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Peptidoglycan synthesis in *Tannerella forsythia*: scavenging is the modus operandi

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Abstract

Tannerella forsythia is a Gram-negative oral pathogen strongly associated with periodontitis. This bacterium has an absolute requirement for exogenous *N*-acetylmuramic acid (MurNAc), an amino sugar which forms the repeating disaccharide unit with amino sugar *N*-acetylglucosamine (GlcNAc) of the peptidoglycan backbone. *In silico* genome analysis indicates that *T. forsythia* lacks the key biosynthetic enzymes needed for the *de novo* synthesis of MurNAc, and thus relies on alternative ways to meet its requirement for peptidoglycan biosynthesis. In the subgingival niche, the bacterium can acquire MurNAc and peptidoglycan fragments (muropeptides) released by the cohabiting bacteria during their cell wall breakdown associated with cell division. *T. forsythia* is able to also utilize host sialic acid (Neu5Ac) in lieu of MurNAc or muropeptides for its survival during the biofilm growth. The evidence suggests that the bacterium might be able to shunt sialic acid into a metabolic pathway leading to peptidoglycan synthesis. In this review, we explore the mechanisms by which *T. forsythia* is able to scavenge MurNAc, muropeptide, and sialic acid for its peptidoglycan synthesis, and the impact of these scavenging activities on pathogenesis.

Introduction

Periodontitis is an inflammatory disease that is characterized by the destruction of the tooth supporting apparatus, which include the periodontal ligament, connective tissue, and alveolar bone. Periodontitis is caused by dysregulated inflammation in response to polymicrobial and dysbiotic subgingival microbiota¹. While more than 700 bacterial species, of which more than 50% yet to be cultivated, are considered as components of periodontal microbiota², a consortium known as the ‘*red complex*’ consisting of the Gram-negative species *Tannerella forsythia*, *Treponema denticola*, and *Porphyromonas gingivalis* is strongly associated with pathogenesis³.

T. forsythia, previously called *Bacteroides forsythus*, was isolated by Anne Tanner from periodontitis patient plaque samples as a slow growing organism on blood agar plates by co-streaking with *Fusobacterium nucleatum* as the helper bacterium⁴. Subsequently, Wyss

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Conflict of Interest

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(1989) showed that exogenous *N*-acetylmuramic acid (MurNAc) was essential for the growth of the organism⁵. MurNAc is an amino sugar that together with *N*-acetylglucosamine (GlcNAc) forms the repeating disaccharide unit of the peptidoglycan backbone⁶. *T. forsythia* is a member of the *Cytophaga-Bacteroides* family and the founding member of the genus *Tannerella*. It is phylogenetically related to *Bacteroides distasonis* and *Bacteroides merdae* to some extent⁷. *T. forsythia* expresses a number of virulence factors that are suggested to play roles in its virulence⁸. This pathogen seems to have adapted to the human oral niche, at least, since the time of prehistoric Neolithic humans⁹. It is not clear what adaptive advantage the loss of *de novo* MurNAc synthesis provided to the bacterium, except for reducing its genome burden somewhat.

While it has been known since the late 1980s that *T. forsythia* has an absolute requirement for the amino sugar MurNAc, the genetic basis of MurNAc auxotrophy became evident with the availability of the *T. forsythia* genome sequence¹⁰, which indicated the absence of orthologs of MurA and MurB enzymes required for the formation of MurNAc from GlcNAc in bacteria¹¹. MurA is a UDP-*N*-acetylglucosamine enolpyruvyl transferase that catalyzes the first committed step of peptidoglycan synthesis by incorporating enolpyruvate from phosphoenolpyruvate (PEP) to UDP-GlcNAc¹¹. The aim of this review is to provide current understanding of the strategies employed by the bacterium to meet its need for peptidoglycan biosynthesis. The review will focus on the components of pathways involved in the scavenging of MurNAc, peptidoglycan fragments, and sialic acid for peptidoglycan production, and will briefly highlight potential effects of these scavenging activities on the host innate immunity.

