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On the question of activity of oxidative branch of pentose phosphate shunt in *pgl* mutant of *Escherichia coli*

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Motivation and Aim: *Escherichia coli* is one of the best investigated organism, which is widely used both in microbiology and for biotechnological production of different biologically active compounds. The special stage in microorganism metabolism investigations started with implementation of ^{13}C metabolic flux analysis (^{13}C-MFA). This approach allows quantitative estimation of actual carbon metabolic fluxes distribution *in vivo*. For example, using ^{13}C-MFA the role of two transhydrogenases encoded by genes *pntAB* and *udhA* was experimentally determined and characterized [1]. However, there are still some unsolved questions, even about central metabolism of *E. coli*. In particular, there are two opposite points of view about flux distribution in 6-phosphogluconolactonase deficient (*Δpgl*) mutant of *E. coli*. For example, applying the flux balance analysis (FBA) to the well known *E. coli* strain BL21, which has *pgl* deficient genotype, investigators assume that oxidative branch of pentose phosphate pathway (oxPPP) is inactive in this strain [2]; this assumption influences on prediction of biotechnological potential of this strain based on genome-scale model. This assumption is based on experimental data of accumulation of product of glucose-6-phosphate dehydrogenase reaction (G6PDH reaction), 6-phosphogluconolactone [3], which is a substrate of 6-phosphogluconolactonase reactions (PGL reaction). In contrast, ^{13}C-MFA of BL21 [4] and *pgl* deficient MG1655 strain mutant [5], carried out also in our laboratory, has demonstrated only insignificant decrease of flux through oxPPP; the spontaneous hydrolysis of 6-phosphogluconolacton is proposed as the simplest explanation of this phenomenon. In this work we hypothesized that flux through oxPP pathway is actually reduced by near two times, but not to zero, in *pgl* mutant.

Methods and Algorithms: For ^{13}C-MFA of *E. coli* strains grown on minimal medium with 20 %/80 % mixture of [1-^{13}C]- and [U-13C]-glucose as sole carbon source. Flux calculation and statistical analysis of calculated carbon flux distribution have been provided the OpenFLUX2 software [6] on the basis of labeling data of proteinogenic amino acids and monosaccharides from cellular polymers.

Results: Comparative ^{13}C-MFA of two *E. coli* strains MG1655 and MG1655 *Δpgl* has shown that the flux solution with the best accordance with experimental data (minimal value of sum of squared residuals, SSR) corresponds to carbon flux through the oxPPP of 18 % of input glucose in case of *Δpgl* mutant in contrast with 25 % in parent strain. The minimal value of SSR is commonly used but conditional criterion. Formally, any flux distribution providing SSR value which passes χ^2-criterion can be recognized as acceptable [7]. However flux calculation under assumption that *pgl* inactivation blocks oxPPP led to only statistically unacceptable solution (even minimal SSR value do not pass χ^2-criterion). We determined that flux through PGL reaction responded to χ^2-criterion on minimal 10 % level. It means that the 2.5-fold decrease in flux through the oxPPP in *pgl* deficient mutant can reflect the real situation.

It is known from literature that inactivation of oxPPP with simultaneous *pntAB* deletion resulted in drastic decrease of growth rate caused by NADPH deficiency. Therefore, we measured growth characteristics for the strains with inactivation of *pntAB* and each of three genes of oxPPP, *zwf*, *pgl*, *gnd*. The *Δzwf ΔpntAB* mutant with completely blocked oxPPP, had growth rate which is reduced in 5.9 times relatively to parent strain MG1655. The *ΔpglΔpntAB* and *ΔgndΔpntAB* mutants demonstrated decrease of growth rate in 1.3 and 1.7 times respectively. This additionally confirms that *pgl* inactivation do not abolish activity of oxPPP as in case of *zwf* inactivation. The interesting fact that mutant *ΔgndΔpntAB* grows 1.3 times slower than *ΔpglΔpntAB* mutant despite of activity of Entner-Doudoroff bypass, indicating, probably, that the first strain has more restriction in NADPH supplementation.
Previously it was proposed that the specific bypass through gluconate can function in *pgl* deficient mutant [8]. In this case the flux through *pgl* is zero, but oxPPP is still functioning due to 6-phosphogluconolactone dephosphorylation to gluconolactone followed by its transport out of the cell and hydrolysis to gluconate which is consumed and phosphorylated to 6-phosphogluconate – substrate for 6-phosphogluconate dehydrogenase (GND). Unfortunately, steady-state ^{13}C-MFA cannot distinguish fluxes trough the PGL and this bypass, because both paths possess the same carbon atom rearrangements. Simultaneous inactivation of major gluconate transporters, *gntT*, *gntKU* and *gntP*, reduces growth of Δ*pgl* mutant and growth of *ΔpglΔpntAB* mutant on glucose. These observations indicate that gluconate bypass can participate in keeping of flux through oxPPP in *pgl* deficient strain.
Conclusions: Genome-scale metabolic models of bacterial metabolism have a big impact in evaluation of the biotechnological potential of the particular bacterium/strain. Although the whole genome sequence is a necessary basis of these models, a lot of experimental data should be taken into account to make them applicable for reliable prediction. In this work we pay attention to erroneous proposal of inactive oxPPP in *pgl* deficient *E. coli* mutant; this proposal is contradicts to ^{13}C-MFA of carbon flux distribution in MG1655Δ*pgl* strain and to growth characteristics of double mutants, with inactivated pntAB transhydrogenases gene and genes of oxPPP. According to ^{13}C-MFA the flux through the oxPPP decreases by no more than 2.5-times in the MG1655Δ*pgl* strain.

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