Microbial reuterin production and its effects on a 3-D model of colonic epithelium

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The human gut symbiont *Lactobacillus reuteri* produces and secretes reuterin, as an intermediate in the reduction of glycerol to 1,3-propanediol. Reuterin formation could be a tool for *Lactobacillus reuteri* to outcompete other species, first because it's a strong antimicrobial agent against pathogens and the commensal gut bacteria but secondly, because it is an electron acceptor in the anaerobic environment of the gut. In our study on glycerol fermentation by human faecal microbiota we found that rapid glycerol fermenting communities exhibited shifts in their *Lactobacillus-Enterococcus* community (De Weirdt *et al.*, 2010). Based on *in vitro* ^{13}C-glycerol batch fermentations by human faecal microbiota, we suggested that within this community, glycerol-degrading lactobacilli were responsible for the rapid reduction of glycerol to 1,3-propanediol because these lactobacilli were most efficiently producing reuterin as an electron accepting intermediate. Reuterin is a very reactive molecule (the core molecule is an aldehyde) and can be dehydrated to acrolein, which is a highly reactive toxin that rapidly binds to the antioxidant glutathione and has a mutagenic potency comparable to formaldehyde. Therefore, it is desirable to assess the physiological effects of microbial reuterin formation on gut epithelial cells. Using confluent 2-D cultures of colon carcinoma Caco-2 cells, we found evidence for a potential reuterin effect on cell viability and metabolic activity. However, the effects were very variable and seemed to be connected with the physiological state of the cancer cells (e.g. differentiation status). Furthermore, 100 μM reuterin affected single colon carcinoma cells (HT-29 and HCT-116), but didn't affect their 3-D aggregates. We are currently investigating the reuterin effects more closely in a fully developed 3-D model of intestinal epithelium, which mimics the parental tissue *in vivo* (Barilla et al., 2010). In addition, the effect of reuterin production on infection by *Salmonella enterica* serovar Typhimurium is being examined. Overall, we hypothesized that *in situ* reuterin production by *Lactobacillus reuteri* will not affect the healthy gut epithelial tissue negatively, while actively protecting the cells against enteric infection.