Peptidoglycan structure and function

Peptidoglycan is a polymer of alternating β -1, 4 linked GlcNAc and MurNAc residues in which MurNAc is attached to L- and D-amino acid-containing stem pentapeptides crosslinking adjacent sugar chains¹¹. MurNAc is a unique sugar found only in the chemical make-up of bacteria. The peptidoglycan layer provides a rigid exoskeleton surrounding the cytoplasmic membrane, protects bacteria from osmotic shock and determines the shape of the bacteria¹². While the basic chemical structure of peptidoglycan is highly conserved among all bacteria, minor variations do exist. These include differences in the chain length of the peptidoglycan, the nature of peptide crosslinks and their amino acid composition and modification (e.g., amidation), and the sites and density of interchain linkages^{13,14}. In Gram-negative bacteria, peptidoglycan forms a thin layer in the periplasmic space and in Gram-positive bacteria it forms a thick layer (cell wall) surrounding the single lipid membrane¹¹. Gram-positive cell wall also contains large amounts of teichoic acid, with wall teichoic acids covalently attached to peptidoglycan^{15,16}. Moreover, in some bacteria post-synthetic modification of peptidoglycan glycan backbone occurs via O-acetylation, de-O-acetylation and Ndeacetylation¹⁷. These modifications to the glycan backbone are generally restricted to the C-2 amine or the C-6 hydroxyl moieties of either aminosugar¹⁷. For instance, Ndeacetylation of GlcNAc is particularly observed amongst Gram-positive species, while N-glycosylation of GlcNAc occurs in *Mycobacterium tuberculosis*^{18,19}. In addition, teichoic acids, other surface polymers such as capsular polysaccharides and arabinogalactans and O-acetylation are observed at the C-6 hydroxyl moiety of MurNAc residues^{20,21}. Such

type of modifications to the glycan residues protect peptidoglycan from both the host-derived (innate immune system) agents such as lysozymes, and the bacterially-derived autolysins involved in peptidoglycan degradation. In Gram-negative bacteria, peptidoglycan is generally linked to the outer membrane via a small molecular weight lipoprotein, the Braun's lipoprotein. Peptidoglycan can differ in composition among species because of MurNAc attached stem peptide composition. While all bacteria have two D-alanine residues at the end of the stem peptide, the third residue on a stem peptide differentiates the two major types of peptidoglycan. In Gram-negative bacteria, this residue is primarily a *meso*-diaminopimelic acid (mDAP) and in Gram-positive bacteria it is commonly a lysine²². There are also variations found in the extent of interpeptide crosslinking and the residues involved in the formation of such interpeptide bridges²². Most Gram-negative bacteria form crosslinks by bonding to the D-alanine at the fourth position with a D-amino group of DAP at the third position on the adjacent mucopeptide forming a characteristic DD, 4–3 cross-bridge linkage²³. The enzymes responsible for crosslinking are L, D-transpeptidases and attach mucopeptides to Braun's lipoproteins on the outer membrane of some Gram-negative bacteria²³.

