Development of Fermentative Production of Pyruvate by Metabolic Engineering.

Tetsu Yonehara, ○Reiko Miyata, Hiroshi Matsuno
Motojiro Goto, Sabro Yahannda
(Toray Industries, Inc.)

Pyruvate is a key organic acid in metabolic pathways. The commercial demand for pyruvic acid has been expanding because of its use as an effective starting material for the synthesis of many drugs and agrochemicals as well as a component of cell cultures.

Fermentative production of pyruvate by metabolic engineering was studied and developed. A new approach to the screening of novel pyruvate - producing yeasts and variations in their vitamin -auxotrophy with regard to the productivity of pyruvate are described. Among the isolates, *Torulopsis glabrata* IFO 0005 proved to be a superior strain for the fermentative production of pyruvate. *T. glabrata* IFO 0005 has an absolute requirement for four vitamins; nicotinic acid (NA), thiamine (B_1), pyridoxine (Pdx) and biotin (Bio) for growth. Consequently, the auxotrophy of multiple vitamins involved in the metabolism of pyruvate proved to be highly effective for the fermentative production of pyruvate from glucose. It has been postulated that the auxotrophy of the four vitamins noted above plays an essential role in maintaining the metabolic balance for pyruvate accumulation. The system for regeneration of NAD^+ from NADH in this fermentation

of *T. glabrata* IFO 0005 is also discussed . *T. glabrata* IFO 0005 had accumulated 67.8g/l of free pyruvic acid at 63 hours (yield, 49.7% ; conversion of pyruvic acid from consumed glucose) on a 3*l* jar-fermentor scale.

In addition, three efficient methods for the isolation of pyruvate - producing mutants of *Torulopsis glabrata* IFO 0005 were established. After mutagenesis of the parent strain, mutants that demonstrated higher fermentative production of pyruvate from glucose than the parent strain. These were of the following three kinds; (a) Mutants requiring acetate for full growth (acetate – leaky auxotrophic mutants) (b) Amino Acid Auxotrophic Mutants (Arginine auxotrophic mutants and L-Isoleucine and L-Valine double auxotrophic mutant) (c) Salt tolerance mutants

(a) acetate – leaky auxotrophic mutants

Mutants requiring acetate for full growth were derived and the acetate - leaky auxotrophic mutants showed higher fermentative production of pyruvic acid from glucose than the parent strain. As a result, the pyruvate decarboxylase (PDC) activities of mutants requiring acetate for full growth were lower than that of the parent strain. Among the mutants, *T. glabrata* ACII-33 had accumulated 60.3g/l of free pyruvic acid at 47 hours (yield, 67.3%) on a 3*l* jar-fermentor scale. This yield of ACII-33 represented an about 15% increase compared to that with the parent strain.

(b) Amino Acid Auxotrophic Mutants

Mutants requiring polypeptone for complete growth were isolated. Among them, Arginine (L-Arg) auxotrophic mutants, and as Isoleucine (L-Ile) and Valine (L-Val) double

auxotrophic mutant exhibited higher fermentative production of pyruvate from glucose than the parent strain. Among them, the best strain as to pyruvate productivity, *T. glabrata* X-15 (Arg^-), accumulated 59.5 g/l of free pyruvic acid (yield, 60.1 %; complete conversion to pyruvic acid from glucose added in 43 hours) on a 3*l* jar-fermentor scale. This yield with strain X-15 represented a 12% increase compared to that with the parent strain.

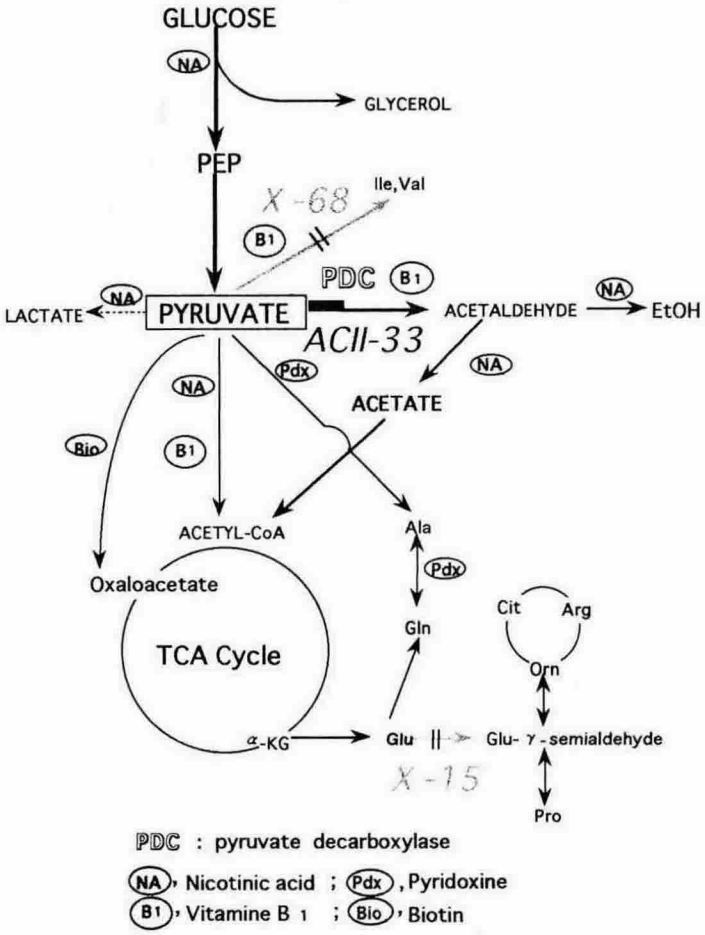

Fig. 1. Metabolism of pyruvate by *T.glabrata* and mutants

(c) Salt tolerance mutants

Mutagenesis of the parent strain with the aim of obtaining salt-tolerant mutants with respect to decreased production of glycerol was performed. After mutagenesis of the parent strain, three strains were found that the accumulation of pyruvate was more than 15 % higher and that of glycerol as by-product was less than 30% lower than the parent strain. Among them , *T. glabrata* NL2a had accumulated 72 g/l of free pyruvic acid (yield 55% ; conversion of pyruvic acid from glucose consumed 69 hours) on a 3*l* jar-fermentor scale. This yield by strain NL2a represented a 20 % increase compared to that with the parent strain.

The optimal practical fermentative conditions for *T. glabrata* IFO 0005 and its mutants in a 3*l* jar fermentor and the determination of several by-products were discussed.

It was also discussed that the establishment of isolation and purification practicl method by reverse osmotic menbrane.

[key words] *Torulopsis glabrata*, pyruvic acid fermentation, vitamin–auxotrophy, nicotinic acid, thiamine, pyridoxine, and biotin, mitochondria anaerobic condition, electron transport chain, acetate- leaky auxotroph, Pyruvatedehydrogenase (PDH) complex, pyruvate decarboxylase (PDC), salt tolerance mutants, isoleucine and valine double auxotroph , Arginine auxotroph, reverse osmotic menbrane