The microbial degradation of pentoses in the human gut is a crucial factor for the utilization of plant-based dietary fibers. A vast majority of gut microbes are able to use these C5-sugars as a carbon and energy source. However, the underlying metabolic pathways are not fully understood. Bioinformatic analysis showed that a large number of abundant gut bacteria lack genes encoding a transaldolase as a key enzyme of the pentose phosphate pathway. Among them was the important human gut microbe *Prevotella copri*, which was able to grow in minimal media containing xylose or hemicelluloses as the sole carbon source. Therefore, we looked for an alternative pathway for pentose conversion in *P. copri* using bioinformatics, enzyme activity assays, and the detection of intermediates of pentose metabolism. It became evident that the organism converted C5-sugars via the sedoheptulose-1,7-bisphosphate pathway (SBPP) to connect pentose metabolism with glycolysis. To circumvent the transaldolase reaction, *P. copri* uses the combined catalysis of a pyrophosphate-dependent phosphofructokinase and a fructose-bisphosphate aldolase. Furthermore, we present strong evidence that the SBPP is widely distributed in important gut bacteria, including members of the phyla Bacteroidetes, Firmicutes, Proteobacteria, Verrucomicrobia, and Lentisphaerae.

Introduction

The human body, especially the digestive tract, is inhabited by a huge quantity of microbes, the so-called microbiota [1,2]. The intestinal microbiota consists of more than $10^{14}$ cells, which culminate in the colon in high densities of $10^{11}$ to $10^{12}$ cells per milliliter [3,4]. Additionally, the community of the gut microbiota is very diverse, consisting mostly of bacteria that can be classified into more than 50 genera [5–7]. Most of these microorganisms belong to the phyla Bacteroidetes and Firmicutes [5,8–10]. Human physiology profits from several beneficial properties of the gut microbiota, including the impact on digestion and the provision of nutrients or the influence on the development and the homeostasis of the host immune system [11–13]. The effects on digestion in relation to human nutrition are highly interesting, especially because intestinal bacteria can enable an expansion of the host’s substrate spectrum. For example, it is well known that a high-fiber

**Abbreviations**

6-PGL, 6-phosphogluconolactone; ATP-PFK, ATP-dependent phosphofructokinase; DHA, dihydroxyacetone; DHAP, dihydroxyacetone phosphate; E4P, erythrose 4-phosphate; EMP, Embden–Meyerhof–Parnas; F6P, fructose 6-phosphate; FBP aldolase, fructose-bisphosphate aldolase; G6P, glucose 6-phosphate; G6PD, glucose 6-phosphate dehydrogenase; GAP, glyceraldehyde 3-phosphate; GPD, α-glycerophosphate dehydrogenase; GPI, glucose 6-phosphate isomerase; PFK, phosphofructokinase; Pi, inorganic phosphate; PPi, pyrophosphate; PPI-PFK, pyrophosphate-dependent phosphofructokinase; PPP, pentose phosphate pathway; R5P, ribose 5-phosphate; RPE, ribulose-phosphate 3-epimerase; RPI, ribose 5-phosphate isomerase; Ru5P, ribulose 5-phosphate; S-1,7-bP, sedoheptulose 1,7-bisphosphate; S7P, sedoheptulose 7-phosphate; TK, transketolase; TPI, triose phosphate isomerase; Xu5P, xylulose 5-phosphate.
diet comprising cereals, nuts, fruits, and vegetables has beneficial effects on health, as their intake has been associated with a reduced prevalence of various diseases such as obesity, type 2 diabetes, or cardiovascular diseases [14,15]. Dietary fibers like cellulose, hemicellulose, and pectin are components of plant material, which are resistant to alimentary enzymes of the human body and therefore reaching the colon mainly undigested [16,17]. In the large intestine, the gut microbiota utilizes these nondigestible food components by fermentation and produces several short-chain fatty acids (SCFA), such as butyrate, propionate, and acetate [11]. These SCFAs are taken up by the large intestinal epithelium and are either utilized by the colonocytes directly or transported into the bloodstream reaching a plethora of possible sites of action [11].

Remarkably, a fiber-rich diet is significantly associated with a Prevotella-dominated gut microbiota [18], while the most abundant species is Prevotella copri [19–21]. It is well known that P. copri is capable of utilizing various dietary fibers [22], channeling the monomeric units of these polysaccharides into its main metabolic pathways, which consists of the Embden–Meyerhof–Parnas (EMP) pathway, fumarate respiration, and the formation of acetate and formate from pyruvate [23]. One of the most important substrates of P. copri is xylan [24,25], which represents the majority of hemicelluloses on earth. Interestingly, hemicelluloses make up 20–40% of total plant biomass [26]. The primary monomer subunits of the heteropolysaccharide xylan are the pentoses xylose and arabinose [27]. However, it has never been shown how these pentoses are metabolized by P. copri. Database analysis indicated that pentose degradation is unlikely to occur via the common pentose phosphate pathway (PPP) due to the lack of a transaldolase (EC 2.2.1.2) encoding gene. Recently, it was shown that two cellulolytic clostridia use an alternative pathway for pentose metabolism referred to as the sedoheptulose 1,7-bisphosphate pathway (SBPP) [28]. The same pathway is also present in the phylogenetically distant amoebozoan Entamoeba histolytica [29]. Here, we provide detailed biochemical evidence that the SBPP is also used by the important gut bacterium P. copri. The data not only provide information on how this organism degrades pentoses, but also on how a pentose metabolism can function in other important gut bacteria that do not possess a transaldolase. The topic is of major interest because a general scheme about the utilization of C5 sugar units of hemicelluloses can contribute to the understanding of the beneficial impact of these dietary fibers on the gut microbiota and their health-promoting effect on the host.

Results

Growth with xylose and hemicelluloses as substrates

One of the most important functions of the human intestinal flora, especially of P. copri, is the degradation of xylan in the colon and the corresponding utilization of xylose as the main component of this heteropolysaccharide. Growth experiments showed that P. copri can degrade xylose, indicating that a biochemical pathway for pentose conversion must be present (Fig. 1). For the cultivation, DMM supplemented with xylose or glucose was used as defined minimal medium. The organism revealed a typical exponential growth phase with both substrates (Fig. 1A). Cultures grown on xylose showed a doubling time of 2.6 h, which was slightly lower compared to glucose containing cultures with a doubling time of 1.8 h. The maximum optical density at 600 nm (OD600) on both substrates was approximately 1.6.

Prevotella copri could also use different hemicelluloses (Fig. 1B) and pectins (not shown) as growth substrates, conforming that the organism is able to degrade various dietary fibers. During growth on wheat arabinoxylan, P. copri had a doubling time of 2.7 h, but when the organism grew on xyloglucan (from tamarind), the doubling time increased to 3.5 h. However, the maximum OD600 on both substrates was similar and reached a value of about 0.9. The addition of xylan from oat spelt led to a turbidity of the culture medium. Therefore, growth was determined according to the protein concentration of the samples. The growth behavior of the xylan containing culture was similar in comparison with the culture grown on xylose (t½ = 2.4 h). Major end products of the degradation of glucose, xylose, and hemicelluloses were succinate, acetate, and formate [23].

Bioinformatic prediction of the pentose metabolism of P. copri

Heteroxylans like arabinoxylan and xylan from oats consist mainly of the pentose units xylose and α-L-arabinofuranose [30,31]. The backbone of xyloglucan consists of β-(1,4)-linked D-glucopyranose residues, 60–75% of which have xylose-based side chains attached [32]. For the degradation of these hemicelluloses by P. copri, a number of enzymes are required, whereby in particular xylanases (EC 3.2.1.8), β-xylidosidases (EC 3.2.1.37), and α-L-arabinofuranosidases (EC 3.2.1.55) are used for the release of C5 sugars [33]. There are also esterases (e.g., EC 3.1.1.72, EC 3.1.1.73) and α-glucuronidases.
(EC 3.2.1.139), which are responsible for the hydrolysis of further side groups [33]. Pentoses are also frequently found in other fibers such as pectin [34].

