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Characterisation and pure culture of putative health-associated oral bacterium BU063 (*Tannerella* sp. HOT-286) reveals presence of a potentially novel glycosylated S-layer

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Abstract-200 words

Tannerella HOT-286 (phylotype BU063) is a recently identified novel filamentous Gram-negative anaerobic oral bacterium cultured for the first time recently in co-culture with *Propionibacterium acnes*. In contrast to the related periodontal disease associated pathobiont *Tannerella forsythia* it is considered a putative health-associated bacterium. In this paper we identified that this organism could be grown in pure culture if N-acetyl muramic acid (NAM) was provided in the media, although surprisingly the genetic basis of this phenomenon is not likely to be due to a lack of NAM synthesis genes. During further microbiological investigations we showed for the first time that *Tannerella* HOT-286 possesses a prominent extracellular S-layer with a novel morphology putatively made up of two proteins modified with an unknown glycan. This data furthers our knowledge of this poorly understood organism and genus that is an important part of the oral and human microbiome.

Keywords: Oral microbiology, *Tannerella*, Surface Layer, Anaerobe, Periodontitis, Glycobiology

Introduction

The oral cavity in humans is home to an average of 250 different species per person, with over 700 identified in total, with only around one-third culturable (Thompson et al. 2015). It is also well established that the relationship between the human host and this bacterial community is key to the homeostasis of health (Curtis et al. 2011; Darveau 2010; Hajishengallis et al. 2012; Ebersole et al. 2016). In the case of the gum diseases gingivitis and periodontitis, alterations in both the host immune response, the subgingival environment and hence the composition of the oral microbial community in this niche are altered in disease. In the case of periodontitis a dysbiotic community has been characterised by culture based approaches, DNA-hybridisation, and next generation sequencing of 16S rRNA genes, highlighting a group of species associated with disease that was termed the 'red-complex' which included the keystone pathogen *Porphyromonas gingivalis*, the spirochaete *Treponema denticola* and the less well studied *Tannerella forsythia* (Ximénez-Fyvie et al. 2000; Socransky et al. 1998; Griffen et al. 2012), alongside several other species whose numbers are now known to be raised in periodontitis (Kumar et al. 2006; Griffen et al. 2012).

The advent of next generation sequencing has resulted in a greater understanding of the oral microbiome in health and disease (Paster et al. 2001; Griffen et al. 2012). In addition to the association of certain species with disease, these in-depth studies of the oral microbiota have highlighted a number of species associated with healthy periodontium. Of note is the previously uncultured species *Tannerella* HOT-286 (phylotype BU063, or *T.* HOT-286, as it will be known as in this paper), a member of the phylum *Bacteroides* which is currently classified as a member of the *Tannerella* genus (de Lillo et al. 2004; Paster et al. 2001). Recently, *T*. HOT-286 was isolated, cultured, and part-characterised for the first time (Vartoukian et al. 2016) and shown to require the presence of a helper strain to grow on solid media. Here, we present further characterisation of *T.* HOT-286, revealing that N-acetyl muramic acid can partially substitute the need for a feeder strain, while we also present morphological studies highlighting the presence of a glycosylated S-layer, an intact type IX secretion genetic locus and a potentially novel glycosylation island in the genome.

Materials and Methods

Bacterial Strains and Culture Conditions

Tannnerella HOT-286 (phylotype BU063, a gift from William Wade, Kings College, London, UK), was grown on solid media alongside or in the absence of *Propionibacterium acnes* ATCC 6919 on Fastidious Anaerobe agar (FA, Lab M, UK), with or without addition of 10 µg/ml N-acetyl muramic acid (NAM, Sigma Aldrich, USA), and 5 % (v/v) oxalated horse blood (Oxoid, USA). *Tannerella forsythia* ATCC 43037 was grown on FA-NAM agar. Antibiotic sensitivity to gentamicin was tested on FA-NAM-blood agar with Test strips from Liofilchem Diagnostici.

Sialidase Assays

The sialic acid conjugate methylumbelliferyl-N-acetyl neuraminic acid (MUNANA) was used to assess whole cell sialidase activity. Reactions were performed in clear, flat-bottomed 96-well plates (Greiner) containing 100 µM MUNANA, phosphate buffer saline (PBS, 137 mM

NaCl, 2.7 mM KCl and 10 mM sodium phosphate, Sigma Aldrich). Live bacteria were at an OD_{600} of 0.01 (*T. forsythia*) or 0.05 (*T.* HOT-286). Reactions were quenched with 100mM sodium carbonate buffer (pH 10.5) and fluorescence emission measured at 450 nm following excitation at 350 nm using a Tecan Infinite M200 microplate reader (Tecan), or observed using a UV transilluminator-GBOX with Genesys software (Syngene).

Fluorescent Staining and Imaging of *T.* HOT-286

T. HOT-286 was cultured as described for 5 days. Bacteria were scraped from the plates, resuspended in PBS, and washed twice by centrifugation at 12000 xg for 3 minutes, followed by resuspension in PBS to an OD_{600} of 1.0 before pelleting and resuspension in 200 µl of 1 x FilmTracer ™ FM 1-43 green to stain cell membranes. Stained bacteria were then fixed using 2 % paraformaldehyde for 10 minutes at 37 °C before washing twice in PBS with 50 µl of this suspension placed on a glass coverslip. Mounting media with DAPI (ProLong Antifade Gold, Life Technologies) was applied before visualisation using an Axiovert 200 fluorescence microscope (Zeiss) and AxioVision image analysis software (version 4.6; Zeiss) under 400 X magnification using blue (DAPI) and green (FilmTracer FM-143) channels. Fiji image analysis software (Schindelin et al. 2012) was used to further process images.

