Development of anaerobic fermentation techniques for measuring the impact of prebiotic supplementation on human gut microbiota in clinical studies

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Introduction: The ability of prebiotic dietary carbohydrates to influence the composition and metabolism of the gut microbiota is central to defining their impact in diverse individuals and populations. Unfortunately, most methods currently used to assess microbial metabolic products in the context of clinical trials are indirect and limited. This study aimed to develop an ex vivo anaerobic fermentation method to overcome this gap and thereby increase the information from prebiotic supplementation trials.

Methods: Stool samples from six volunteers were analyzed to detect differences in microbiome and metabolome based on comparison of different methods: sample collection vial (standard vial and two different BD anaerobic collection vials); storage (-80℃ or refrigerated); duration of fermentation (0, 4, 8, 16, 32, 48hr); and carbon source for growth (2'-fucosyllactose, glucose or galacto-oligosaccharides). All samples were grown anaerobically, measuring optical density (OD) and pH throughout culture.

Results: Neither the collection vial, nor cryopreservation influenced composition. Fermentation reached its maximum growth and minimum pH by 16hr, which was used as the end point for metagenomics and metabolomics, while 8hr identified intermediate metabolites. Metagenomic analysis distinguished samples at 16hr by subject and then by prebiotic. However, from metabolomic analysis, subjects were the primary driver of differences at 0hr, whereas the prebiotic became the dominant influence by 16hr.

Discussion: We identified a feasible and valid approach for prebiotic fermentation analysis of individual samples in large clinical studies: Collection of stool microbiota using standard vials; cryopreservation prior to testing; and collecting fermentation read-out at 8 and 16hr. Our findings that microbial community composition is primarily affected by the host, whereas microbial metabolism is most affected by the prebiotic at 16hr, supports the value of fermentation analysis to test prebiotic impact.