Since the pentose metabolism in \textit{P. copri} is poorly understood, the genome was screened for the presence of genes encoding enzymes of the PPP and enzymes involved in xylose and arabinose metabolism (Fig. 2). From this data, we propose that L-arabinose released from dietary fibers is taken up into the cell by a Na\textsuperscript{+}-dependent symporter (PREVCOP_04056) and is converted to L-ribulose by a L-arabinose isomerase (PREVCOP_04059; EC 5.3.1.4). A L-ribulose kinase (PREVCOP_04060; EC 2.7.1.16) is responsible for the phosphorylation and the resulting L-ribulose 5-phosphate is converted to D-xylulose 5-phosphate (Xu5P) by the catalytic activity of the L-ribulose 5-phosphate 4-epimerase (PREVCOP_04058; EC 5.1.3.4). Interestingly, all genes encoding these proteins are found in one gene cluster. Xylose is transported into the cell via a xylose-proton symporter (PREVCOP_05160). The compound is then converted into xylulose by a xylose isomerase and phosphorylated forming Xu5P catalyzed by a xylulokinase (PREVCOP_06339; EC 2.7.1.17). However, it is not known how \textit{Prevotella} spp. convert xylose into xylulose since a typical xylose isomerase is missing. It is assumed that the isomerization can be performed by another isomerase (e.g., PREVCOP_04059; EC 5.3.1.4 or PREVCOP_05606; EC 5.3.1.14; [24]). Other essential proteins such as a transketolase (TK, PREVCOP_04062; EC 2.2.1.1), \textalpha{}-ribulose-phosphate 3-epimerase (RPE, PREVCOP_03582; EC 5.1.3.1), and ribose 5-phosphate isomerase (RPI, PREVCOP_04063; EC 5.3.1.6) were detected (Table 1). Furthermore, it became evident that enzymes of the oxidative part of the PPP (glucose-6-phosphate dehydrogenase; EC 1.1.1.49 and 6-phosphogluconate dehydrogenase; EC 1.1.1.44) are absent. In addition, one of the key enzymes of the nonoxidative part of the PPP, the transaldolase, was missing as well. With this exceptional finding that there is no transaldolase, the possible existence of an alternative pentose metabolism in \textit{P. copri} was considered.

One potential pathway to circumvent the reaction of the transaldolase in \textit{P. copri} is the combined catalysis of a phosphofructokinase (PFK) that could be able to phosphorylate sedoheptulose 7-phosphate (S7P) and an aldolase that cleaves sedoheptulose 1,7-bisphosphate (S-1,7-bP) to erythrose 4-phosphate (E4P) and dihydroxyacetone phosphate (DHAP) [28]. Strikingly, the genome of \textit{P. copri} codes for two PFKs, from which one is predicted to be ATP- (ATP-PFK; PREVCOP_04138; EC 2.7.1.11) and the other pyrophosphate (PPi)-dependent (PPi-PFK; PREVCOP_03899; EC 2.7.1.90) (Table 1). The only gene encoding an aldolase involved in the central carbon metabolism of \textit{P. copri} is the fructose-bisphosphate aldolase (FBP aldolase, PREVCOP_06123; EC 4.1.2.13). Hence, the candidates to catalyze the two-step conversion of S7P were the ATP-PFK or PPi-PFK in combination with the FBP aldolase. The functional verification of the combination of these enzymes in \textit{P. copri} would also contribute to uncover the pentose conversion in gut bacteria missing a transaldolase.

Based on bioinformatic data and growth experiments, we postulate that pentose conversion in \textit{P. copri} starts with the isomerization of ribulose 5-phosphate (Ru5P) to ribose 5-phosphate (R5P) by RPI and the further
conversion to Xu5P by RPE (Fig. 3). Both products are subsequently converted by the rearrangement of a glycolaldehyde moiety from Xu5P to R5P into glyceraldehyde 3-phosphate (GAP) and S7P, which is catalyzed by the TK. GAP enters the Embden-Meyerhof-Parnas (EMP) pathway, whereas S7P is transformed to S-1,7-bP by the PPi- or the ATP-PFK. S-1,7-bP is then cleaved by the FBP aldolase into E4P and DHAP, whereby DHAP is also fed into the EMP pathway by the triosephosphate isomerase (TPI; PREVCOP_06387; EC 5.3.1.1).

Another glycolaldehyde group is transferred from Xu5P to E4P by the TK that leads to the formation of GAP and fructose 6-phosphate (F6P).

**Functional verification of the SBPP in *P. copri***

To verify the predicted pentose metabolism, the pathway, starting from the substrate R5P, was reconstructed by enzyme activity assays using the corresponding proteins of *P. copri* DSM 18205. For this approach, the following enzymes were necessary: RPI, RPE, TK, ATP-PFK, PPI-PFK, and FBP aldolase. The corresponding genes from *P. copri* were fused with the DNA sequence encoding a N- or C-terminal Strep-Tag and cloned into the expression vectors pASK-IBA5 or pASK-IBA3. The recombinant proteins were produced heterologously in *Escherichia coli* DH5α and purified by Strep-Tactin®/C210 (IBA Lifesciences, Gottingen, Germany) affinity chromatography. These purified proteins were analyzed by SDS/PAGE and western blotting (Fig. 4). The molecular masses of the recombinant proteins matched the estimated masses based on the amino acid sequences.

Starting from the substrate R5P, the activities of the RPI, RPE, and TK were confirmed using the auxiliary enzymes TPI and α-glycerophosphate dehydrogenase (GPD) (Fig. 5A). The coupled enzyme assay indicated that the RPI isomerized R5P to Ru5P, which was further converted to Xu5P by the RPE. Subsequently, Xu5P and R5P were transformed into GAP and S7P by the TK. The isomerization of GAP to DHAP was catalyzed by the TPI, and DHAP was reduced by the GDP to α-glycerophosphate (α-GP) with NADH as reductant. The latter reaction was verified experimentally using the distance tree tool of NCBI BLASTP, it was verified that the identified homologs are orthologs; identification numbers of the proteins correspond to the UniProt database (https://www.uniprot.org).
quantified photometrically by the decrease of absorption at 340 nm due to the oxidation of NADH. Omitting one of the enzymes mentioned above did not result in any detectable reaction. The experiment showed that the heterologously produced enzymes RPI, RPE, and TK from \textit{P. copri} were active and catalyzed the expected reactions.

After the verification of the activity of the first three enzymes of the PPP, the fate of the intermediate S7P was of major interest since a transaldolase was absent. As mentioned above, we suggest that S7P is converted to S-1,7-bP by the catalytic activity of the ATP-PFK or the PPi-PFK. In accordance with our hypothesis, the phosphorylation of S7P could be confirmed by the release of inorganic phosphate (P\textsubscript{i}) from PPi catalyzed by the recombinant PPi-PFK. The increasing amount of released P\textsubscript{i} correlated with the increase in absorption over time, reflecting the phosphorylation activity of the PPi-PFK in the coupled enzyme assay (Fig. 5B). In contrast to the PPi-PFK, the ATP-PFK was not able to phosphorylate S7P under the applied assay conditions. Hence, the ATP-PFK is probably not involved in the phosphorylation of S7P to S1,7-bP within the SBPP. In accordance with these findings was the fact that the purified PPi-PFK showed an approx. 370-fold higher specific activity (147.9 ± 5.5 U·mg\textsuperscript{-1}) and an approx. 600-fold higher catalytic efficiency (145 ± 7.8 s\textsuperscript{-1}) than the ATP-PFK (0.4 ± 0.04 U·mg\textsuperscript{-1} and 0.24 ± 0.04 s\textsuperscript{-1}) for the phosphorylation of S7P (Table S1). The second reaction of interest within the pathway of pentose utilization in \textit{P. copri} was the conversion of S-1,7-bP. Therefore, we tested the cleavage of S-1,7-bP into DHAP and E4P by the recombinant FBP aldolase using the auxiliary...
Important gut bacteria rely on the SBP pathway

L. S. Garschagen et al.
enzyme GPD. Due to the NADH-dependent reduction of DHAP by GDP, the reaction could be determined photometrically (Fig. 5C). The final glycolaldehyde shift from Xu5P to E4P, catalyzed by the TK and resulting in F6P and GAP, was detected using the auxiliary enzymes glucose 6-phosphate isomerase (GPI) and NADP-dependent glucose 6-phosphate dehydrogenase (G6PD). By using these auxiliary enzymes, the resulting product of the TK reaction, F6P, was converted to glucose 6-phosphate (G6P) and further transformed to 6-phosphogluconolactone (6-PGL). The reaction was followed by the reduction of NADP⁺ to NADPH (Fig. 5D). Thus, the entire pentose conversion from R5P to F6P was reconstructed (Fig. 5D).