Electron Microscopy

Samples for analysis by TEM were taken from 5-day agar cultures of *T.* HOT-286, resuspended in PBS, washed twice by centrifugation at 10000 xg for 2 minutes and resuspended in PBS. Bacteria were centrifuged as above, and pellets fixed in 3 % glutaraldehyde, 0.1 M sodium cacodylate for 18 hours at 4 °C, washed twice with 0.1 M sodium cacodylate, and post fixed in 2 % osmium tetroxide for 1 hour. Fixed bacterial pellets were rinsed with deionised water followed by sodium cacodylate buffer, and dehydrated using graded ethanol washes (15 minutes per wash at 75 %, 95 %, 100 %, and 100 % over anhydrous $CuSO_4$). Samples were cleared in epoxypropane (EPP) and incubated in 50/50 araldite resin:EPP mixture overnight. This mixture was replaced with fresh araldite resin mixture twice over 8 hours, before being embedded and cured in a 60 °C oven for 48-72 hours. Ultrathin sections ~85nm thick, were cut on a Leica UC 6 ultramicrotome onto 200 mesh copper grids, stained for 30 mins with saturated aqueous uranyl acetate followed by Reynold's lead citrate for 5 mins. Sections were examined using a FEI Tecnai Transmission Electron Microscope at an accelerating voltage of 80 Kv. Electron micrographs were recorded using a Gatan Orius 1000 digital camera and Gatan Digital Micrograph software.

SDS-PAGE and (Glycoprotein) Staining

Crude whole cell lysates of *T. forsythia* and *T.* HOT-286 were prepared from 5 day agar cultures by resuspension in PBS and heating at 95 °C for 5 minutes. Following this, protein concentration of lysates was established using a bicinchoninic acid (BCA) assay kit (Thermo Fisher Scientific). 25 µg of each lysate was mixed with SDS-loading buffer and on an SDS-PAGE gel. Proteins were visualised using InstantBlue (Expedeon) or Pro-Q Emerald 300 glycoprotein gel stain kit (Invitrogen) as indicated.

Mass Spectrometry for Protein Identification

SDS-PAGE gels were rinsed with deionised water and bands of interest excised and placed in low-bind microfuge tubes (Fisher Scientific). Gel bands were equilibrated by three cycles of immersion in 200 µl of 200 mM ammonium bicarbonate in 40 % (v/v) acetonitrile (37 °C for 30 minutes), centrifuged (13000 xg) and supernatant discarded. Gel pieces were then submerged in 100 % (v/v) acetonitrile and incubated for 30 minutes at 37 °C, centrifuged at 13000 xg and supernatant discarded. The gel pieces were then dried in a Vacuo Concentrator Plus (Eppendorf) before reduction in 200 µl of 50mM ammonium bicarbonate plus 10 mM dithiothreitol (DTT) at 55 °C for 60 minutes, centrifuged and supernatant discarded. Gel pieces were than alkylated with 50 mM ammonium bicarbonate plus 55 mM iodoacetamide (20 minutes, 25 °C, in dark) before washing 3x in ammonium bicarbonate buffer, and resuspension in ammonium-acetonitrile (50 %) (37 °C, 15 min), centrifuged and dried in a speedvac. In-gel trypsinisation (100 µl) was performed in ammonium bicarbonate (10 % acetonitrile) at 37 °C for 16-20 hours, centrifuged at 13000 xg and the solution collected and pooled with supernatant from a further treatment of the gel slice with 100 % acetonitrile plus 5 % formic acid (37 °C, 10 min). Next a further round of formic acid, acetonitrile elution was performed as above and all supernatants pooled before concentration via speedVac and resuspension in 30 µl of 100 % acetonitrile. Finally, 5 µl of trypsinised protein was subjected to MS/MS using a maXis ultra-high resolution time of flight mass spectrometer (Bruker). Peptide fragment data was collected and analysed by searches against MASCOT and NCBI databases alongside the *T.* HOT-286 genome sequence (NZ_CP017038.1).

Bioinformatics

Amino acid sequence alignments were performed using Multalin (Corpet 1988) and annotated with Adobe Illustrator. ScanProsite (https://prosite.expasy.org/scanprosite/) was used to detect putative glycosylation sites (D-[ST]-[AILMTV]) and SnapGene Viewer used to generate the glycosylation island. BLAST searches were performed using BlastP (https://blast.ncbi.nlm.nih.gov/Blast.cgi) against the *T.* HOT-286 genome (NZ_CP017038.1).