Dependency of *T. forsythia* on MurNAc

Peptidoglycan biosynthesis is a highly conserved and complex multistep process unique to bacteria. It begins in the cytoplasm with the biosynthesis of the nucleotide-linked sugar precursors and culminating in formation of a sugar-pentapeptide intermediate with help of enzymes encoded by *mur* genes and D-Ala-D-Ala ligase Ddl (Fig. 1); this includes the formation of UDP-GlcNAc from fructose-6-phosphate, formation of UDP-MurNAc from UDP-GlcNAc, and assembly of the peptide stem leading to UDP-MurNAc-pentapeptide (UDP-MurNAc-pp)^{6,11}. The cytoplasmic steps are followed by the synthesis of lipid-linked UDP-MurNAc-pp and UDP-GlcNAc intermediates on the inner-side and polymerization on the outer-side of the cytoplasmic membrane to assemble the peptidoglycan. Briefly, these steps begin with the ligation of UDP-MurNAc-pp to an undecaprenyl (C₅₅) lipid carrier by a membrane-associated enzyme (MraY) to form lipid I intermediate. A ligase enzyme (MurG) then ligates UDP-GlcNAc to lipid I to form the final lipidated disaccharide-pentapeptide lipid II at the cytoplasmic side of the inner membrane²⁴. The lipid II precursor is then 'flipped' by a flippase (MurJ) to the outer-side of the inner membrane, where lipid II subunits are polymerized into glycan strands via transglycosylation (TG) reactions mediated by penicillin binding proteins (PBPs)²⁵. Following TG reaction, the glycan strands are subsequently covalently linked by peptide bonds through transpeptidation reaction (TP)²⁵. A number of excellent review articles on this topic are available^{6,26–29} and our intent here is to highlight and predict why *T. forsythia* is unable to synthesize its own MurNAc from simple sugars and depends on exogenous MurNAc or mucopeptides for peptidoglycan synthesis. As mentioned above, in most bacteria *de novo* synthesis of peptidoglycan begins with the metabolic conversion of fructose-6-phosphate, a glycolytic intermediate, to UDP-*N*-acetylglucosamine (UDP-GlcNAc) (Fig. 1). The first reaction in this process catalyzed by glutamine-fructose-6-phosphate aminotransferase (GlmS) converts fructose-6-P to glucosamine-6-P using glutamine as a nitrogen source. Next, glucosamine-6-P is converted to glucosamine-1-P by a phosphoglucosamine mutase (GlmM), which is followed by conversion of glucosamine-1-P to UDP-GlcNAc by GlcNAc-1-P uridylyltransferase (GlmU). Once UDP-GlcNAc becomes

available, the first committed step to peptidoglycan synthesis is the transfer of an enolpyruvate residue from phosphoenolpyruvate (PEP) to the third position of UDP-GlcNAc by MurA enzyme (UDP-N-acetylglucosamine enolpyruvyl transferase, a fosfomycin sensitive enzyme). The second stage of the biosynthetic pathway is the MurB enzyme (UDP-N-acetylenolpyruvylglucosamine reductase) catalyzed reduction of the enolpyruvate moiety to D-lactate, yielding UDP-N-acetylmuramate (UDP-MurNAc). UDP-GlcNAc is thus converted to UDP-MurNAc by enzymes MurA and MurB (Fig.1). With respect to *T. forsythia*, the seminal work of Wyss⁵ early on showed that *T. forsythia* was able to sustain its growth only when MurNAc was supplemented in the medium; the bacterium did not grow on other amino sugars, including GlcNAc. Later, with availability of *T. forsythia* genome sequence, it was noted that the bacterium does not possess orthologs of MurA and MurB enzymes¹⁰. Interestingly, based on amino sugar synthesis pathway analysis of *T. forsythia* genome by KEGG database (Kyoto Encyclopedia of Genes and Genomes) orthologs of Glm enzymes are also not found in the bacterium. Since MurNAc amino sugar is not synthesized by the human host, the pathogen likely obtains this sugar from the environment, which includes the cohabiting microbiota. To the best of our knowledge, no other bacterium has such a strict requirement for MurNAc because of the lack of de novo synthesis of this amino sugar. Interestingly, a salvage pathway has been reported in *Pseudomonas putida* that bypasses de novo biosynthesis of UDP-MurNAc³⁰. This pathway is dependent on the activities of two enzymes, an anomeric sugar kinase (AmgK) and a MurNAc α-1-phosphate uridylyl transferase (MurU). These enzymes directly shunt external MurNAc into peptidoglycan biosynthetic pathway. Predicted homologs of AmgK and MurU enzymes are found in a number of Gram-negatives including *T. forsythia*³⁰. Studies ongoing in our lab are exploring if a similar salvage pathway that bypasses de novo MurNAc synthesis is operative in *T. forsythia*.

How is environmental MurNAc imported by *T. forsythia*?