In summary, the postulated pentose conversion via the SBPP (Fig. 3) could be confirmed, using purified enzymes. All reactions starting from the substrate R5P including the highly interesting combined action of the PPI-PFK and the FBP aldolase were detected.

However, activity of the key enzymes PPI-PFK and FBP aldolase could also be detected in xylose-grown Prevotella copri cells were lysed and after centrifugation, the soluble cytoplasmic fraction was used for the experiments. Cytoplasm, PPI-PFK, PPI, NADH, and the auxiliary enzyme GDP were added to the first assay (Fig. 6A). After the addition of S7P, the oxidation of NADH was measured at a rate of 80 ± 30 nmol-min⁻¹-mg⁻¹ extract protein. This reaction was based on the cleavage of S-1,7-bP by the cytoplasmic FBP aldolase, whereby the product DHAP was reduced to α-GP by the auxiliary enzyme GAPDH. In the second assay, FBP aldolase instead of PPI-PFK was added (Fig. 6B). Again, oxidation of NADH was observed (120 ± 50 nmol-min⁻¹-mg⁻¹ extract protein), indicating that the cytoplasm contained an active PPI-PFK capable of phosphorylating S7P. S-1,7-bP was then metabolized as described above. The potential transaldolase activity in the cytoplasm of P. copri was investigated using the auxiliary enzymes GPI and G6PD and the substrates S7P and GAP. Thus, the product of the transaldolase reaction, F6P, would be converted to G6P and would be detectable by the NADP⁺-dependent oxidation of G6P. After the addition of S7P, no transaldolase reaction was observed (Fig. 6C). F6P was subsequently added to the assay as a positive control. In summary, both activities of the two key enzymes of the SBPP were detected in the cytoplasm, indicating that the proteins of the SBPP are produced in P. copri. Furthermore, the lack of transaldolase activity confirmed the bioinformatic analyses that a transaldolase encoding gene is missing and the experiments proved that pentoses are converted via the SBPP.

In addition to the photometrical assays, it was possible to confirm the SBPP for the degradation of pentoses in P. copri by the detection of relevant intermediates via HPLC analyses. For that, a reaction mixture was prepared that contained the necessary enzymes described above in stepwise additions. The first enzymatic reaction was initiated by the addition of R5P to the mixture containing the RPI and RPE, which resulted in the conversion of R5P into Xu5P via the intermediate Ru5P. The structural isomers R5P and Ru5P could not be separated chromatographically and both intermediates showed up as one peak after 63.8 min. In contrast, Xu5P was detected as a single peak at 60.5 min. (Fig. 7A). After the addition of TK to the reaction mixture, S7P eluted at 51.3 min (Fig. 7B) and the activity of the PPI-PFK was demonstrated by the appearance of the S-1,7-bP peak at 12.5 min after adding the enzyme (Fig. 7C). After FBP aldolase was supplemented to the assay, the final intermediate of the alternative pentose metabolism F6P was detected at 9.1 min (Fig. 7D). The
The experiments demonstrated both the cleavage of S-1,7-bP to DHAP and E4P catalyzed by the FBP aldolase and the transformation of these products to F6P and GAP catalyzed by the TK. The intermediate products GAP, DHAP, and E4P could not be detected, either because they were not separated chromatographically by the applied HPLC methods or because they were below the detection limits due to the enzyme activities. Again, no ATP-PFK-catalyzed phosphorylation of S7P was found under the applied test conditions.

**Function of BVU_3333 from Bacteroides vulgatus**

The experiments with *P. copri* showed that this bacterium does not metabolize pentoses via the regular PPP. Instead, the SBPP is used, which is based on the combined activity of PPI-PFK and FBP aldolase as key elements of this pathway. However, a gene in the close relative *Bacteroides vulgatus* was identified whose corresponding amino acid sequence showed high homologies to the transaldolase from *Bacillus subtilis*, which is assigned to the transaldolase subfamily 4[35]. The corresponding gene from *Bacillus subtilis* was overexpressed in *E. coli* and elevated transaldolase activity was found in the crude cell extracts of the recombinant strain [36]. To analyze whether the homologous protein from *Bacteroides vulgatus* (BVU_3333) can also act as a transaldolase, the corresponding protein was heterologously overproduced in *E. coli* and purified (Fig. 8A, B). A coupled optical enzyme assay was performed with BVU_3333 and the auxiliary enzymes GPI and G6PD as well as the...
substrates S7P and GAP. The enzyme combination indicated that BVU_3333 had transaldolase activity and formed E4P and F6P from S7P and GAP (Fig. 8C). The auxiliary enzymes led to the formation of 6-PGL and the simultaneous reduction of NADP$^+$ to NADPH, which was quantified photometrically. The enzyme activity was proportional to the amount of BVU_3333 used in the assay, and the highest activity of the transaldolase in this test system was 0.2 U/mg protein. A conversion of F6P to GAP and dihydroxyacetone (DHA) was not observed (Fig. 8C). Thus, the enzyme does not belong to the group of F6P aldolase. These results showed that BVU_3333 acts as a transaldolase, indicating that Bacteroides vulgatus possesses a normal PPP.

Distribution of the SBPP in intestinal bacteria

As shown above, the SBPP (P. copri) and the PPP (Bacteroides vulgatus) were found in important representatives of the intestinal flora. In this context, it is to note that the degradation of C5 sugar is of crucial importance, as the process directly contributes to the utilization of pentose-rich dietary fibers to form health-promoting SCFAs. The question arose whether the presence of the SBPP is a rare case and found only in a few organisms or whether the metabolic pathway also occurs in other intestinal bacteria. To answer this question, we performed a detailed BLASTP analysis in a variety of abundant human gut bacteria. All available genomes of the respective species from the NCBI and IMG databases were used for the BLASTP search. This approach eliminated the possibility that genes encoding transaldolases were missed because the genetic information was located in an unsequenced gap of unfinished genomes. Thus, all species, listed in Table 2 and Table S2, were represented by at least two sequenced strains or two independent sequencing projects for one strain. The only exception was Catenibacterium mitsuokai, since only one genome sequence was available. BLASTP analyses were performed using biochemically characterized transaldolases and F6P aldolases as reference proteins (Table S2; subfamilies 1–5, [35]). The approach led to the discovery of further