Results

Culture of *T.* HOT-286 is Possible in the Presence of N-acetyl muramic acid

Tannerella HOT-286 was originally isolated and grown in co-culture with *Propionibacterium acnes*, via an unknown secreted factor which seems to cross-feed *T*. HOT-286 on agar (Vartoukian et al. 2016). However, given that the closest cultured relative of *T*. HOT-286 is *Tannerella forsythia*, which itself was isolated and grown in co-culture with *Fusobacterium nucleatum* (Tanner et al. 1986) until its requirement for exogenous NAM was discovered (Wyss 1989), it followed that growth of *T.* HOT-286 might also be boosted by NAM. Therefore, *T*. HOT-286 was grown on FA-NAM (on which our lab routinely grows *T. forsythia*), in the presence or absence of *P. acnes*. Cultures were grown for 5-7 days before repeated subculture to fresh FA-NAM. Monocultures of *T.* HOT-286 were established and passaged for more than five serial subcultures, forming grey-beige colonies, slightly smaller than those grown in the presence of *P. acnes*. The presence of *P. acnes* resulted in colonies of more uniform size and appearance (~1-1.5 mm), while in its absence *T*. HOT-286 colonies appeared to vary in size (0.5-1 mm, figure 1A). We have been unable to achieve growth in liquid broth media to date or on agar in the absence of NAM. In addition we also observe that in contrast to the gentamicin-resistant *T. forsythia, T*. HOT-286 is susceptible to

gentamicin with an estimated MIC of 32-48 μg/ml, a fact that hindered our initial studies given we routinely add gentamicin to long term *T. forsythia* cultures to supress contamination (Fig S1A).

T. HOT-286 Forms Chains/Filaments of Individual Organisms with Visible Cell-Cell Boundaries, as Revealed by Fluorescence Microscopy

We then examined the gross cellular morphology of the *T*. HOT-286 from co- and mono-culture conditions by Gram staining (figure 1A, right), regardless of the presence or absence of *P. acnes*, with cells seen as long filamentous cells of varying lengths (20-40 μm) and 1 μm across.

To more clearly observe the cellular morphology of *T*. HOT-286 grown under both conditions, a fluorescent stain for bacterial membranes (FilmTracer ™ FM 1-43 green) and a stain for nucleic acid (DAPI, blue) were used to enable imaging by fluorescence microscopy. This approach highlighted variable morphology between individual organisms in both conditions. Notably, individual organisms vary in length (5-10 μm) and in many cases form filamentous structures with cells connected at their poles, apparently separated by distinctly visible membranes or septa (figure 1B). Nucleic acid appeared to be distributed in a variety of different positions- either distributed throughout the cell or localised centrally in the organism (figure 1B). Similar images were obtained for *T*. HOT-286 grown under both conditions and there is no indication at this stage of any consistent discernible difference in morphology in our experiments.

T. HOT-286 is Sialidase Negative

A notable feature of the so-called red complex periodontal pathogens and indeed many oral dwelling bacteria-such as *Streptococcus oralis*-is the expression of sialidases, enabling cleavage of sialic acid from host glycoproteins, and this has a number of implications for virulence (Aruni et al. 2011; Stafford et al. 2012; Kurniyati et al. 2013). Sialidase activity appears to be particularly important for *T. forsythia*, where mutant strains deficient in the NanH sialidase display decreased attachment and invasion of host cells and reduced biofilm formation on host glycoproteins (Honma et al. 2011; Roy et al. 2011). Given this, we tested *T.* HOT-286 for sialidase activity using the fluorogenic sialic acid-substrate MUNANA, and found *T.* HOT-286 to be sialidase negative under the same conditions as tested for *T. forsythia*, conditions which we routinely use in the lab (supplementary figure S1B) (Frey et al. 2018).

T. HOT-286 Appears to Possess an Extracellular Glycosylated S-layer

T. HOT-286 is currently classed as a member of the genus *Tannerella*, with *T. forsythia* being the only other member of this genus that can be cultured. *T. forsythia* notably expresses a unique glycosylated S-layer (Posch, Sekot, et al. 2012). Therefore, we examined the protein profile of *T.* HOT-286 in comparison with the *T. forsythia t*ype strain (ATCC 43037) using SDS-PAGE on crude lysates. It was observed that similar to the *T. forsythia* 43037 strain, *T.* HOT-286 possesses two highly expressed high molecular weight (Mw) proteins at ~200-250 KDa (figure 2), which although smaller than those seen in *T. forsythia* may represent the S-layer proteins. Therefore, we also considered it likely that the high molecular weight proteins observed in *T.* HOT 286 might also be S-layer proteins. Assessment of the *T.* HOT-286 protein profile using glycoprotein staining was also carried

out on both co- and mono-cultured *T.* HOT-286, and showed that while there are several potential differences in the protein profile under the differing culture conditions (notably at approx. 125kDa), the two high M_w proteins appear still to be present and glycosylated under both conditions (figure 2, right). To investigate the identity of these proteins the bands (labelled with asterisks in the *T.* HOT-286 gel (figure 2) were excised, processed, and analysed by mass spectrometry followed by MASCOT searches against the NCBI databases and the predicted proteome of *T.* HOT-286 (NZ_CP017038.1) and revealed that the MS/MS tryptic profile of the higher M_w protein matched the BCB_RS0675 (TfsB) protein with 20 % coverage. Similarly the lower band identified a match with the BCB_RS0680 (TfsA) protein with 25 % coverage over the whole protein sequence (figure, S2). Reassuringly these proteins are encoded by genes that are adjacent on the chromosome of *T.* HOT-286, as would be expected for co-transcribed S-layer genes and as is the case for *T. forsythia* 43037. In addition, as would be predicted for glycosylated proteins they migrate abberantly in SDS-PAGE compared to their predicted Masses of 134 kDa (TfsA) and 155 kDa (TfsB).