In *E. coli* and many bacteria, the phosphoenolpyruvate-dependent MurP PTS system (phosphotransferase system) is responsible for the phosphorylation and import of MurNAc³¹. MurP has the MurNAc-specific domain and requires enzyme EI, histidine protein HPr, and the phosphoryl transfer protein EIIA (EIIGlc) for function (Fig. 2). Utilization of MurNAc transported as MurNAc-6-P occurs through the action of MurQ etherase, allowing metabolic products to enter either a glycolytic pathway or a biosynthetic pathway to generate peptidoglycan amino sugar GlcNAc (Fig. 2). In the case of *T. forsythia*, despite its clear ability to utilize exogenously supplied MurNAc, orthologs of MurP or a PTS-type system are not found³². Instead, the bacterium has been shown to use a novel non-PTS type system for the transport and utilization of exogenous MurNAc³². It consists of an integral membrane protein for MurNAc transport (TfMurT), a sugar kinase involved in MurNAc phosphorylation (TfMurK), and an etherase (TfMurQ) involved in the metabolic conversion of MurNAc (Fig. 2). The presence of TfMurTK and MurQ etherase in *T. forsythia* suggests an active involvement of MurNAc and peptidoglycan recycling in this bacterium. Orthologs of MurQ are found in Gram-negative bacterial species, mostly γ-proteobacteria, and most Gram-positive bacteria such as in the bacillales, clostridia, and lactobacillales, where cell wall recycling has been proposed similar to *E. coli*³³. TfMurT and TfMurK proteins do not possess PTS-type signatures and are expressed from a genetic locus involved in

peptidoglycan recycling as implied from the presence of a peptidoglycan permease (AmpG)-encoding ORF on this cluster (Fig. 2). In an *E. coli* mutant host lacking both the MurP protein and a functional PTS-system (*murP*⁻ *pts*) co-expression of TfMurT and TfMurK is able to restore the bacterial growth on MurNAc³². These data showed that TfMurT and TfMurK function as a unique bipartite MurNAc transporter in a PTS-independent manner. PTS-independent sugar transporters are not as common as PTS-dependent systems. The TfMurTK system is the first evidence of a PTS-independent MurNAc transporter system thus far, and although so far it is unique to *T. forsythia*, we predict that this type of transporter is present in a range of Gram-negative bacteria, especially of the phylum Bacteroidetes present in the oral cavity or the gut³². This notion is based on genome mining data³², indicating the presence of genetic loci coding for TfMurTK and MurQ homologs in *Bacteroides fragilis* NCTC9343, *Prevotella saccharolytica* JCM17484, *Bacteroides caccae* CL03T12C61, and *Bacteroides uniformis* ATCC 8492. Moreover, as in *T. forsythia*, genes encoding Mur homologs in these bacteria are present tandemly as a three-gene cluster closely linked to a putative peptidoglycan recycling locus (Fig. 2B).