---

Fig. 7. HPLC analysis of intermediates of the pentose metabolism in Prevotella copri. The standard enzyme assay (1 mL final volume) contained R5P, TPP, MgCl$_2$, PPI, and Tris/HCl buffer pH7. Depending on the reaction, the corresponding enzymes (50 µg) were added to the reaction mixture and were incubated for 30 min at RT. Fifty microlitre of samples was diluted and analyzed by HPLC. (A) HPLC chromatogram of the enzyme assay containing RPI and RPE. A peak for Xu5P at 60.5 min and an overlayed peak for R5P and Ru5P at 63.8 min were found. (B) HPLC chromatogram of the enzyme assay with RPI, RPE, and TK. An additional signal for S7P at 51.3 min was detected as a product of the TK reaction. (C) HPLC chromatogram of the enzyme assay containing RPI, RPE, TK, and PPI-PFK. S7P, C5 sugars, and S-1,7-bP eluted at 8.7, 10.1, and 12.5 min, respectively. (D) HPLC chromatogram of the enzyme assay with RPI, RPE, TK, PPI-PFK and FBP aldolase. The signal for F6P at 9.1 min was identified as the final product of the metabolic pathway.
important representatives of the human intestinal microbiota that did not possess genes for the production of transaldolases. These organisms included frequently occurring bacteria of the intestinal flora such as *Akkermansia muciniphila*, *Prevotella stercorea*, and *Parabacteroides johnsonii*. Also, *Faecalibacterium prausnitzii* can be classified into this group since 31 of 34 strains were transaldolase negative. In addition, species without transaldolases were found in other important genera. This class of bacteria included *Alitipes shahii*, *Butyrivibrio crossotus*, *Bilophila wadsworthia*, *Coprococcus eutactus*, *Holdemania filiformis*, *Pseudoflavonifractor* ssp., *Roseburia inulinivorans*, *Ruminococcus bromii*, and *Victivallis vadensis*. A differentiated distribution was discovered in the prominent genus *Bacteroides*, as some species had no transaldolases (e.g., *B. coprocola*, *B. coprophilus*, *B. eggerthii*, *B. finegoldii*, and *B. plebeius*) while other important representatives (e.g., *B. fragilis*, *B. ovatus*, *B. thetaotaomicron*, and *B. uniformis*) contained a transaldolase of the subfamily type 4 (Table S2). A second group of human gut bacteria contained homologs of putative F6P aldolases or enzymes with high homology to type 4 transaldolases from *Bacillus subtilis* (P19669) and *Bacteroides vulgatus* (BVU_3333). These two groups of aldolases are difficult to distinguish because they share a high degree of structural similarity and sequence identity [36]. However, the molecular basis of the different reaction specificities (transferase vs. aldolase) was solved recently [37]. The critical difference between the active sites of the enzymes is the presence of either a transaldolase-specific Glu (Gln in F6P aldolases) or a F6P aldolases-specific Tyr (Phe in transaldolases). Based on these findings, the proteins identified by BLAST were subjected to multiple alignments and compared with aldolases of known activity. In this way, it could be shown that the putative aldolases from *C. mitsuokai*, *Clostridium leptum*, *Megamonas hypermegale*, *Enterococcus faecalis*, and *Enterococcus faecium* had to be classified in the group of F6P aldolases (Fig. 9). The best-known representatives of this group of enzymes are FsaA and FsaB from *E. coli*, but their physiological function is still unknown [36]. It can therefore be assumed that the above-mentioned intestinal bacteria do not have the regular PPP.

At this point, we cannot state that these bacteria possess the SBPP because it is possible that the identified F6P aldolases can cleave S7P to E4P and DHA, which could be channeled into the central carbon metabolism [38]. For the analyzed lactic acid bacteria
Streptococcus salivarius, Lactobacillus lactis, Lactobacillus ruminis, Enterococcus faecium, and Enterococcus faecalis, even another pathway might be realized to circumvent the transaldolase reaction. Heterofermentative and facultative homofermentative lactic acid bacteria use the phosphoketolase pathway, whereby the phosphoketolase cleaves Xu5P to GAP and acetyl phosphate \[39\]. In contrast, strictly homofermentative lactic acid bacteria use the regular PPP \[40\]. There is also evidence that the phosphoketolase forms acetyl phosphate and E4P from F6P, providing the essential precursor E4P for the synthesis of aromatic amino acids \[41\]. During growth on C6 sugars, the oxidative PPP might be utilized to produce C5 metabolites required for anabolism.

Besides the absence of a transaldolase, the presence of a PPI-PFK is obviously essential for a functional SBPP. Therefore, all genomes that were used for the identification of transaldolase enzymes were also tested for the presence of a PPI-PFK (Table 2). Enzymes of different groups of PPI-PFK with known activity were used for comparison (Table S2) \[42\]. In all genomes of strains shown in Table 2, genes were found that encode enzymes with high similarity to biochemically characterized PPI-PFKs.

In summary, we propose that the SBPP is widespread and occurs in various groups of bacteria from the important phyla Bacteroides and Firmicutes (e.g., Bilophila wadsworthia), Verrucomicrobia (e.g., A. muciniphila), and Lentisphaerae (e.g., V. vadensis).

**Discussion**

Bioinformatic analysis and biochemical experiments on the enzymatic and metabolite level showed that the gut...
Important gut bacteria rely on the SBP pathway