Bioinformatics Reveals Putative Glycosylation Sites, an Intact T9SS and a Potentially Novel Glycan Addition Island in *T.* HOT-286

In order to interrogate the S-layer proteins more closely and also to consider whether *T.* HOT-286 has the predicted genetic capacity for glycosylation of its S-layer proteins a preliminary bioinformatics study was performed.

Firstly, upon closer inspection of the S-layer sequences, the two S-layer protein encoding genes share 52 % and 59 % aa identity to the *TfsAB* genes of *T. forsythia* ATCC 43037. Alignment of the two sets of protein sequences reveals higher conservation in the C-terminal region, where significantly, the *T.* HOT-286 sequence contains archetypal type IX (C-terminal, CTD) secretion domain motifs (figure 3A) and a predicted cleavage site. These CTD features are also shared with many other proteins in *T.* HOT-286, indicating that the T9SS capability may also be shared between *T. forsythia* and *T.* HOT-286. This notion is further strengthened by the finding that homologues of the core T9SS genes (Lasica et al. 2017) are present in the *T.* HOT-286 genome, namely sov (BCB71_RS09030), the *PorKLMN* locus (BCB71_RS04065-80), and *PorT* (BCB71_RS01770), among others such as *gldH* (BCB71_RS01705).

In addition, the S-layer proteins of *T. forsythia* 43037 are glycosylated at specific D(S/T)(A/I/L/M/T/V) motifs within the S-layer proteins (Posch et al. 2011). Of note, the *tfsA* and *tfsB* genes of *T*. HOT-286 also contain a number of these motifs (12 and 21 in each, respectively, PROSITE search results, supplementary figure. S2), while notably there is a lack of tryptic peptides covering any of these glycosylation motifs in the MS/MS experiments-probably due to the presence of an unknown post-translational modification that is not in the MS databases. Furthermore, the elegant work of the Schaeffer group (Posch, Pabst, et al. 2012; Posch, Sekot, et al. 2012; Posch et al. 2011) showed that in *T. forsythia* 43037, these motifs are O-glycosylated with a large branched glycan that is terminated in either a pseudaminic or legionaminic acid sugar residue, and that the ability to place this glycan is encoded for by specific gene clusters for each sugar (pse or leg) (Posch et al. 2011). However, examination of the *T.* HOT-286 genome reveals that while a large putative glycosylation island exists that spans the loci BCB71_RS10385 to _RS10440 and contains homologues to *wecC* (UDP-N-acetyl-D-mannosamine dehydrogenase), *wecB* (UDP-N-acetylglucosamine 2-epimerase) and a putative flippase, it does not contain genes that might

encode a putative *leg* or *pse* transferase or synthase system (figure. 3B). Rather, a number of putative glycosyl and methyl transferases (GTase and MTases) are present, some of which resemble fucosyl transferases (FucT).

Unsurprisingly, and given the import of glycans and carbohydrates to the *Bacteroidetes* in terms of other colonisation and other factors, *T.* HOT-286 seems to contain a large number of predicted TonB-dependent receptors (>40), and a range of glycosyl hydrolases, transferases and esterases according to the CaZy database (Lombard et al. 2014), but notably does not seem to target sialic acid in the same manner as *T. forsythia*, as it does not possess a classical Nan cluster (Stafford et al. 2012).

Visualisation of *T.* HOT-286 by TEM Reveals Cell Wall Structure indicative of an outer S-Layer and Possible Intracellular Structures

In order to further probe the ultrastructure of *T.* HOT-286 and also to examine if a potential S-layer might exist on the surface of *T.* HOT-286, as it does in *T. forsythia*, we performed TEM on ultra-thin sections of *T.* HOT-286. Processing of *T*. HOT 286 samples included sectioning and resulted in several different views of the organism (i.e. transverse and longitudinal cross-sections of *T.* HOT-286, figure 4) and was compared to previous images we had of *T. forsythia*.

Strikingly, the cell wall of *T.* HOT-286 has a distinct triple-layered cell wall spanning approximately 50 nm (figure 4). On the surface of the cell sits what we assume, based on experience of *T. forsythia,* is a glycoprotein composed S-layer, that we propose is made up of the putative Tfs proteins. In comparison to the more zig-zagged, tooth-like structure of the *T. forsythia* S-layer, the *T.* HOT-286 surface layer appears to resemble more of a striated 'blanket-stitch' like composition, with a regular repeating pattern reminiscent of *T. forsythia* 43037 and suggestive of a regular lattice (figure. 4 left and centre).

In addition to the visible S-layer, these images reveal some interesting features of *T.* HOT-286. Firstly, that given its filamentous nature it is of note that neither the S-layer or the outer membrane seem to intersect the cellular joints between these cells- i.e. are continuous (figure. 3, bottom). In addition, there appear to be several intracellular structures in these images, for example there appears to be evidence of a structure resembling a potential spore (figure 4 right, ES), while there also seems to be evidence of possible intracellular membranous structures (striations- IS), while finally other cells seem to contain potential storage granules (figure 4, right).

