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Deliverable 3.2

Synthetic pathways in E. coli

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1. Executive summary

We have engineered *E. coli* for the implementation of three of the photorespiration bypass shunts: (A) the arabinose 5-phosphate shunt; (B) the erythrulose shunt; and (C) the tartronyl-CoA shunt. Our approach was to construct gene deletion strains, whose growth is dependent on the activity of a pathway or a sub-pathway (pathway segment). We were able to demonstrate the activity of the primary segment of the erythrulose shunt. The arabinose 5-phosphate seems to be limited by unfavorable kinetics. Work on the tartronyl-CoA shunt is undergoing.

Because of IP protection issues, only general findings have been summarized in this report. Collected results will be published in more detail in the near future via peer reviewed journals and/or once IP protection is pursued.

2. Cooperation between participants

The work described here was performed by partner MPIMP. Enzymes were supported by partners MPI-TM and WIS. All partners supported this work in discussions and suggestions.

3. Core report

We worked on the implementation of three synthetic photorespiration pathways in *E. coli*, in order to test and maximize pathway activity within an *in vivo* platform. The three pathways were (A) the arabinose 5-phosphate shunt; (B) the erythrulose shunt; and (C) the tartronyl-CoA shunt (Figure 1).

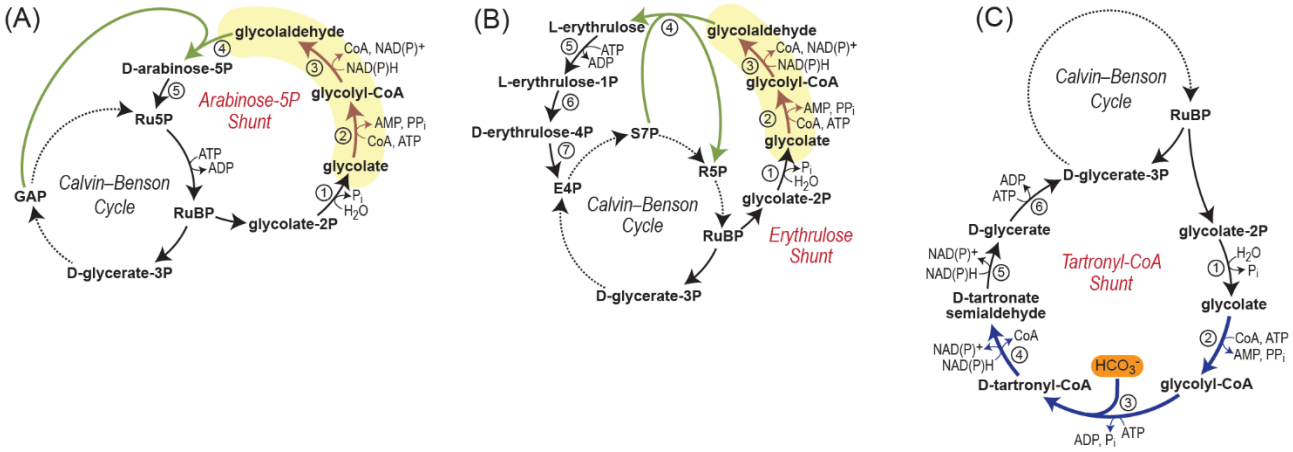

Figure 1. The three photorespiration shunts we aimed to establish in *E. coli*. Key reactions are shown in color: reactions in green are the glycolate reduction module; green arrows correspond to aldolase reactions that assimilate glycolaldehyde; and blue arrows correspond to the glycolate carboxylation module.

3.1. Glycolate reduction module

To select for the activity of the glycolate reduction module, we generated several strains the growth on which depends on the availability of glycolaldehyde. We confirmed that these strains can indeed grow only when supplemented with glycolaldehyde. Overexpression of the enzymes of the glycolate reduction module, as provided by partner WIS, should have enabled the strain to grow with glycolate replacing glycolaldehyde in the medium. So far this selection failed. This is attributed to the existence of strong metabolic sinks for glycolaldehyde that prevents its accumulation. We are currently in the process of deleting all such deleterious metabolic sinks, before continuing with the selection schemes.

3.2. Arabinose 5-phosphate shunt

We have generated a selection strain the growth on which depends on the reverse activity of the arabinose 5-phosphate shunt. Indeed, upon expression of pathway enzymes we could detect growth, indicating activity in the reverse direction of the pathway. We have generated several selection strains for the activity of the arabinose 5-phosphate shunt in the forward direction. We were able to select for the activity of only sub-segment of the pathway in the required direction, where selection of the operation of the entire pathway failed. Analyzing the thermodynamics of the pathway, we identified an energetic barrier that can be breached only via high concentration of glycolaldehyde. Nevertheless, such accumulation glycolaldehyde is deleterious due to the reactivity of the compound. Hence, we decided to switch to the erythrose shunt.

3.3. Erythrulose shunt

First, we generated several selection strains, the growth of each is dependent on the activity of a different enzyme of the erythrulose shunt. Upon overexpression of the appropriate enzyme, we were able to establish growth of all of these strains, thus confirming high activity of all pathway enzymes. We then generated a

selection strain in which the activity of the erythrulose shunt is essential for providing cellular building blocks for upper metabolism. Upon expression of the pathway enzymes and supplementation with the appropriate feedstock we observed growth, confirming the operation of the pathway.

3.4. Tartronyl-CoA shunt

We generated a set of selection strains, each displaying different dependency on glycerate – the product of this synthetic pathway – for growth. We have characterized these strains comprehensively and found that together they span almost two orders of magnitude sensitivity towards glycerate, making them ideal to detect different rates of glycerate production. We have cloned the enzymes of the tartronyl-CoA shunt, as provided to us by partner MTI-MP, but so far failed to obtain growth of even the most sensitive strain. Enzyme activity measurements in the cell extract confirm that some of the pathway enzymes are quite inactive. We are currently working to solve this problem together with partner MTI-MP.

4. Conclusions

We were able to demonstrate the activity of the primary segment of the erythrulose shunt. The necessary activity of the glycolate reduction module requires further deletions of enzymes that deleteriously consume this metabolite. Work on the tartronyl-CoA is undergoing, focusing on addressing the problem of low activity of some of the pathway enzymes.