L. S. Garschagen et al.

**Ecol**

AWAKQGNDRAQQIVDADKLVAVNIGLEILKLV--GRISTFDARLVDSTDAISHAKAKR 114

**Bsub**

---------------DLRLKQ5TVDV--GSVSAEQV---ISLKAEEMIEEGKE 73

**Bvul**

-------------KHYIECINIVD--GDVSAEQV---IAINYEGMEKEBE 76

**Cmit**

--------------------------------GHRMEREIKGIDRSLHIOQV---IAEAEDEIKVEAHIK 75

**Mhyp**

-------------NHFAEIRKIIKRESSLHVQV---TTTTEKEIREAHG 75

**Efl**

------------AQMEIKITTQG--ASLHVQV---VGQTEEMLEDAQT 74

**Efc**

----------------SHSMKEIKKIQ--IPHIHOQ---IGRTEBEMEADRV 74

**Clep**

-------------DVLANIKEFPP--EIPVFGQV---VAKIDTEVMAEARK 73

**FsaA**

--------------------------------VVLPLQHEAMGQRLFAQV---MTEAEGMVNDALK 73

**FsaB**

-------------EVLRLQKAIDEGILFAPQ---MSRDQAMVEEAKR 74

**Ecol**

LIKYNDAGISNDRILKILKLASTWQGIRAEQLEKEGINCLNLFLFSFAQARACAEAVFL 174

**Bsub**

------------LAPNITKVMPMTDGLKAARLTDLGIKTNVTLFNQANAILARAGAY 126

**Bvul**

-------------LNPHIVKVCPEDIGAIKAIYFNSKIGIRTNCNTLVFSAGQALLAAGAKAY 129

**Cmit**

------------EVDSDKVYKVPPTYIEDKAMIKLAEKGNVTATAVYDLMQAYNAALAGADY 129

**Mhyp**

-------------KIDKDVXIKVPTVGLAAAMLQALKKEFNTATATIMQGMFLAMEQADGF 128

**Efl**

-------------QLQGQETPIKIFPVNEAGLAIAIKQQLKANYRITAIYTEFPGYLAIAAGADY 128

**Efc**

-------------ELKDTDFIKVPVQAGLQAIKLLKEGYLIQTGAITYLQYLAINGADY 128

**Clep**

-------------AGKNNIVKXLPATREAVKAIHLHAEIHTCATAVOTALQSCFQCATAGADY 126

**FsaA**

-------------IIADIVKVPVTAELAIAKIMLKEAEPITLGAVIALGQSLALAGAY 127

**FsaB**

-------------ALPGIVKXIPVTSEGLAIAIKLKEGTITLGTAVASAAGGLAALAGAY 127

**Ecol**

ISPFVGRDLWYKANTQDKKEAPADPVVSVSEQYQYKEHGYETVMGASFRNIGEIL 234

**Bsub**

-----------------GVHGLDILISEVQKIFDHIHGDTQIIASAISHPQVHT 173

**Bvul**

-----------------CNDGYGLVAVQILEYQTADKQTQVLAISIRNTLHL 176

**Cmit**

-------------NDFPEMLHSLRVEDGDKILAASFKFGQVQR 176

**Mhyp**

-------------DINSDEIAFPAKIAEYNGTKILASFKNMGQVN 176

**Efl**

-------------TIDSQKVEHLAAEIRKTNASKILASFKNVAQIN 175

**Efc**

-------------GIDAMKVDSLRTQETRQPTKILAASFKNCEQVT 176

**Clep**

-------------GYDGLVTELQLCDVDIEEQLPTEVLASIEKQPQIEV 173

**FsaA**

-------------GGSGGQTVTDLNLKMHAPQAKVLAASFKTPRQAL 174

**FsaB**

-------------GGDGTIVQELTLLEMHPASFMVLASFKTPRQAL 174

Fig. 9. Sequence alignment of aldolases from different intestinal bacteria to identify transaldolases and F6P aldolases using reference sequences. The classification was performed according to reference [37], using the crucial amino acids Glu (transaldolase specific) or Gin (F6P aldolase specific) and Phe (transaldolase specific) or Tyr (F6P aldolase specific). As an example, the following amino acid sequence alignments are shown: *Catenibacterium mitsuokai* (Cmit; IMG gene ID 644182441), *Clostridium leptum* (Clep; IMG gene ID 283506833), *Megamonas hypermegele* (Mhyp; IMG gene ID 252393509), *Enterococcus faecalis* (Efc; IMG gene ID 650493676) and *Enterococcus faecium* (Efc; IMG gene ID 286042724). The transaldolases of *Escherichia coli* (Ecol, UniProt ID P0A870), *Bacteroides vulgatus* (Bvul, BU_3333), *Bacillus subtilis* (Bsub, UniProt ID P19669) and the F6P aldolases A and B from *E. coli* (FsaA, UniProt ID P32669) were used as reference proteins. The decisive amino acids are marked with a red box. The figure was created with the program CLUSTAL OMEGA (www.clustal.org/omega). An "*" (asterisk) indicates positions which have a single, fully conserved residue. A ":" (colon) indicates conservation between groups of strongly similar properties. A "." (period) indicates conservation between groups of weakly similar properties.

Bacterium *P. copri* uses the SBPP for the degradation of C5 sugars and for the synthesis of pentose phosphates as metabolites required for anabolism during growth on glucose. Here, the transaldolase reaction, which is a crucial step in the PPP, is replaced by the combined activity of PPI-PFK and FBP aldolase. Furthermore, there is no evidence that an alternative pathway for the pentose metabolism exists because the corresponding genes for the phosphoketolase pathway or the Weinberg/Dahms pathway [43,44] are missing. In addition, there are no genes encoding a xylose reductase and a xylitol dehydrogenase [45], which
indicates that the ‘xylose oxidoreductase’ pathway is absent. Interestingly, the lack of a transaldolase was similarly found in the parasitic amoeba *E. histolytica* and two cellulolytic clostridia. Susskind *et al.* [29] provided the first evidence that the combination of a PPi-PFK and a FBP aldolase could substitute the missing transaldolase reaction in *E. histolytica*. For bacteria, the first proof of the so-called SBPP was provided for two cellulolytic clostridia. Koendjibharie *et al.* [28] showed that the PPi-PFKs of *Pseudomonas thermosuccinogenes* and *Hungateiclostridium thermocellum* can convert S7P to S-1,7-bP and proposed that the FBP aldolase is responsible for the cleavage of S-1,7-bP, which was already shown in plants using the Calvin cycle [46,47], in *Methyllococcus capsulatus* [48] and in *Myobacterium smegmatis* [49]. Furthermore, the phosphorylation of S7P by PPi-PFKs was already demonstrated for *Methyllococcus capsulatus* [50], *Enterococcus faecalis* [51], and *Methylomicrobium alcaliphilum* [52].

Overall the SBPP is almost identical to the regenerative part of the Calvin cycle with respect to reactions and intermediates (Fig. 10). The only difference between the metabolic pathways is the synthesis or conversion of S-1,7-bP. In the Calvin cycle, this compound is dephosphorylated to S7P in an irreversible phosphatase reaction, leading the metabolic flux direction toward the synthesis of pentose phosphates. In contrast, an important function of the regular PPP and the SBPP is to ensure that the pentose conversion can occur in both directions. In addition, to utilize pentoses as energy and carbon sources, the pathways are also essential to provide precursor molecules for nucleotide and amino acid biosynthesis [53]. The nonoxidative PPP is reversible and the flux direction depends on the concentration of C5 and C3/C6 metabolites in the cytoplasm of the cells. The same might be true for the SBPP since all enzymes catalyze reversible reactions. Under standard conditions, the change of free energy for the PPi-PFK reaction is \(-8.7 \text{ kJ mol}^{-1}\) [54]. However, taking the estimated cellular steady-state concentrations of the educts and products of the reaction into account (in *E. histolytica* PPi \(\sim 0.45 \text{ mM}\), Pi \(\sim 5.4 \text{ mM}\), S7P \(\sim 0.43 \text{ mM}\), S-1,7-bP \(\sim 1.1 \text{ mM}\) [55]), the \(\Delta G\) value of the reaction turns to \(-4 \text{ kJ mol}^{-1}\), which is close to equilibrium [54]. Hence, also the SBPP might be reversible and could fulfill both essential functions namely the catabolism of pentoses and the production of C5 metabolites required for anabolism during growth on other substrates. This calculation might also explain why the ATP-PFK is obviously not used for S7P phosphorylation since the \(\Delta G\) is \(-18.5 \text{ kJ mol}^{-1}\) under standard condition and the entire flux of carbon would solely be channeled through S-1,7-bP into glycolysis whereby the production of pentose phosphates from glucose or other C6 sugars would be thermodynamically difficult.

PPi is produced in a variety of reactions for the synthesis of cellular polymers such as DNA, RNA, and fatty acids as well as for the loading of tRNAs with amino acids in the course of protein biosynthesis. These anabolic reactions often operate close to equilibrium. Therefore, many organisms hydrolyze PPi by pyrophosphatases to shift the overall reaction equilibrium toward product formation. In this way, a considerable amount of metabolic energy is released as heat. However, it is possible that some of the energy of the PPi anhydride bond is conserved by coupling the hydrolysis of PPi to the phosphorylation of an intermediate of the cellular metabolism. A classic example for this kind of metabolic energy conservation is the reaction of the PPi-PFK that phosphorylates F6P in a variety of archaea, bacteria, and some eukaryotes within glycolysis [42,56,57]. Here, we show that the PPi-PFK is also involved in the catabolic conversion of pentose phosphate intermediates to link C5 metabolism to glycolysis. However, stoichiometric calculations indicate that the PPi formation as a by-product of the anabolism alone cannot sustain the PFK reaction in the SBPP. Growth experiments show that approx. 40 mmol xylose per g dry weight is consumed by *P. copri* (unpublished results). The PPi requirement in SBPP corresponds to a quotient of one PPi per three pentose molecules so that a total of approximately 13.3 mmol PPi per g dry weight is required. However, the PPi supply from anabolism and maintenance metabolism is probably insufficient to sustain a PPi-dependent pentose metabolism. Furthermore, xylose fermentation in nongrowing *P. copri* cells is possible (unpublished results), indicating that PPi synthesis not absolutely depends on the biosynthesis of cell material. Hence, the necessity of an alternative mechanism of PPi generation is evident. A couple of PPi producing mechanisms are possible [58,59]. In *E. histolytica*, a phosphoenolpyruvate (PEP) carboxykinase was found, which produces PPi instead of ATP during the carboxylation of PEP to oxaloacetate [60]. This reaction could also take place in *P. copri* and could provide sufficient quantities of PPi since the enzyme is essential for fumarate respiration and very high activity was found in the cytoplasm of this organism [23]. It is also possible that two enzymes work together, which catalyze inverse reactions leading to so-called futile cycles. Examples are the simultaneous reaction of acetyl-CoA synthetase (PREVCOP_04832) and phosphotransacetylation/acetate kinase (PREVCOP_04866 and
PREVCOP_04865) or the combined action of pyruvate phosphate dikinase (PREVCOP_04292) and pyruvate kinase (PREVCOP_04707) [59]. In both cases, 1 ATP would be consumed leading to the formation of 1 PPi. The third possibility is the process of glycogen cycling, where a glucose 1-phosphate adenylyltransferase catalyzes the ATP-dependent conversion of glucose 1-phosphate (Gluc1P) to ADP-glucose (ADP-Gluc) plus PPi [58,59]. ADP-Gluc is then used for glycogen synthesis. In a reverse reaction, a glycogen phosphorylase hydrolyzes glycogen to Gluc1P [61]. Since *P. copri* is able to synthesize and to hydrolyze glycogen [23], this process could lead to a net PPi production.

