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[P03-389] Purification and characterization of the sheath-forming protein secreted by the filamentous bacterium *Haliscomenobacter hydrossis*

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Purpose: *Haliscomenobacter hydrossis* is a filamentous bacterium composed of connected cylindrical cells covered by a microtube-like sheath. The overabundance of *Haliscomenobacter* strains in activated sludge causes poor settling and filamentous bulking. The increase in porosity (water content) owing to the mesh structure formed by bacterial filaments may contribute to bulking. This study aimed to investigate whether the properties of the filament surface (sheath) also contribute to bulking.
Methods: *H. hydrossis* cultured on glucose and tuna extract was used for sheath preparation. The cells were selectively lysed via lysozyme and detergent (sodium dodecyl sulfate [SDS]) treatments to obtain the sheath. The sheath was purified via electroelution in the presence of SDS, followed by washing with water. The purified sheath was digested with trypsin, and the digest was subjected to amino acid sequence analysis after separation via high-performance liquid chromatography. To identify the gene encoding the sheath-forming protein, the genomic data were analyzed based on the determined partial amino acid sequences.
Results and Discussion: Glucose and tuna extract were suitable carbon and nitrogen sources, respectively, to cultivate *H. hydrossis*. The microtube structure of the purified sheath was confirmed via scanning probe microscopy, revealing its tolerance to lysozyme and SDS. The sheath was also stable in the presence of 2-mercaptoethanol, dithiothreitol, urea, guanidine, lithium bromide, ethylenediaminetetraacetic acid, ascorbic acid, and oxalic acid. Notably, the sheath was solubilized using 1-butyl-3-methylimidazolium chloride (an ionic liquid). Various amino acids were detected in the sheath, and the sheath was susceptible to proteinases, including trypsin. These findings indicated that the sheath was protein-based, unlike other polysaccharide-based bacterial sheaths. The N-terminal and internal amino acid sequences were successfully determined from the purified sheath and its trypsin digest. These sequences were found in four previously unidentified genes in the genome. All genes contained putative signal peptides for inner membrane transport and a putative T9SS motif for outer membrane transport in the N- and C-terminal regions, respectively. They encoded proteins approximately 140 kDa in size. Filamentous structures rich in the beta-sheet were commonly predicted by AlphaFold 3, suggesting that at least one of the identified genes encodes the sheath-forming protein. Unlike those in common fibrous proteins, no regular amino acid sequence repeats were observed in the potential sheath-forming proteins. Interestingly, precipitation of the purified sheath via centrifugation was difficult, suggesting that dispersibility imparted by the sheath is a potential cause of bulking.
Conclusions: Our findings suggest that *H. hydrossis* prefers tuna extract as a nitrogen source. Its sheath exhibits low sedimentation, which possibly exacerbates bulking, and is composed of a protein degradable by proteinases and soluble in ionic liquids. It remains stable in the presence of detergents, chaotropic reagents, and chelating reagents. Moreover, the identified

sheath-forming protein is a potential new type of bacterial-origin fibrous protein (natural fiber).