Discussion

In this paper we report further characterisation of the novel health-associated strain *Tannerella* HOT-286 and uncover for the first time that-like its closest known relative *T. forsythia*-growth is boosted by the presence of N-acetyl muramic acid in the growth media. In the case of *T. forsythia* this is due to a lack of NAM synthesis genes *murA* and *murB*, therefore this finding was surprising since *T*. HOT-286 contains both *murA* and *murB* homologues (BCVB1_RS025110 and _08115, respectively), implying the ability to synthesise its own NAM. However, we have no information on levels of expression of the *murA* or *B* genes in *T.* HOT-286 nor whether other metabolic defects exist in this organism that are compensated for by either NAM or the presence of a cross-feeding strain. Of note it appears that *T.* HOT-286 does possess genes to allow uptake of NAM from the

environment, as it has an intact *murQTK* gene cluster (BCVB1_RS0926950-60), giving it the genetic potential to uptake NAM from its environment. In addition, we assume these genes are expressed given the ability of NAM to boost *T.* HOT-286 growth on agar plates. However, in contrast to *T. forsythia* it does not appear that *T.* HOT-286 has the ability to use sialic acid as a carbon source, as it both lacks sialidase activity but also the entire nan operon- the latter fact noted in the single cell incomplete genomes published previously (Beall et al. 2014). In addition, in contrast to *T. forsythia*, which we routinely grow in the presence of gentamicin during long growth experiments to suppress contamination, *T.* HOT-286 has an MIC for gentamicin of 32-48 μg/ml. Overall, these findings may facilitate improved understanding of this strain and open the way for pure culture and genetic studies.

Upon basic analysis of the *T.* HOT-286 proteome via SDS-PAGE we revealed that this strain possesses two highly expressed high molecular weight proteins, reminiscent of *T. forsythia*, which upon MS/MS analysis were revealed to be the predicted TfsA and TfsB, putative S-layer proteins. These two proteins also appear to be glycosylated and migrate in SDS-PAGE at a much higher level than the predicted masses with amino sequences that contain predicted glycosylation motifs, suggestive of highly glycosylated proteins. However, the nature of this glycan, which we assume, based on knowledge of *T. forsythia* might be a highly branched complex glycan, is currently unknown. While not visible in the whole cell SDS-PAGE samples analysed here (i.e. not enriched for outer membrane proteins), one might also expect this glycan to be present on a range of other proteins- as is the case in *T. forsythia* and other bacteria and certainly there are a number of proteins containing the D(S/T)(A/I/L/M/T/V) motifs in the predicted proteome. Notably though, unlike *T. forsythia* the *T.* HOT-286 strain does not contain putative genes for legionaminic or pseudaminic acid synthesis or transfer, rather it contains a putative glycosylation island that suggests the presence of fucose, but for which we currently have no biochemical evidence. As mentioned above, this organism also contains a large amount of putative carbohydrate acquisition TonB dependent transporters, but also a range of putative carbohydrate active enzyme genes, with the presence of two putative fucosidases (BCBV71_RS01570 and 08535) suggestive of fucose scavenging as part of its lifestyle. One might speculate that this altered glycan structure is related to the non-pathogenic lifestyle of *T.* HOT-286 in terms of the type of immune response it might elicit since one postulated role for the *T. forsythia* S-layer glycan is modulation/ dampening of host immune responses (Settem et al. 2013; Sekot et al. 2011) but this is open to future confirmation. We also highlight the presence of a core Type IX secretion system (T9SS) in this organism, first discovered in relation to gliding motility in *Flavobacterium* spp. but most notably employed by the keystone periodontal organism *Porphyromonas gingivalis* for secretion and surface attachment of virulence associated proteases known as gingipains (Lasica *et al.,* 2017). In *T. forsythia* the T9SS is essential for S-layer assembly and virulence factor deposition on the surface (e.g. BspA) iand we expect the same to apply got T-HOT 286, thus supporting further the presence of this system in *Tannerella* and other oral *Bacteroidetes* spp, potentially representing a unique feature of this group of organisms.

Finally we reveal several novel features of the morphology of T. *HOT-286* strain in terms of how its' S-layer surface structure relate to its' filamentous structure- i.e. the S-layer seems to form a continuous layer or sheath around the cellular filaments (Fig. 4). This structure and the presence of potential internal granules/ compartments are reminiscent of environmental sulphur reducing Gram-negative filamentous *Triothrix* spp. (Williams et al., 1987) or may

potentially be spore-like structures. In addition we note several striations that may represent internal membranous structures, such as those used by type I methanotrophs in metabolic processes (Choi et al., 2003). However, at present we have no data relating to the function or nature of these features, but it is clear they differentiate T. HOT-286 from its' close relative *T. forsythia.*

Overall, despite these differences in cell morphology, gentamicin sensitivity and metabolic capability, both strains have a glycosylated S-layer that seems to be suggestive of a novel *Tannerella* specific trait that it is not displayed by related *Bacteroidetes* such as *Porphyromonas*, *Prevotella*, or *Bacteroides* spp.

In summary we have furthered knowledge of this important genus of bacterium, prominent not only in the oral microbiome but also identified as part of the gut microbiome (Song et al. 2017; Ardissone et al. 2014; Renouf & Hendrich 2011) and expanded our overall knowledge of human-dwelling bacterial species.

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Conflicts of Interest

We have no conflicts of interest to declare.