Since a sufficient source of PPi in SBPP using organisms is subject of further research and debate, it is currently speculative to assign the SBPP a specific evolutionary advantage. Especially because the bioinformatic analysis indicates a widespread distribution of the pathway in very different genera, and moreover, the occurrence varies within a genus and even between closely related organisms. Nevertheless, the SBPP is mostly connected to anaerobic fermentation and glycogen metabolism [53,54]. Therefore, it interrelates with organisms that rely mainly on ATP formation from substrate level phosphorylation and that live in habitats with a changing availability of nutrients [62]. In complex ecosystems like the human gut, it is conceivable that the SBPP is an advantageous feature to thrive in certain microhabitats, where a reversible and therefore flexible central carbon metabolism is required to easily and rapidly adapt to changes in the obtainable carbon source. Here, the energetic benefit of using PPi instead of ATP for central sugar phosphorylation reactions makes equally sense as the overall usage of PPi generating futile cycles that, as part of energy spilling reactions, accelerate the metabolic rate of the organisms [63]. Either way, the SBPP is a previously rather overlooked but seemingly widespread alternative for pentose metabolization in many important microorganisms, of which many numbers among the beneficial inhabitants of the human gastrointestinal tract. This illustrates that further investigations into the metabolism and the general carbon flux of *P. copri* and other gut bacteria are necessary to understand the SBPP and the overall PPi/Pi metabolism of these remarkable organisms.

**Methods**

**Materials**

If not otherwise indicated, all chemicals, substrates, and reagents were obtained from Carl Roth GmbH (Karlsruhe, Germany) or Sigma-Aldrich (St. Louis, MO, USA). Auxiliary enzymes were purchased from Sigma-Aldrich or Mega-zyme (Bray, Ireland). DNA polymerases, restriction
Bacterial strains and culture conditions

*Prevotella copri* DSM 18205 was obtained from the Deutsche Sammlung für Mikroorganismen und Zellkulturen (DSMZ; Braunschweig, Germany). The organism was cultivated anaerobically in defined minimal medium complemented with 30 mM xylose or glucose (DMMX or DMMG) under an N₂/CO₂ (80/20%) atmosphere [64]. Prior to inoculation, L-cysteine (0.5 g/L) was added. Wheat arabinoxylan (Megazyme), tamarind xyloglucan (Megazyme), and xylan from oat spelts (Sigma-Aldrich) in a concentration of 5 g L⁻¹ were added. Barium ions present in the commercial R5P and S7P preparations were precipitated by adding concentrated sulfuric acid (3M). Barium ions were selected from the data of the MetaHIT project [69] and the review of Rinninella *et al.* [70].

Overexpression and purification of proteins

The *P. copri* genes encoding R5P-isomerase (prevcop_04063), ribulose-phosphate 3-epimerase (prevcop_03582), transketolase (prevcop_04062), ATP-dependent phosphofructokinase (prevcop_04138), PPI-dependent phosphofructokinase (prevcop_03899), fructose-bisphosphate aldolase (prevcop_06125), and transaldolase from *Bacteroides vulgatus* (bvu_3333) were amplified by PCR using Q5 High-Fidelity DNA Polymerase (New England Biolabs) and specific primers (Table 3) with genomic DNA of *P. copri* DSM 18205 or *Bacteroides vulgatus* DSM 1447 as template. The PCR products contained the endonuclease restriction sites for XbaI and NcoI (prevcop_04138, prevcop_03899) or BsaI (prevcop_04063, prevcop_03582, prevcop_04062, prevcop_06123, bvu_3333). The DNA fragments were ligated into the vectors pASK-IBA3 or pASK-IBA5 (Table 3) and transferred to *E. coli* DH5α.

For protein overexpression, the cultures were grown to an OD₆₀₀ of 0.4–0.6 in 1 L LB medium at 37 °C and 200 r.p.m. and protein production was induced by the addition of 200 ng mL⁻¹ anhydrotetracycline. The temperature and the shaking power were downregulated to 25 °C and 120 r.p.m. for 10 h. Cells were harvested at 9000 g for 15 min and washed with buffer W (100 mM Tris/HCl, 150 mM NaCl, pH 8). After sonication, the cell lysate was centrifuged at 13 000 g and 4 °C for 10 min to separate cell debris. The proteins were purified by Strep-tactin Superflow⁺ affinity chromatography as described by the manufacturer (IBA GmbH) and quantified according to Bradford (1976) [66]. SDS/PAGE and western blots were performed as described in reference [71] and [72], respectively.

Bioinformatic analysis of the pentose metabolism in *P. copri* and other important gut bacteria

The genomes of *P. copri* strains DSM18205 and CAG:164 (accession no. PRJNA30025 and PRJEB734) were manually screened by *BLAST*P analysis [68] to reconstruct the pentose metabolism. As reference, amino acid sequences from biochemically characterized enzymes were used. To investigate whether the SBPP also occurs in other important intestinal bacteria, *BLAST*P programs from NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and IMG/G (https://img.jgi.doe.gov/) were used to identify homologous proteins in gut bacteria (Table S2). A collection of well-characterized enzymes served as references (Table S2). The ϵ-value thresholds for transaldolase and PPI-PFK homologs were set to ϵ < e⁻²⁰ and ϵ < e⁻⁵⁰, respectively. Abundant intestinal bacteria were selected from the data of the MetaHIT project [69] and the review of Rinninella *et al.* [70].

Measurement of enzyme activities

Preparation of cytoplasmic fractions of *P. copri*

Cytoplasmic fractions were prepared from *P. copri* cells grown in 500 mL DMMX medium with 30 mM xylose as substrate to the late exponential phase. Cell harvesting, lysis, and cytoplasm separation were performed as described in reference [23].

Photometric assays

Barium ions present in the commercial R5P and S7P preparations were precipitated by adding concentrated sulfuric...
Plasmids and primers.

| Plasmid or primer | Description or sequence | Source or restriction/recognition site |
|-------------------|-------------------------|---------------------------------------|
| pASK-IBA5         | Vector with inducible tetracycline promoter/operator, ampicillin resistance cassette, f1 origin, MCS, and Strep-Tag for N-terminal fusion to a recombinant protein | IBA, Göttingen, Germany               |
| pASK-IBA3         | Vector with inducible tetracycline promoter/operator, ampicillin resistance cassette, f1 origin, MCS, and Strep-Tag for C-terminal fusion to a recombinant protein | IBA, Göttingen, Germany               |
| pASK-IBA3_prevcop_04138 | pASK-IBA3 derivative containing prevcop_04138 from Prevotella copri DSM 18205 | This study                            |
| pASK-IBA3_prevcop_03999 | pASK-IBA3 derivative containing prevcop_03999 from P. copri DSM 18205 | This study                            |
| pASK-IBA5_prevcop_04063 | pASK-IBA5 derivative containing prevcop_04063 from P. copri DSM 18205 | This study                            |
| pASK-IBA5_prevcop_03982 | pASK-IBA5 derivative containing prevcop_03982 from P. copri DSM 18205 | This study                            |
| pASK-IBA5_prevcop_04062 | pASK-IBA5 derivative containing prevcop_04062 from P. copri DSM 18205 | This study                            |
| pASK-IBA5_prevcop_06123 | pASK-IBA5 derivative containing prevcop_06123 from P. copri DSM 18205 | This study                            |
| pASK-IBA5_bvu_3333 | pASK-IBA5 derivative containing buv_33333 from Bacteroides vulgatus DSM 1447 | This study                            |

