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Comprehensive analysis of polyamine transport and biosynthesis in the dominant human gut bacteria: Potential presence of novel polyamine metabolism and transport genes

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ABSTRACT

Recent studies have reported that polyamines in the colonic lumen might affect animal health and these polyamines are thought to be produced by gut bacteria. In the present study, we measured the concentrations of three polyamines (putrescine, spermidine, and spermine) in cells and culture supernatants of 32 dominant human gut bacterial species in their growing and stationary phases. Combining polyamine concentration analysis in culture supernatant and cells with available genomic information showed that novel polyamine biosynthetic proteins and transporters were present in dominant human gut bacteria. Based on these findings, we suggested strategies for optimizing polyamine concentrations in the human colonic lumen via regulation of genes responsible for polyamine biosynthesis and transport in the dominant human gut bacteria.

1. Introduction

Polyamines (putrescine [Put], spermidine [Spd], spermine [Spm]) are aliphatic amines possessing two or more amino groups. They are widely distributed in eukaryotic (Pegg, 2009) and prokaryotic cells (Tabor and Tabor, 1985). In the mammalian colonic lumen, polyamines are present at millimolar concentrations (Matsumoto and Benno, 2007), and it was previously reported that these polyamines are derived from gut bacteria (Matsumoto et al., 2012; Noack et al., 2000). Polyamines in the colonic lumen are transferred into the bloodstream via the colonic mucosa (Kibe et al., 2014), after which they have various effects on the body. For example, high concentrations of polyamines are found in cancer cells because of their role in cell proliferation. The possibility of treating cancer by reducing gut bacterial polyamine levels via administration of antibiotics is being investigated (Johnson et al., 2015). However, polyamines in the intestinal tract have various beneficial effects on mammalian health, such as increased longevity (Kibe et al., 2014; Matsumoto et al., 2011), recovery of injured mucosa (Lux et al., 1980), and favorable effects on cognitive function (Kibe et al., 2014). As intestinal polyamines are derived from gut bacteria, colonic luminal polyamine concentration is determined by bacterial polyamine metabolism. The known pathways for bacterial polyamine biosynthesis and transport are summarized in Fig. 1 (Kurihara and Suzuki, 2015; Michael, 2015; Michael, 2016a,b; Sugiyama et al., 2016). Recently, Kibe et al. reported that even though Put increased in the colonic lumen, the abundance of known Put biosynthetic genes (*speB*, *adi*, and *ncpah*; Fig. 1) were unchanged in mice gut microbiota (Kibe et al., 2014). These results suggest that in addition to the previously described polyamine biosynthetic pathway (Fig. 1), there is a novel gene or set of genes facilitating Put biosynthesis in the gut bacteria. Therefore, identification of new genes for polyamine biosynthesis and transport is indispensable for optimization of polyamine concentrations in human colonic lumen, enabled by regulation of genes facilitating polyamine metabolism in gut bacteria.

A "human gut microbial gene catalog" ranking the dominant microbial species/genera in the human gut has been described (Qin et al., 2010). Recently, we reported that Gifu anaerobic medium (GAM) was useful for the cultivation of 32 species of dominant human gut bacteria and for comparison of their metabolite profiles (Gotoh et al., 2017). The polyamine concentration in stationary phases of *Alistipes* (Hamana et al., 2008), *Bacteroides* (Hamana et al., 2008; Hosoya and Hamana, 2004) and *Parabacteroides* (Hamana et al., 2008) species, which are members of human gut bacteria, have been previously reported. However, in some bacteria, the polyamine profile in cells and in culture supernatants differ based on their growth phase (Hanfrey et al., 2011; Sakanaka et al., 2016; Sugiyama et al., 2016): cellular polyamine