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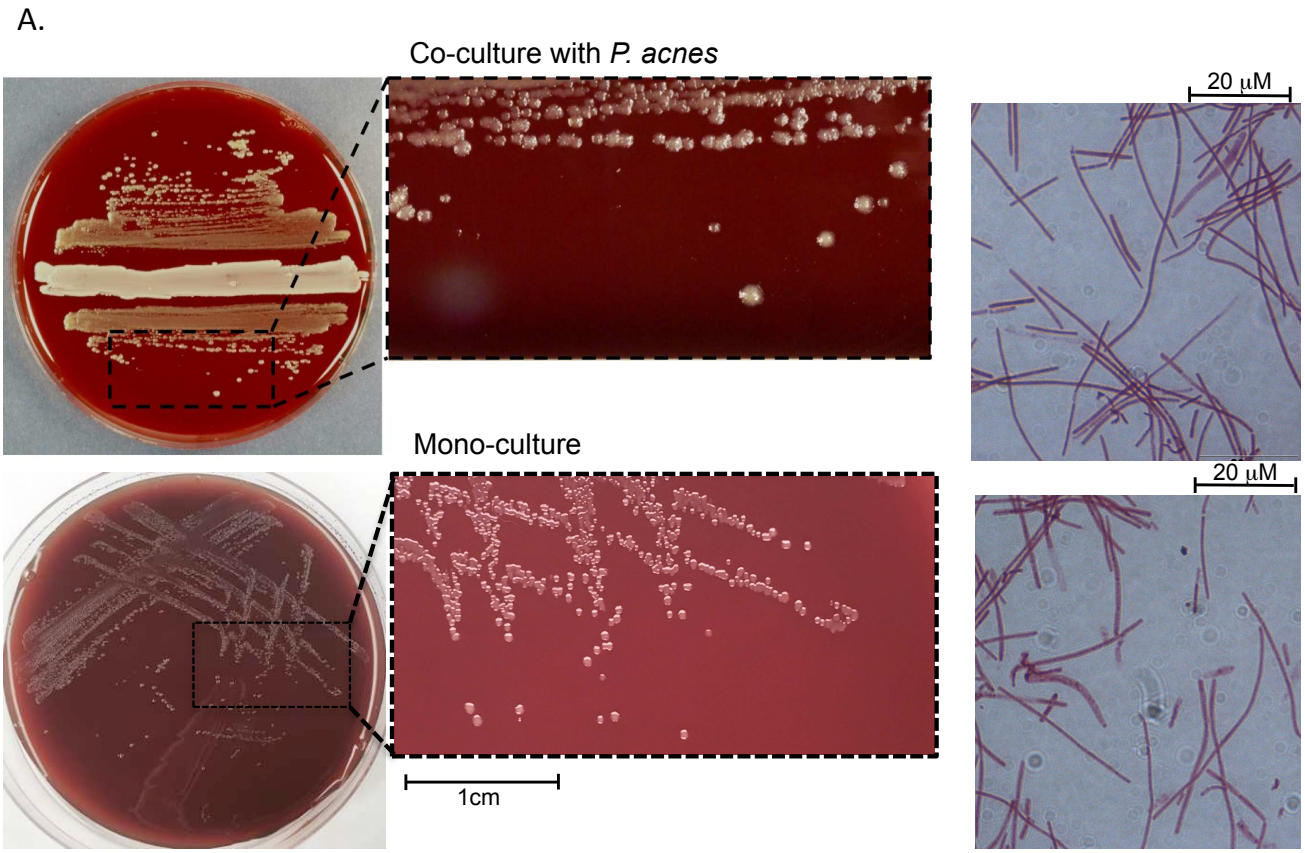
Figure 1


