrobia)
- Odoribacter splanchicus DSM 20712 (Bacteroidetes)
- Calditerrivibrio nitroreducens DSM 19672 (Deferrribacteres)
- Desulforudis salinus DSM 206956 (Chrysogogenes)
- Magnetococcus sp. MC-1 (Proteobacteria)
- Bordetella pertussis Tohoma 1 (β-Proteobacteria)
- Uncultured Termite Group1 Rs-D17 (Elusimicrobia)
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Fig 8

- Bacteroides uniformis
- Alistipes putredinis
- Ruminococcus torques L2-14
- Roseburia intestinalis M50/1
- Eubacterium siraeum
- Bacteroides capillosus
- Bacteroides eggerthii
An alternative spermidine biosynthetic route is critical for growth of Campylobacter jejuni and is the dominant polyamine pathway in the human gut microbiota

Colin C. Hanfrey, Bruce M. Pearson, Stuart Hazeldine, Jeongmi Lee, Duncan J. Gaskin, Patrick M. Woster, Margaret A. Phillips and Anthony J. Michael

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