

Poster Presentation

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## A counter-selectable marker for *Bacillus*

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### Background

A putative gene, denoted *ysbC*, was previously identified by genome sequencing of a *Lactococcus lactis* strain, but the gene was not annotated in the databases, and no function of the predicted encoded polypeptide was identified. Studies show that the *ysbC* encodes a membrane associated orotate transporter enabling orotate to be taken up by the cell and used as a pyrimidine precursor. It was further shown that orotate transporters are quite rare in bacteria and that most bacillus species do not possess such a function.

### Results

In order to exploit the functional properties of the *ysbC* gene it was investigated if an orotate analogue fluoro-orotate could be transported by YsbC. Fluoro-orotate is a toxic pyrimidine precursor which will be incorporated in the host DNA and very efficiently stop further DNA synthesis and divisions of the cell. *Lactococcus* strains with or without the *ysbC* gene was tested for resistance against fluoro-orotate on minimal plates. Only *Lactococcus* strains without the *ysbC* plasmid was able to grow on minimal plates with fluoro-orotate. The conclusion is that orotate (and fluoro-orotate) can only be transported over the cell membrane when the specific orotate transporter encoded by *ysbC* is present.

The gene was transferred to *Bacillus subtilis* to investigate if the orotate transporter would be functional in this background. Experiments in *Bacillus subtilis* using a plasmids with the *ysbC* gene show that transformants can not grow

on minimal plates supplemented with Fluoro-orotate. Further experiments presented on the poster show that the *ysbC* gene can be of use in general bacillus recombinant technology as an efficient counter selectable marker.