Survival through recycling environmental peptidoglycan fragments

Many Gram-negative and Gram-positive bacteria recycle a large proportion of their peptidoglycan components during their growth and cell division (septation). This is a beneficial trait possibly all bacteria possess. However, the magnitude of recycling in a species may differ depending on the need. Given that Gram-positive bacteria contain much larger portion of peptidoglycan in their cell wall, peptidoglycan recycling and recovery is thought to be more important and beneficial to a Gram-positive species compared to a Gram-negatives species³⁴. Peptidoglycan recycling is even more critical for the survival of *T. forsythia* as the bacterium is unable to synthesis its own peptidoglycan precursors. The dependency of *T. forsythia* on exogenous growth factors and peptidoglycan precursors became evident early on through the experiments of Tanner and co-workers demonstrating that the bacterium growth required *Fusobacterium nucleatum* co-streaking as a helper species on agar plates⁴ or culturing with *F. nucleatum* in co-cultures separated by a dialysis membrane³⁵. Subsequently, the dependency of the bacterium on peptidoglycan precursors became evident when it was demonstrated by Wyss⁵ that the bacterial growth could be restored with MurNAc. In an *in vivo* situation, it is reasonable to conclude that *T. forsythia* utilizes peptidoglycan precursors which are released by the cohabiting bacteria during their cell wall recycling. Generally, in bacteria about two-thirds of peptidoglycan is degraded during cell wall turnover in cell division by lytic transglycosylases and endopeptidases, releasing GlcNAc-1, 6-anhydro-MurNAc-L-Ala-D-Glu-mesoDAP-D-Ala (anhydro-muropeptide) as a major degradation product. In most Gram-negative bacteria, the released muropeptides are then recycled into the cytoplasm via the AmpG-like permeases^{6,27,28}. Orthologs of peptidoglycan recycling enzymes have been identified in some Grampositive bacteria as well, such as in *Bacillus subtilis*, and *Clostridium acetobutylicum*^{34,36}. In some bacteria, peptidoglycan fragments (muropeptides) function as important messengers in bacterial communication, and as anti-dormancy and spore resuscitation signals in some Gram-positive bacteria^{37–39}. In many Gram-negative bacteria, but not *E. coli*, muropeptide fragments entering the cytoplasm due to cell wall damage or cell wall recycling regulate induction of chromosomal AmpC-type β -lactamases, which can provide resistance to β -

lactam antibiotics²⁷. For the purpose of peptidoglycan synthesis, after entering the cytoplasm, muropeptides are further broken down by amidases and carboxypeptidases to release GlcNAc, anhMurNAc, murein tripeptides, and D-Ala, which then enter the peptidoglycan biosynthetic pathway^{6,27,28}. In the oral cavity, peptidoglycan fragments released from the cohabiting bacteria as a result of their cell wall turnover or death are expected to be available to *T. forsythia*. Importantly, scavenging on these fragments by *T. forsythia* is even more significant given that the human host does not make the peptidoglycan amino sugars. In support, direct evidence that *T. forsythia* can utilize muropeptides derived cohabiting bacteria was recently provided by our group⁴⁰. We showed that the growth of *T. forsythia* can be sustained on muropeptides extracted from *F. nucleatum* for multiple passages⁴⁰. We predict that dependence of *T. forsythia* on *F. nucleatum* peptidoglycan dictates a close physical association between these two organisms in the dental plaque occurs⁴¹, and synergistic effects on cobiofilm activity and virulence^{42,43}. This prediction is based on previous studies that showed that these organisms form synergistic biofilms *in vitro*⁴³, induce synergistic alveolar bone loss mice in a mixed oral infection setting⁴², and are found in close proximity to each other in a human subgingival plaque biofilms⁴¹. Interferingly, the *T. forsythia* AmpG permease (Tf AmpG) is under the direct control of a transcriptional regulator⁴⁰, GppX, a unique hybrid two-component system (TCS) regulator comprising of an N-terminal histidine kinase (HK) sensor domain fused to a central receiver and C-terminal response regulator (RR) domain with a putative AraClike helix-turn-helix DNA binding motif⁴⁴. *T. forsythia* mutant lacking GppX with a functional AmpG permease is severely growth impaired on muropeptides as the sole peptidoglycan precursor source⁴⁰. It remains to be determined how GppX senses environmental muropeptide to regulate the expression of AmpG promoter. In bacteria, in general, peptidoglycan is regulated by different mechanisms depending on the stage of cell propagation, growth phase and morphogenesis of cell shape⁴⁵. With respect to regulation via TCS systems, WalRK and PhoPR of *Bacillus subtilis* are prime examples of TCS regulators involved in the regulation of peptidoglycan metabolism⁴⁶.