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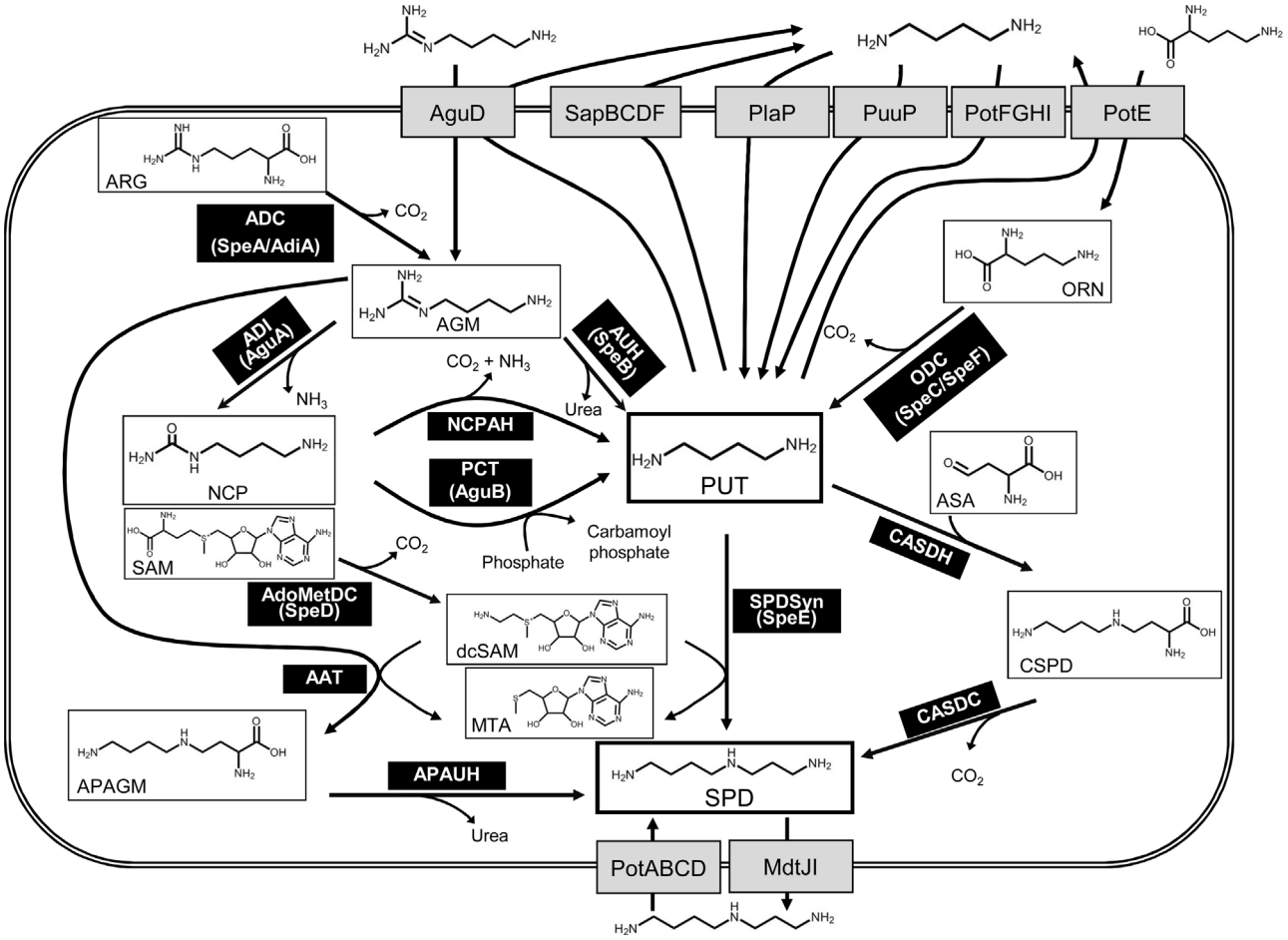