A.
Co-culture with *P. acnes*
20 μM
Mono-culture
20 μM
1cm

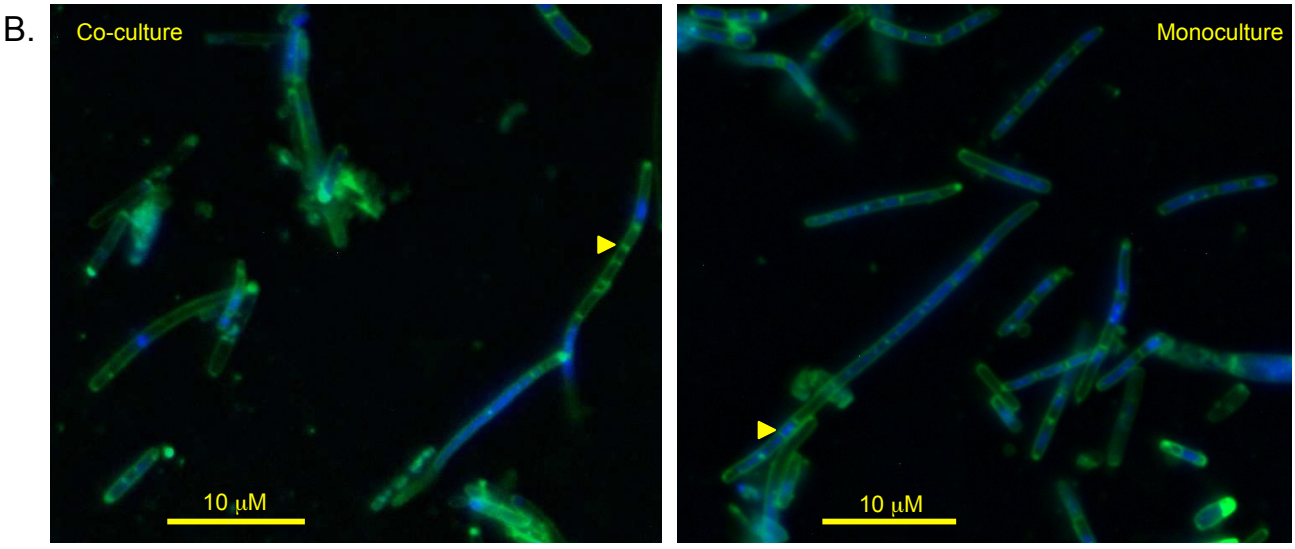

B.
Co-culture
Monoculture
10 μM
10 μM

Figure 2.

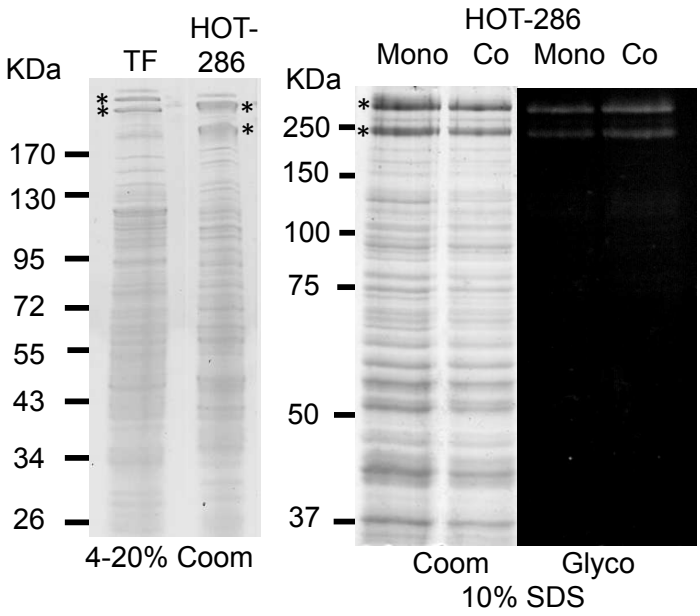

KDa
TF
HOT-286
170
130
95
72
55
43
34
26
4-20% Coom
HOT-286
Mono
Co
Mono
Co
KDa
250
150
100
75
50
37
Coom
Glyco
10% SDS

Figure 3.

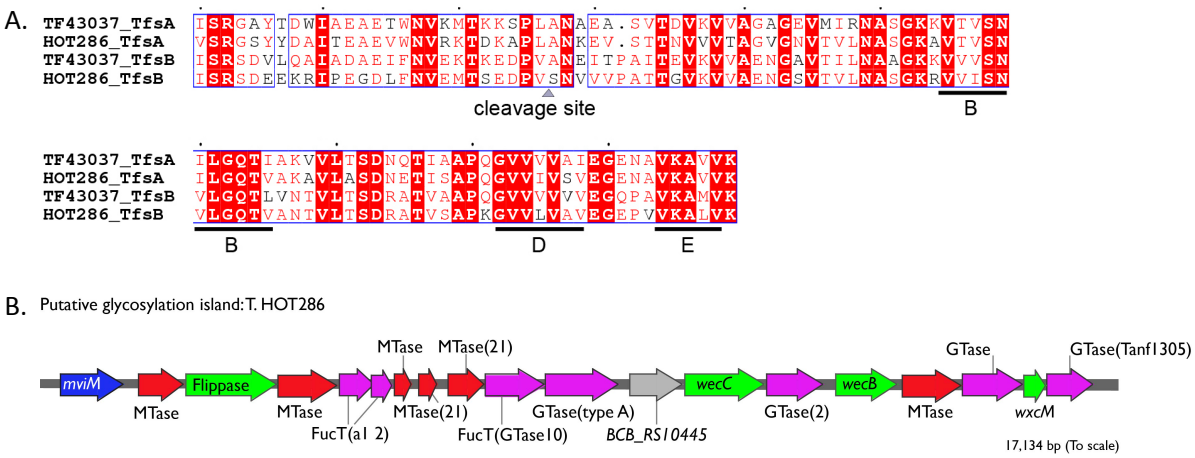

A.
TF43037_TfsA
HOT286_TfsA
TF43037_TfsB
HOT286_TfsB
cleavage site
B
B
D
E
B. Putative glycosylation island:T. HOT286
mviM
MTase
Flippase
MTase
FucT(a1 2)
MTase
MTase(21)
MTase(21)
FucT(GTase10)
GTase(type A)
BCB_RS10445
wecC
GTase(2)
wecB
MTase
GTase
wxcM
GTase(Tanf1305)
17,134 bp (To scale)

Figure 4.

