Influence of the biomass growth conditions on the bioconversion of glycerol to 3-hydroxypropanoic acid and 1,3-propanediol by resting cells of *Lactobacillus reuteri*

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Abstract

The recent interest in biodiesel production resulted in the raise of glycerol availability. Under this context, the development of processes involving the conversion of glycerol into high added value compounds has gained interest. In particular, bioconversions involving mild operational conditions (neutral pH, low temperature, and atmospheric pressure) are often advantageous compared to chemical synthesis. Glycerol can be converted to 3-hydroxypropanoic acid (3-HP), a platform molecule which can be used in the synthesis of many materials such as resins, adhesives, or plastics. This bioconversion can occur in a few microorganisms via two steps reaction starting with the dehydratation of glycerol to 3-hydroxypropionaldehyde (3-HPA) by coenzyme B12-dependant glycerol dehydratase. Then, 3-HPA is converted to 3-HP via an oxidative pathway and to 1,3-propanediol (1,3-PDO) via a reductive pathway.

Among the microorganisms possessing this enzymatic machinery, *Lactobacillus reuteri* (1) is an interesting candidate given its GRAS status and its ability to synthesize coenzyme B12. Resting cells of *L. reuteri* can be used to produce 3-HP from glycerol with a quite good productivity. However, this process is limited by the toxicity of 3-HPA and 3-HP, whose accumulation in the medium causes a rapid inhibition of the bacterial activity. Thus, the efficiency of the process depends both on the capacity of *L. reuteri* to convert glycerol into 3-HP and its resistance to the presence of 3-HP and 3-HPA.

We studied the influence of the cultivation conditions on the ability of *L reuteri* DSM 17938 (2) to produce 3-HP from glycerol in a two step process (Figure 1): the growth of the microorganism and the bioconversion phase by resting cells. *L reuteri* was cultivated in different growth media based on MRS and by-product of biorefinery (3) before being transferred into the bioconversion medium containing only glycerol. Then, the bioconversion kinetics were measured by following the production of 3-HPA, 3-HP and 1,3-PDO (quantified by HPLC).

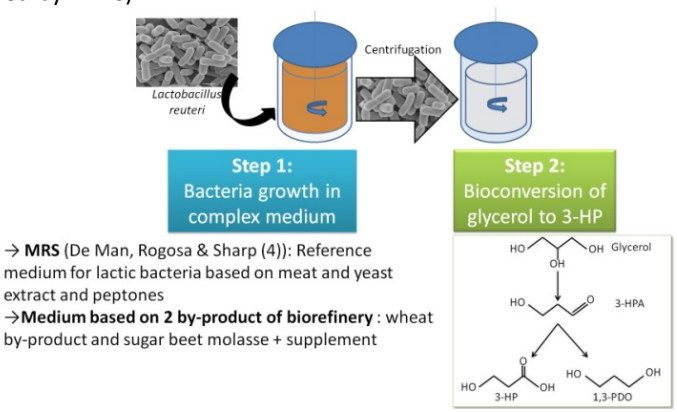

Figure 1: Two step process of 3-HP production

The composition of the growth medium had a significant impact on the metabolism of glycerol. The addition of glutamate and arginine allowed to double the production of 1,3-PDO and 3-HP from 3-HPA. Further experiments using flow cytometry are needed to assess whether this is due to a better resistance to acid stress or to a metabolic shift. This work will enable us better understanding the metabolism of *L. reuteri* and optimize the growth conditions for the bioconversion of glycerol into 3-HP.

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Key references

(1) Dishisha, T., Pereyra, L.P., Pyo, S.-H., Britton, R.A., Hatti-Kaul, R., 2014. Flux analysis of the Lactobacillus reuteri propanediol-utilization pathway for production of 3-hydroxypropionaldehyde, 3-hydroxypropionic acid and 1,3-propanediol from glycerol. Microbial Cell Factories 13, 76. doi:10.1186/1475-2859-13-76

(2) Burgé, G., Saulou-Bérion, C., Moussa, M., Pollet, B., Flourat, A., Allais, F., Athès, V., Spinnler, H.E., 2015b. Diversity of Lactobacillus reuteri Strains in Converting Glycerol into 3-Hydroxypropionic Acid. Appl Biochem Biotechnol 177, 923–939. doi:10.1007/s12010-015-1787-8

(3) Han, Y.W., Watson, M.A., 1992. Production of microbial levan from sucrose, sugarcane juice and beet molasses. Journal of Industrial Microbiology 9, 257–260. doi:10.1007/BF01569633

(4) J. C. De Man, M. Rogosa, M. Elisabeth Sharpe, 1960 A medium for the cultivation of *lactobacilli*, Journal of applied Microbiology doi:10.1111/j.1365-2672.1960.tb00188.x