Fig. 1. Polyamine biosynthetic and transport pathways in bacteria.
Polyamine biosynthetic and transport pathways previously described in bacteria are integrated and illustrated. Gray squares indicate transporters (importer, antiporter, and exporter) that were previously reported in *Escherichia coli* or *Enterococcus faecalis*. Black squares with white letters show abbreviated names of enzymes experimentally identified in *E. coli*, *En. faecalis*, *B. thetaiotaomicron*, *Thermus thermophilus*, *Vibrio cholerae*, or *Pseudomonas aeruginosa*. The abbreviations used are as follows: AAT, agmatine aminopropyltransferase (Ohnuma et al., 2005); ADC, arginine decarboxylase (Moore and Boyle, 1990; Stim and Bennett, 1993); ADI, agmatine deiminase (Llacer et al., 2007); AdoMetDC, *S*-adenosylmethionine decarboxylase (Tabor and Tabor, 1987); AGM, agmatine; AguD, putrescine-agmatine antiporter (Suarez et al., 2013); APAGM, aminopropylagmatine; APAUH, aminopropylagmatine ureohydrolase (Ohnuma et al., 2005); ARG, arginine; ASA, aspartate-β-semialdehyde; AUH, agmatine ureohydrolase (Satishchandran and Boyle, 1986); CASDC, carboxyspermidine decarboxylase (Hanfrey et al., 2011; Lee et al., 2009; Sakanaka et al., 2016); CASDH, carboxyspermidine dehydrogenase (Hanfrey et al., 2011; Lee et al., 2009); CSPD, carboxyspermidine; dcSAM, decarboxylated *S*-adenosylmethionine; MdtJI, spermidine exporter (Higashi et al., 2008); MTA, 5′-deoxy-5′-methylthioadenosine; NCP, *N*-carbamoylputrescine; NCPAH, *N*-carbamoylputrescine amidohydrolase (Nakada and Itoh, 2003); ODC, ornithine decarboxylase (Kashiwagi et al., 1991; Morris and Pardee, 1965); ORN, ornithine; PCT, putrescine carbamoyltransferase (Llacer et al., 2007); PlaP, low-affinity putrescine importer (Kurihara et al., 2011); PotABCD, ATP-binding cassette type spermidine preferential importer (Furuchi et al., 1991); PotE, putrescine-ornithine antiporter (Kashiwagi et al., 1992; Kashiwagi et al., 1997); PotFGHI, ATP-binding cassette type putrescine specific importer (Pistocchi et al., 1993); PuuP, high-affinity putrescine importer (Kurihara et al., 2009); SAM, *S*-adenosylmethionine; SapBCDF, putrescine exporter (Sugiyama et al., 2016); SPD, spermidine; SPDSyn, spermidine synthase (Tabor et al., 1986).

concentration is higher in growing phase than that in stationary phase, probably because polyamines are important for cell proliferation. Therefore, the measurement of polyamine concentrations in the cell and culture supernatant in different growth phases is necessary for a better insight into bacterial polyamine biosynthetic and transport activity. Furthermore, polyamine biosynthesis and transport should be analyzed in dominant human gut bacterial species other than those belonging to the *Alistipes*, *Bacteroides* and *Parabacteroides* genera (Table 1) for a comprehensive understanding of polyamine homeostasis in the human intestinal lumen.

In the present study, we measured polyamine concentration in the cell and culture supernatants of 32 species of dominant human gut bacteria cultured in GAM at different growth phases. Furthermore, by using *in silico* analysis, we estimated the possibility of gut bacteria harboring the novel polyamine biosynthetic and transport proteins.

2. Materials and methods

2.1. Materials

GAM bouillon, putrescine dihydrochloride, spermidine trihydrochloride, and spermine tetrahydrochloride were purchased from Nissui pharmaceuticals (Tokyo, Japan), Wako Pure Chemicals (Osaka, Japan), Nacalai Tesque (Kyoto, Japan), and MP Biomedicals (Solon, OH), respectively.

2.2. Strains and growth conditions

Bacterial strains were obtained from the Japan Collection of Microorganisms (JCM), the American Type Culture Collection (ATCC), and the German Collection of Microorganisms and Cultures (DSMZ). Bacterial strains used in this study are listed in Table 1. The conditions used for their cultivation have been previously described (Gotoh et al., 2017).