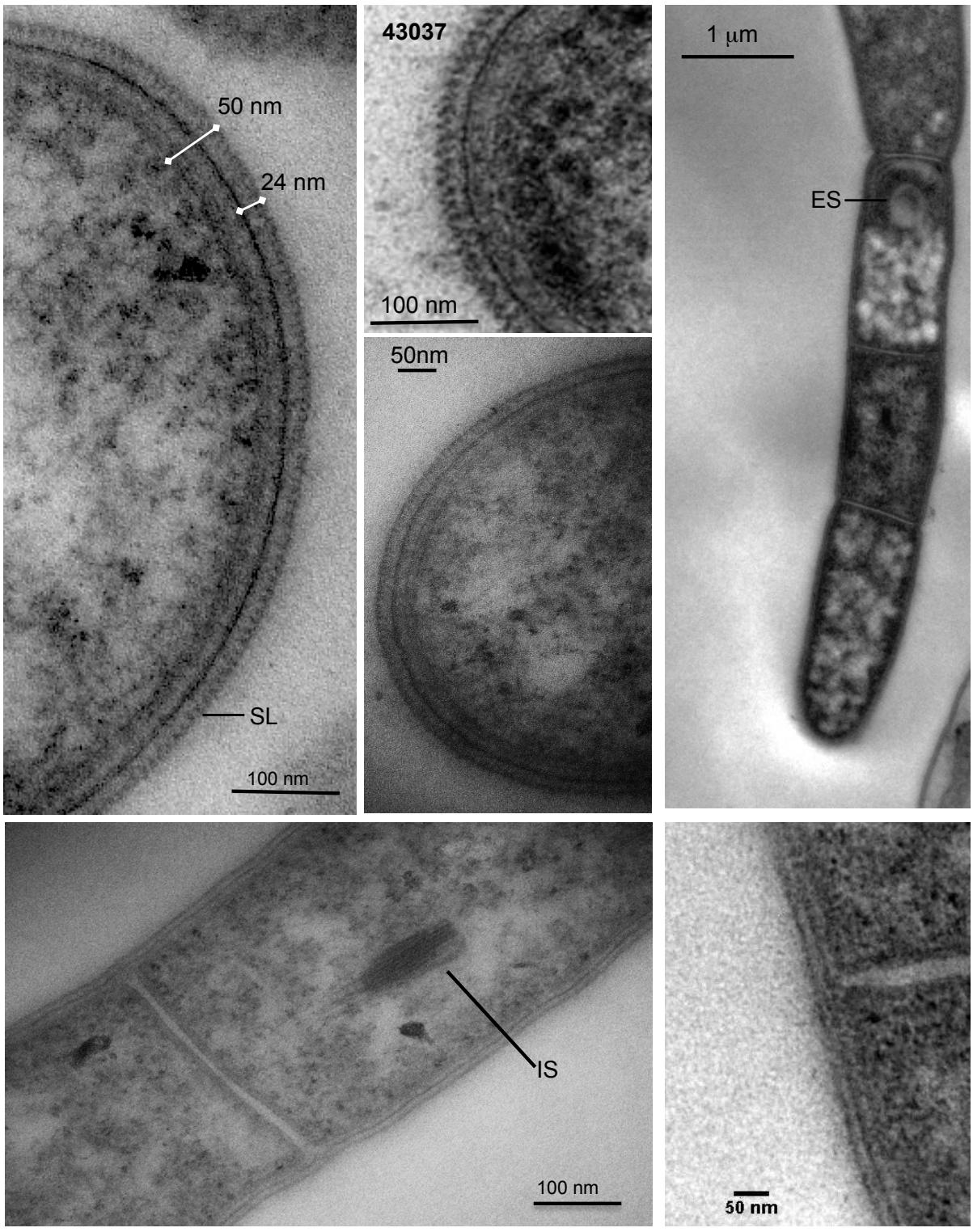
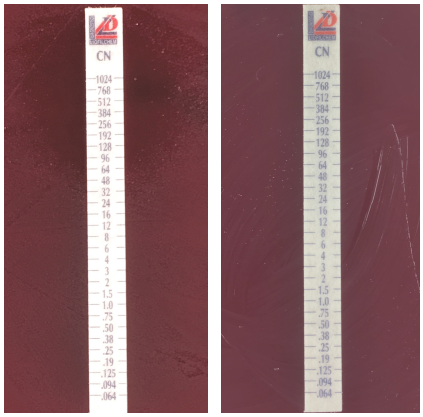


Figure S1.

A. *T.* HOT-286 *T. forsythia*



B.

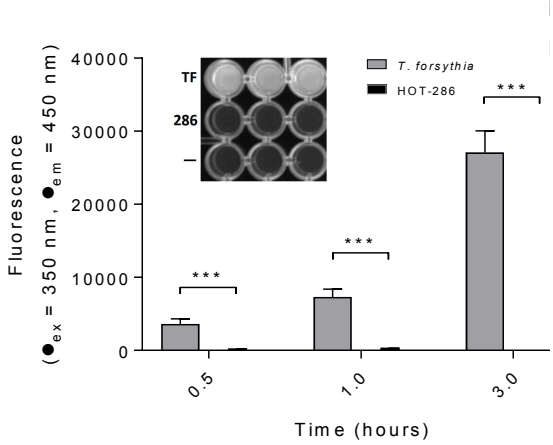


Figure S2.

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PANTRIHFNINNCVVYDTDRTTVPSNLYTVRDREGRYLVVPMYAGDLTPQWMRLEDIENANRTPSDQWFV
TKVNDGSGISKIHLTNREFNNIRIEFVQVYNDYHLFKGVWHRIDENGLGRDYSPIMGRNYVNLAGFTVVP
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>HOT286_TfsB
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