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Characterisation of small intestinal *Streptococcus* and *Veillonella* populations

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Introduction: The human gastrointestinal tract is rich in nutrients from ingested food, which are used as energy source by the host and the resident microbiota. The small intestine is the first region where food components come in contact with the intestinal bacteria. Therefore, the small intestinal microbiota is expected to have an important influence on initial diet digestion and host physiology. However, little is known about the small intestinal microbiota, which is mainly due to sampling difficulties. Nonetheless, small intestinal samples can be collected from ileostomy subjects, which are individuals that had their colon surgically removed. At the end of surgery, the terminal ileum is connected to an abdominal stoma, which offers an opportunity for sampling of ileal contents. High throughput 16S ribosomal RNA profiling of stoma effluent revealed a bacterial community enriched in *Streptococcus* spp. and *Veillonella* spp. This study focuses on in-depth characterization of these bacterial populations and assessment of their diversity as well as aims to elucidate their functional properties and interactions.

Methods: Fresh ileostomy effluent was collected and maintained at 4C under anaerobic conditions. Ileostomy effluent was plated on Mitis Salivarius (MS) agar and Veillonella Selective Agar (VSA) for selective isolation of *Streptococcus* spp. and *Veillonella* spp., respectively. Following incubation at 37C, colonies from each plate were randomly picked and separately stored. Identification of the isolates employed 16S rRNA gene sequencing and subsequent classification. The 16S rRNA gene sequences were grouped into phylotypes based on a threshold of 98% sequence identity to assess the diversity among the bacterial isolates. The diversity at the strain level was assessed by employing amplified fragment length polymorphism (AFLP) analysis.

Results: A total of 120 isolates were obtained from MS agar of which 92 were identified as *Streptococcus*, while the remaining isolates were classified as *Enterococcus*. Out of 42 isolates obtained from VSA, 23 were assigned to the genus *Veillonella*. The remaining VSA isolates were identified as members of the genera *Enterococcus* (13), *Proteus* (4), and *Escherichia* (2). Grouping of the 16S rRNA gene sequences into phylotypes based on a threshold of 98% sequence identity revealed that for *Streptococcus* as well as *Enterococcus* 3 phylotypes could be identified. Isolates belonging to *Veillonella*, *Proteus*, and *Escherichia* were represented by a single phylotype. Furthermore, AFLP analysis demonstrated that for *Streptococcus* and *Enterococcus* 6 and 5 genomic lineages, respectively, could be distinguished.

Conclusion: *Streptococcus*, *Veillonella*, as well as *Enterococcus* isolates were successfully obtained. Phylogenetic analysis of the isolates revealed multiple phylotypes for *Streptococcus* and *Enterococcus* that could be further divided into multiple genomic lineages, which demonstrates the high diversity of the small intestinal microbiota. The genome sequences of representative isolates are currently determined and will be mined to elucidate the functional properties of these isolates as well as their potential microbial interactions that shape the small intestinal microbiota, with a special focus on *Streptococcus* spp. and *Veillonella* spp.