Primer for cloning

| Primer | Sequence |
|--------|----------|
| Prevcop_04138.fw | TCTATCTAGA ATGGCAAAGAGTTAAG |
| Prevcop_04138.rev | Xbal |
| Prevcop_03999.fw | TCTATCTAGA ATGGAAAAGATGCATTG |
| Prevcop_03999.rev | Ncol |
| Prevcop_04063.fw | ATGGTAGGCTTC AGCGCCGAAGTTAAGACAGTTGGATGGAAT |
| Prevcop_04063.rev | Bsal |
| Prevcop_03982.fw | ATGGTAGGCTTC ATATCAGCCTGAAAGAATTCTTCTGAG |
| Prevcop_03982.rev | Bsal |
| Prevcop_04062.fw | ATGGTAGGCTTC ATATCAGCCTGAAAGAATTCTTCTGAG |
| Prevcop_04062.rev | Bsal |
| Prevcop_06123.fw | ATGGTAGGCTTC ATATCAGCCTGAAAGAATTCTTCTGAG |
| Prevcop_06123.rev | Bsal |
| Bvu_3333.fw | ATGGTAGGCTTC ATATCAGCCTGAAAGAATTCTTCTGAG |
| Bvu_3333.rev | Bsal |

Acid. Subsequently, the dissolved compounds (R5P, S7P) were neutralized using 6 N NaOH. The activity of enzymes involved in the pentose metabolism of P. copri was determined photometrically based on the absorption characteristics of NAD(H) and NADP(H) or by measuring inorganic phosphate. The basic assay contained 1 mL 50 mM Tris/HCl buffer pH 7 (0.2 mM thiamine pyrophosphate; TPP), 10 mM MgCl₂, 2 mM R5P, and 20 µM of the indicated enzymes. Since the initial substrate was R5P, the activities were determined via coupled enzyme tests.

The activities of RPI, RPE, and TK were detected photometrically at 340 nm using 2 units (U) of the auxiliary enzymes PPI (from rabbit muscle, Sigma-Aldrich) and GPD (from rabbit muscle, Sigma-Aldrich). To measure the reduction of DHAP, 125 µM NADH was added to the assay. The reaction was started by the addition of TK. The activity of the PPI-PFK was detected according to reference [73] by photometric measurement of the released inorganic phosphate based on the phosphorylation of S7P using PPI (2 mM) at 850 nm. For the detection, 25 µL of samples was taken from the enzyme assay every 5 min and mixed with 1 mL molybdate reagent (15 mM ammonium molybdate, 100 mM zinc acetate, pH 5) and 250 µL of 10 % (w/v) ascorbic acid. The mixture was incubated for 15 min at 30 °C and then the chromophore absorbance was measured at 850 nm. A calibration curve with Pi (potassium dihydrogen phosphate and dipotassium phosphate) pH 7 (0–1 mM) was used to calculate the released phosphate concentration. The background reaction was subtracted using the enzyme assay without R5P as a blank. The aldolase reaction was analyzed by the reduction of DHAP using 2 U of the auxiliary enzyme GPD at 340 nm independence of 125 µM NADH. By adding the FBP aldolase, the reaction was started. The activity of the TK for the glycolaldehyde shift from Xu5P to E4P, resulting in F6P and GAP, was measured using 2 U of the auxiliary enzymes GPI (from E. coli; Megazyme) and G6PD (from Leuconostoc mesenteroides; Megazyme). The basic assay (buffer, TPP, MgCl₂, R5P, PPI, RPI, RPE, TK, FBP aldolase, and PPI-PFK) was incubated for 30 min at room temperature (RT). After adding the auxiliary enzymes, the reaction was started by the addition of 125 µM NADP⁺. For the activity detection of the ATP-PFK, ATP (2 mM) was used instead of PPI.

The enzyme BVU_3333 from Bacteroides vulgatus was tested for transaldolase and F6P aldolase activity by coupled photometric enzyme assays. For the determination of transaldolase activity the rearrangement of the C3 moiety
from S7P to GAP was detected by transforming the resulting F6P into G6P and 6-PGL with the help of the auxiliary enzymes GPI and G6PD. The basic enzyme assay (600 μL total volume) contained 50 mM HEPES buffer pH 7, 5 mM MgCl2, 0.5 mM S7P, 0.5 mM GAP, 2 U G6PD, 250 μM NADP+, and 0–24 μg BVU_3333. To verify whether BVU_3333 had F6P-aldolase activity, the cleavage of F6P into GAP and DHA was detected using the auxiliary enzyme glyceraldehyde 3-phosphate dehydrogenase (from rabbit muscle; Sigma-Aldrich) by the NAD+–dependent conversion of GAP into 1,3-bisphosphoglycerate. The reduction of NAD+ was determined photometrically at 340 nm. The basic enzyme assay (1 mL in total) contained 50 mM HEPES buffer pH 7, 5 mM MgCl2, 5 mM F6P, 2 U glyceraldehyde 3-phosphate dehydrogenase, 250 μM NAD+, and 0–15 μg BVU_3333.

Detection of intermediates by HPLC

The activities of enzymes of the pentose metabolism in P. copri were analyzed by detecting the intermediates and products of the enzymatic reactions using a SpectraSYSTEM HPLC system (Thermo Fisher Scientific Inc.) supplied with a degasser (SpectraSystem SCM1000), a pump (SpectraSystem P4000), an autosampler (SpectraSYSTEM AS3000) and a refractive index (RI) detector (Shodex RI-101; Showa Denko Europe GmbH, Munich, Germany). Substances were separated by a Shodex column Asahipak NH2P-50G 3E (250 × 3.0 mm) combined with the guard column Asahipak column NH2P-50G 3A (10 × 3.0 mm) and the line filter Asahipak NH2-P-LF (75 × 8.0 mm) at 40 °C using 15 or 130 mM NaH2PO4 pH 4.4 as mobile phase. The 1 mL basic assay contained 50 mM Tris/HCl buffer pH 7, 0.2 mM TPP, 10 mM MgCl2, 5 mM F6P, 5 mM PPI, and 50 μg of the enzymes as indicated. The enzyme assays were incubated for 30 min at RT with the exception of the FBP aldolase assay, which was incubated for 2.5 h at RT and contained 85 μg FBP aldolase.

For the detection of products of the enzymes RPI, RPE, and TK, 15 mM NaH2PO4 was used as mobile phase. To shorten the retention time and to detect the dephosphorylated sugar S-1,7-bP, 130 mM NaH2PO4 was used for the reactions of PPI-PFK and FBP aldolase as mobile phase. Fifty microlitre of samples was taken from each reaction mixture and mixed with 50 μL pure ethanol. Precipitates were separated by centrifugation (20 000 g, 1 min), and the supernatant was diluted with 100 μL of the mobile phase. For data analysis, the software CHROMQUEST 5.0 (Thermo Fisher Scientific Inc.) was used.

Acknowledgements

The authors thank Natalie Thum-Schmitz (Institute of Microbiology and Biotechnology, University of Bonn, Germany) for technical assistance. We also thank Luisa Becher for her help with the biochemical analysis of BVU_3333. This work was supported by funds from the BMBF project BaPro (031B0846A). The funding agency was not involved in the research. Open access funding enabled and organized by Projekt DEAL.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

LSG planned experiments, performed experiments, analyzed data, and wrote the paper. TF analyzed data and wrote the paper. UD planned experiments, analyzed data, wrote the paper, and supervised the study.

