GENETIC CHANGES DURING A LABORATORY ADAPTIVE EVOLUTION PROCESS ALLOWED FAST GROWTH IN GLUCOSE TO AN ESCHERICHIA COLI STRAIN LACKING THE MAJOR GLUCOSE TRANSPORT SYSTEM

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Key words: PTS system, resequencing, laboratory evolution, mutation, deletion, adaptation, rppH, glycolysis.

Introduction. *Escherichia coli* strains lacking the *ptsHIcrr* operon can accumulate high amounts of phosphoenolpyruvate which can be diverted to the synthesis of commercially relevant products (1). However, these strains present decreased growth rates in glucose due to its inefficient transport and metabolism (2). A short term adaptive laboratory evolution process gave rise the strain PB12 selected after 120hrs, whose growth increase 400% and has been employed for the high yield aromatic compounds production (1,2). Analysis of the genetic changes in the PB12 strain, will allow a better understanding of its growth adaptation and in the design of improved metabolic engineering strategies for enhancing carbon diversion into the aromatic pathway utilizing PTS$^-$ strains.

Methods. Whole genome analyses were performed using two different sequencing methodologies: the Roche NimbleGen Inc. comparative genome sequencing technique and high throughput sequencing with Illumina Inc. GAIIx. Central carbon metabolism gene expression by RT- qPCR technique was also accomplished. Gene knockouts were performed using the method described by Datsenko (3).

Results. Whole genome analyses using the Roche NimbleGen Inc. comparative genome sequencing technique and high throughput sequencing with Illumina Inc. GAIIx, allowed the identification of the genetic changes that occurred in the PB12 strain. Several non-synonymous mutations mapped in regulatory genes and in other putative regulatory loci were detected. In addition, a chromosomal deletion of 10,328 bp was detected that removed 12 genes, among them, the *rppH*, *mutH* and *galR* genes.

Conclusions. The chromosomal deletion is apparently the main reason for the faster growth of the evolved PB12 strain. The *rppH* inactivation in the parental PB11 strain increased substantially its growth rate by increasing glycolytic mRNA genes stability. Furthermore, *galR* inactivation allowed carbon transport by GalP into the cell. The deletion of *mutH* in a strain that already lacks PTS is apparently responsible for the appearance of several point mutations.

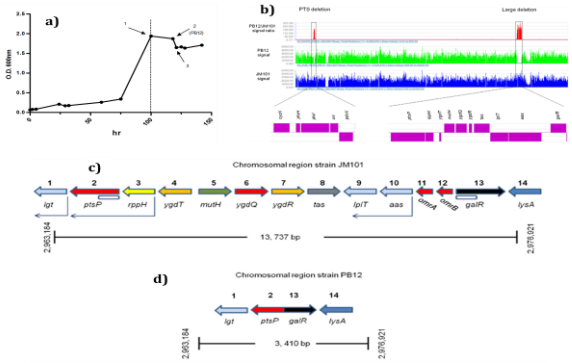

Fig.1 a)Isolation of the evolved PB12 strain. b)Comparative genomic maps of the parental JM101 and evolved PB12 strains. c), d) Chromosomal gene arrangement in JM101 and PB12 strain.

Regulatory genes			Possible regulatory genes		
Strain	**μ**	**%**	**Strain**	**μ**	**%**
PB11	**0.13 ± 0.001**	**100**	**PB12**	**0.44 ± 0.016**	**100**
PB11Δ*barA*	0.12 ± 0.001	92	PB12Δ*barA*	0.46 ± 0.001	105
PB11Δ*rppH*	0.34 ± 0.019	261	PB12Δ*yjjU*	0.34 ± 0.003	77
PB11Δ*yjjU*	0.16 ± 0.005	123	PB12Δ*rssA*	0.43 ± 0.018	98
PB11Δ*rssA*	0.17 ± 0.003	130	PB12Δ*rna*	0.43 ± 0.035	98
PB11Δ*rna*	0.13 ± 0.007	100	PB12Δ*ypdA*	0.44 ± 0.003	100
PB11Δ*ypdA*	0.13 ± 0.002	100			

Table 1. Specific growth rates (μ) of different derivative mutant strains generated in this work.

Acknowledgements.
CONACyT, grant 105782; PAPIIT, grant IN205811. The funders had no role in study design, data collection and analyses.

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