Scavenging on host sialic acid for survival in biofilms

An interesting discovery was made by Stafford and co-workers that showed that *N*-acetylneuraminic acid (Neu5Ac) or sialic acid can be substituted for MurNAc when *T. forsythia* is grown in biofilms⁴⁷. This suggested that *T. forsythia* must have the ability to convert sialic acid into MurNAc and subsequently peptidoglycan. *T. forsythia* possesses a dedicated sialic acid utilization system, which includes sialidase (NanH) for releasing terminal sialic acid from host glycoconjugates, sialic acid transporter (NaT/NanO) and sialic acid catabolism (NanA and NaE enzymes)⁴⁷ (Fig. 3). Sialic acid is a nine-carbon sugar that is located at the terminal positions of eukaryotic glycoconjugates such as salivary mucins or glycoproteins that coat mucosal surfaces⁴⁸. Therefore, within the biofilm community as part of subgingival plaque *T. forsythia* could survive by harvesting sialic acid. Since, the bacterium acquires the ability to utilize sialic acid only during the biofilm phase, suggests that the pathway from sialic acid to MurNAc and peptidoglycan is activated during the transition from planktonic to biofilm growth phase. Here we make a provocative but a plausible prediction that the availability of sialic acid acts as a trigger for the biofilm formation, relieving the bacterium's dependence on peptidoglycan fragment and competition

with cohabiting bacteria. Many pathogenic bacteria have evolved mechanisms to harvest and utilize host sialic acid as a nutritional source, or a molecule for decorating their surfaces to evade or modulate the host immune response^{48–50}. Moreover, metabolic pathways exist in some bacteria that allow them to direct sialic acid into peptidoglycan synthesis^{48–53}. As mentioned above, while sialic acid is nutritionally important for the growth of *T. forsythia* in biofilms It was observed that compared to the parental strain a sialic acid transport deficient mutant was significantly less fit to survive on oral epithelial cell monolayers⁵⁴. Not only epithelial cells, but other bacteria with which *T. forsythia* is known interact, such as *F. nucleatum* can provide sialic acid. We and others have shown that many strains of *F. nucleatum* express surface sialic acid^{55,56}, and specifically in the case of *F. nucleatum* strain sialic acid is a component of LPS O-chain⁵⁵. It is predicted that sialic acid after entering the cell via NanOU/NanT transporter is converted to GlcNAc-6P by the action of NanA, NanK, and NanE enzymes encoded by the sialic acid utilization Nan operon⁴⁷ (Fig. 3). GlcNAc-6P can either enter the energy utilization pathway, or shunted into a peptidoglycan biosynthetic pathway as depicted in the Fig.3. Strikingly, as mentioned above, orthologs of Glm enzymes required for the entry of GlcNAc into the MurNAc/peptidoglycan synthesis pathway have not been detected in the *T. forsythia* genome and it remains to be determined how this pathway of sialic acid to peptidoglycan synthesis is completed in the cell.

Conclusions

The nutritionally fastidious bacterium *T. forsythia* depends on exogenous MurNAc and muropeptide fragments, which are likely made available to the bacterium by the cohabiting bacteria in the oral cavity. During its biofilm lifestyle, the bacterium is able to forage on host sialic acid for survival. There is mounting evidence suggesting that via an alternate metabolic pathway the bacterium is able to convert sialic acid (Neu5Ac) into amino sugars GlcNAc and MurNAc, leading to peptidoglycan synthesis. Despite its clear ability to utilize exogenously supplied MurNAc, *T. forsythia* lacks homologs of PTS-type MurNAc transporters present in bacteria. It utilizes a novel transporter for the uptake of MurNAc across the inner membrane, which comprises an inner membrane integral protein TfMurT and a sugar kinase TfMurK. To the best of our knowledge, this is the only known PTS-independent MurNAc transporter in bacteria. In addition to the utilization of MurNAc, the bacterium is also able to utilize muropeptides via the permease AmpG, whose expression is under the control of a hybrid TCS regulator GppX.