References

1 Bäckhed F, Ley RE, Sonnenburg JL, Peterson DA & Gordon JI (2005) Host–bacterial mutualism in the human intestine. Science 307, 1915–1920.
2 Tancrede C (1992) Role of human microflora in health and disease. Eur J Clin Microbiol Infect Dis 11, 1012–1015.
3 Gill SR, Pop M, DeBoy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, Gordon JI, Relman DA, Fraser-Liggett CM & Nelson KE (2006) Metagenomic analysis of the human distal gut microbiome. Science 312, 1355–1359.
4 Thursby E & Juge N (2017) Introduction to the human gut microbiota. Biochem J 474, 1823–1836.
5 Eckburg PB, Bik EM, Bernstein CN, Purdom E, DeThelefsen L, Sargent M, Gill SR, Nelson KE & Relman DA (2005) Diversity of the human intestinal microbial flora. Science 308, 1635–1638.
6 Tannock GW (2001) Molecular assessment of intestinal microflora. Am J Clin Nutr 73, 410–414.
7 Fanaro S, Chierici R, Guerrini P & Vigi V (2003) Intestinal microflora in early infancy: composition and development. Acta Paediatr 92, 48–55.
8 Hold GL, Pryde SE, Russell VJ, Furrie E & Flint HJ (2002) Assessment of microbial diversity in human colonic samples by 16S rDNA sequence analysis. FEMS Microbiol Ecol 39, 33–39.
9 The Human Microbiome Project Consortium (2012) Structure, function and diversity of the healthy human microbiome. Nature 486, 207–214.
10 Wang X, Heazlewood SP, Krause DO & Florin THJ (2003) Molecular characterization of the microbial species that colonize human ileal and colonic mucosa by using 16S rDNA sequence analysis. J Appl Microbiol 95, 508–520.
Important gut bacteria rely on the SBP pathway

L. S. Garschagen et al.

11 Flint HJ, Scott KP, Louis P & Duncan SH (2012) The role of the gut microbiota in nutrition and health. *Nat Rev Gastro Hepatol* **9**, 577.

12 Hooper LV & Macpherson AJ (2010) Immune adaptations that maintain homeostasis with the intestinal microbiota. *Nat Rev Immunol* **10**, 159–169.

13 Sekirov I, Russell SL, Antunes LCM & Finlay BB (2010) Gut microbiota in health and disease. *Physiol Rev* **90**, 859–904.

14 Dhingra D, Michael M, Rajput H & Patil RT (2012) Dietary fibre in foods: a review. *J Food Sci Technol* **49**, 255–266.

15 Kendall CW, Esfahani A & Jenkins DJ (2010) The link between dietary fibre and human health. *Food Hydrocoll* **24**, 42–48.

16 Cummings J, Pomare EW, Branch WJ, Naylor CP & Macfarlane GT (1987) Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut* **28**, 1221–1227.

17 Trowell H (1976) Definition of dietary fiber and hypotheses that it is a protective factor in certain diseases. *Am J Clin Nutr* **29**, 417–427.

18 Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA, Bewtra M, Knights D, Walters WA, Knight R et al. (2011) Linking long-term dietary patterns with gut microbial enterotypes. *Science* **334**, 105–108.

19 Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, Fernandes GR, Tap J, Bruls T, Batto J-M et al. (2011) Enterotypes of the human gut microbiome. *Nature* **473**, 174–180.

20 Ferrocino I, Di Cagno R, De Angelis M, Turroni S, Vannini L, Bancalari E, Rantsiou K, Cardinali G, Neviani E & Cocolin L (2015) Fecal microbiota in healthy subjects following omnivore, vegetarian and healthy subjects. *PLoS One* **10**, e0128669.

21 Lin A, Bik EM, Costello EK, Dethlefsen L, Haque R, Relman DA & Singh U (2013) Distinct distal gut microbiome diversity and composition in healthy children from Bangladesh and the United States. *PLoS One* **8**, e53838.

22 Fehlner-Peach H, Magnabosco C, Raghavan V, Scher JU, Tett A, Cox LM, Gottsegen C, Watters A, Gordon J, Segata N et al. (2019) Distinct polysaccharide utilization profiles of human intestinal *Prevotella copri* isolates. *Cell Host Microbe* **26**, 680–690.

23 Franke T & Deppenmeier U (2018) Physiology and central carbon metabolism of the gut bacterium *Prevotella copri*. *Mol Microbiol* **109**, 528–540.

24 Dodd D, Mackie RI & Cann IK (2011) Xylan degradation, a metabolic property shared by rumen and human colonic bacteroidetes. *Mol Microbiol* **79**, 292–304.

25 Tan H, Zhao J, Zhang H, Zhai Q & Chen W (2018) Isolation of low-abundant Bacteroidales in the human intestine and the analysis of their differential utilization based on plant-derived polysaccharides. *Front Microbiol* **9**, 1319.

26 McKendry P (2002) Energy production from biomass (part 1): overview of biomass. *Bioresour Technol* **83**, 37–46.

27 Bastawde KB (1992) Xylan structure, microbial xylanases, and their mode of action. *World J Microbiol Biotechnol* **8**, 353–368.

28 Koendjibarie JG, Hon S, Pabst M, Hoofman R, Stevenson DM, Cui J, Amador-Noguez D, Lynd LR, Olson DG & van Kranenburg R (2020) The pentose phosphate pathway of cellulolytic clostridia relies on 6-phosphofructokinase instead of transaldolase. *J Biol Chem* **295**, 1867–1878.

29 Susskind BM, Warren LG & Reeves RE (1982) A pathway for the interconversion of hexose and pentose in the parasitic amoeba *Entamoeba histolytica*. *Biochem J* **204**, 191–199.

30 Hettrich K, Fischer S, Schröder N, Engelhardt J, Drechslers U & Lotth F (2006) Derivatization and characterization of xylan from out spelts. In Macromolecular Symposia 232, pp. 37–48. Wiley-VCH Verlag, Weinheim.

31 Kiszonas AM, Fuerst EP & Morris CF (2013) Wheat arabinoxylan structure provides insights into function. *Cereal Chem* **90**, 387–395.

32 Fry SC (1989) The structure and functions of 1-deoxysugars. *J Exp Bot* **40**, 1–11.

33 Dodd D & Cann IK (2009) Enzymatic deconstruction of xylan for biofuel production. *GCB Bioenergy* **1**, 2–17.

34 Mohnen D (2008) Pectin structure and biosynthesis. *Curr Opin Plant Biol* **11**, 266–277.

35 Samland AK & Sprenger GA (2009) Transaldolase: from biochemistry to human disease. *Int J Biochem Cell Biol* **41**, 1482–1494.

36 Schürmann M & Sprenger GA (2001) Fructose-6-phosphate aldolase is a novel class I aldolase from *Escherichia coli* and is related to a novel group of bacterial transaldolases. *J Biol Chem* **276**, 11055–11061.

37 Sautner V, Friedrich MM, Lehwess-Litzmann A & Tittmann K (2015) Converting transaldolase into aldolase through swapping of the multifunctional acid-base catalyst: common and divergent catalytic principles in F6P aldolase and transaldolase. *Biochemistry* **54**, 4475–4486.

38 Schürmann M, Schürmann M & Sprenger GA (2002) Fructose 6-phosphate aldolase and 1-deoxy-D-xylulose 5-phosphate synthase from *Escherichia coli* as tools in enzymatic synthesis of 1-deoxysugars. *J Mol Catal B Enzym* **19**, 247–252.
Important gut bacteria rely on the SBP pathway
Important gut bacteria rely on the SBP pathway

L. S. Garschagen et al.

67 Miller JH (1972) Experiments in Molecular Genetics. Cold Spring Harbor Laboratory, New York.
68 Altschul SF, Gish W, Miller W, Myers EW & Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215, 403–410.
69 Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T et al. (2010) A human gut microbial gene catalogue established by metagenomic sequencing. Nature 464, 59–65.
70 Rinninella E, Raoul P, Cintoni M, Franceschi F, Miggiano GAD, Gasbarrini A & Mele MC (2019) What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet and diseases. Microorganisms 7, 14.
71 Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680–685.
72 Towbin H, Staehelin T & Gordon J (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 76, 4350–4354.
73 Saheki S, Takeda A & Shimazu T (1985) Assay of inorganic phosphate in the mild pH range, suitable for measurement of glycogen phosphorylase activity. Anal Biochem 148, 277–281.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Kinetic parameters of the PPI-PFK and ATP-PFK of P. copri.

Table S2. Distribution of the SBPP and PPP in different human gut bacteria. Highlighted in gray are species using the SBPP.