UNRAVELLING THE LELOIR PATHWAY IN BIFIDOBACTERIUM BIFIDUM

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Bifidobacteria are important probiotic bacteria which are considered beneficial for the human health. These effects are due to the regulation of the intestinal microbial homeostasis and the inhibition of pathogens. Moreover, they are able to ferment various galacto-oligosaccharides (GOS) via the classic Leloir pathway and a unique metabolic pathway. The Leloir pathway, that enables *Bifidobacterium bifidum* to ferment lactose, is well described; the various enzymes and reactions involved in the metabolism are however not well studied.

Galactose-1-phosphate uridylyltransferase (GalT2 – EC 2.7.7.12) converts galactose-1-phosphate and UDP-glucose into glucose-1-phosphate and UDP-galactose. However, an alternative route has been reported that directly converts galactose-1-phosphate into UDP-galactose. This reaction, which uses UTP and releases pyrophosphate, is catalyzed by a promiscuous UTP-hexose-1-phosphate uridylyltransferase (GalT1 - EC 2.7.7.10). Thus far, hard evidence about which gene is responsible for this conversion was still lacking. The described route is absent in most bacteria and is similar to the more widespread UTPglucose- 1-phosphate uridylyltransferase (UgpA - EC 2.7.7.9). A reaction that is also present in *B. bifidum*.

In this study the various uridylyltransferases in *B. bifidum* have been annotated and characterized. In order to reduce possible interference of native enzymes from the expression host with the assay, these enzymes were overexpressed in an *E. coli* mutant lacking the Leloir pathway.

The kinetic parameters and substrate specificity of these enzymes in the cell extract was determined. To this end, a chemoenzymatic assay was developed. The enzyme activity of the UTP-sugar-1-phosphate uridylyltransferases can be determined by measuring the phosphate release in a coupled assay with a pyrophosphatase. The reaction of phosphate with malachite green reagent can be spectrophotometrically detected. This assay proves to be very sensitive since phosphate is detected in the micro molar range.