Conceivably, *T. forsythia* muropeptide scavenging might impact the local immune environment since peptidoglycan components such as iE-DAP (dipeptide, D- γ -glutamylmeso-DAP; NOD1 ligand) and MDP (muramyl dipeptide; NOD2 ligand) are immunostimulatory molecules as they can activate innate immune NOD-like receptors^{57–60}. Dysregulation of NOD receptor signaling leads to several inflammatory diseases such as Crohn's disease and Blau disease^{61–63}. Paradoxically, though, muropeptide scavenging by *T. forsythia* is expected to reduce the availability of free peptidoglycan fragments in the subgingival environment, and result in dampening of NOD-mediated inflammation. Simultaneously, sialic acid scavenging by *T. forsythia* might contribute to inflammation in other ways as well. For example, desialylation of host sialoglycans such as mucins, immunoglobulins, and pattern recognition receptors (TLRs) can cause functional

dysregulation of these molecules and impact immunity in various ways^{49,50,64,65}. It would be of interest to determine how sialic acid and mucopeptide utilization pathways together contribute to periodontal inflammation.

In summary, *T. forsythia*, a nutritionally fastidious periodontal pathogen relies on novel ways to meet its requirement for peptidoglycan synthesis. This review summarizes three mechanisms by which this requirement is likely met in the oral cavity by the bacterium; a novel non-PTS transporter system MurTK for uptake of environmental MurNAc, scavenging on environmental mucopeptides excreted by cohabiting bacteria, and foraging on host sialic acid. Understanding these mechanisms might open up the possibility of developing small molecule compounds targeted against peptidoglycan biosynthesis in the bacterium to reduce its burden in patients with periodontitis.

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Fig. 1.

Peptidoglycan biosynthesis reference pathway. Enzymes catalyzing each reaction are in italics font; homologs of enzymes (*MurA*, *MurB* and *GlmU*) highlighted in red are not found in *T. forsythia*. Gln, glutamine; UTP, uridine triphosphate; PEP, phosphoenol pyruvate; NADPH, nicotinamide adenine dinucleotide phosphate (reduced form); L-Ala, L-alanine; D-Glu, L-glutamic acid; A₂pm, di-aminopimelic (DAP) acid; DLys; D-lysine.

Fig. 2.

(A) Schematic model of a MurNAc transport and utilization pathway in *E. coli* and *T. forsythia* (adapted from Ruscitto et al 2016). Cytoplasmic Memb, cytoplasmic membrane; PEP, phosphoenolpyruvate; E1, enzyme E1; HPR, histidine protein; EIIA, enzyme IIA. Predicted orthologs are shown in parenthesis (*gene IDs*) and dashed line arrows connecting intermediates indicate enzyme orthologs involved are not detected.

(B) Schematic representation (to scale) of MurTKQ containing loci from a range of human dwelling Bacteroidetes. Key: XthA, predicted Xanthan lyase; Ser/Thr, kinase, predicted serine/threonine kinase; Gtf, contain predicted glycosyltransferase domains; LytB, predicted amidase enhancer; AmpG, predicted muropeptide transporter; Hyp, no known homologies found; Ferredoxin, contains 4Fe4s cluster, FAD-binding motif and homology to ferredoxin proteins; AfuC, predicted α-L-fucosidase; β-lactamase, predicted β-lactamase; MurT, predicted homolog of TfMurT homolog; MurK, predicted homolog of TfMurK; MurQ, predicted homolog of MurQ etherase. Gene annotations are predicted from PATRIC/Refseq annotations and use of BlastP.

Fig. 3.

Predicted model of peptidoglycan and sialic acid scavenging pathway in *T. forsythia*. Genetic organization of peptidoglycan and sialic acid scavenging locus is shown on top. The gene IDs for peptidoglycan scavenging components are in parenthesis (adapted from Ruscitto et al, 2017). Sialic acid scavenging locus is adapted from Roy et al, 2012. Dashed line arrows indicate biochemical steps are unknown as orthologs of the canonical enzymes involved in the metabolic conversion of various intermediates are not detected in the bacterium. Gtf, predicted glycosyltransferase; LytB, predicted amidase enhancer; AmpG, muropeptide transporter; YbbC, hypothetical protein.