Appl Microbiol Biotechnol (2001) 57:451–459
DOI 10.1007/s002530100804

MINI-REVIEW

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Biotechnological production of pyruvic acid

Received: 23 April 2001 / Received revision: 30 July 2001 / Accepted: 17 August 2001 / Published online: 22 September 2001

Abstract Pyruvic acid is an important organic acid widely used in the chemical and drug, as well as agrochemical, industries. Compared with the chemical method, biotechnological production of pyruvic acid is an alternative approach because of the low cost. An overview of biotechnological production of pyruvate, including direct fermentative production employing eukaryotic and prokaryotic microorganisms, production by a resting cell method and an enzymatic method as well as the recovery of pyruvate, is discussed. A multi-vitamin auxotrophic yeast strain, *Torulopsis glabrata*, has been used in the commercial production of pyruvate; emphasis is therefore placed on the mechanism and characteristics of pyruvate production by this strain.

Introduction

Pyruvic acid, also known as 2-oxopropanoic acid, α-ketopropionic acid or acetylformic acid, is the most important α-oxocarboxylic acid. It plays a central role in energy metabolism in living organisms. Industrially, it is used mainly as a starting material in the biosynthesis of pharmaceuticals, such as L-tryptophan, L-tyrosine and alanine, as well as L-DOPA, by known methods (Uchio et al. 1976). It is also employed in the production of crop protection agents, polymers, cosmetics and food additives. Calcium pyruvate also has a strong effect in reducing fat because it can accelerate the metabolism of fatty acids in the human body (Roufs 1996). Other applications of pyruvate are shown in Table 1. As it is widely used in drug, agrochemical, chemical and food industries, the commercial demand for pyruvic acid has been expanding (Yonehara and Miyata 1994a).

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Table 1 More applications of pyruvic acid or pyruvate

Application	Reference
Significantly increases fat and weight loss	Stanko et al. 1992
Improves exercise endurance capacity	Stanko et al. 1990
Effectively reduces cholesterol	Stanko et al. 1994
Serves as a potent antioxidant	DeBoer et al. 1993
Reduces anoxic injury and free radical formation	Borle and Stanko 1996

On an industrial scale, pyruvic acid is produced by dehydration and decarboxylation of tartaric acid (Howard and Fraser 1932). In this process, pyruvic acid is distilled from a mixture of tartaric acid and potassium hydrogen sulfates at 220°C; the crude acid obtained is then distilled under vacuum. This process is simple to realize but not cost-effective; the total cost is estimated to be about US $8,000–9,000/ton according to the actual market price of raw materials (Li 2000). Pyruvic acid is, therefore, too expensive to be widely used over a long period. For example, although pyruvic acid is a potential flavoring agent that imparts a sour taste to foods, it is hardly ever used to replace other organic acids because of the high price.

Compared with the chemical method, production of pyruvic acid by using biotechnological methods is an alternative approach to reduce the production cost. There are three methods for biotechnological production of pyruvate: the direct fermentation method, the resting cell method, and the enzymatic method. Of these, direct fermentative production of pyruvate from a carbon source (such as glucose) has merits in terms of both cost-effectiveness and the high purity of the product. However, as pyruvate is located at a vital junction of cell metabolism, it is usually difficult to obtain strains that can accumulate large amounts of pyruvate extracellularly. Many scholars have tried to identify microorganisms with high pyruvate-producing ability, but it was not until 1989 that significant progress was achieved in the fermentative production of pyruvate. Some yeast strains, belonging to the genus *Torulopsis*, that produced more than 50 g/l pyru-

vate were identified in a screen carried out by the researchers of Toray Industries, Japan (Miyata et al. 1989a). This result indicates that the fermentative production of pyruvate can be commercialized. Pyruvate production using the fermentative method, with a scale of about 400 tons per year, was industrialized by Toray Industries in 1992 and the fermentation has now been scaled-up to 50 m^3 fermentors (Yonehara et al. 2000)

This review summarizes the development of biotechnological production of pyruvate, with emphasis on the fermentative production of pyruvate by yeast. The literature cited comprises most of the publications in this field since 1976, including many Japanese patents. For detailed discussion of pyruvate production using a multivitamin auxotrophic *Torulopsis* strain, and *Escherichia coli* defective in energy metabolism, reviews written in Japanese by the groups of Yonehara (Yonehara and Miyata 1994b; Yonehara et al. 2000) and Yokota (Yokota and Tomita 1995; Yokota 1997), respectively, can be consulted.

Biotechnological production of pyruvate: an overview

Direct fermentative production of pyruvate by eukaryotic microorganisms

Yeast is the most commonly used microorganism that directly accumulates pyruvate in the medium using glucose or other carbon source as the substrate. Table 2 summarizes the development in this field contributed by Japanese researchers. As can be seen from Table 2, strains belonging to the species of *Torulopsis* with the phenotype of multi-vitamin auxotrophy, such as *T. glabrata* IFO 0005, without doubt show excellent ability in producing pyruvate (Yonehara and Yomoto 1987a; Yonehara and Miyata 1994a). The physiological mechanism for the accumulation of pyruvate by a multi-vitamin auxotrophic strain of *Torulopsis* is shown in Fig. 1. Thiamine is a co-factor of the pyruvate dehydrogenase (PDH) complex and of pyruvate decarboxylase (PDC). Nicotinic acid, biotin, and pyridoxine are co-factors of PDH, pyruvate carboxylase (PC), and transaminase, respectively. As a multi-vitamin auxotrophic strain cannot synthesize these vitamins itself, under conditions of deficiency for these four vitamins, pyruvate is accumulated due to the decreased activity of PDH, PDC, PC, and transaminase. In order to accumulate large amounts of pyruvate when cultivating the multi-vitamin auxotrophic strain *T. glabrata*, it is very important to maintain a concentration balance between thiamine, nicotinic acid, pyridoxine and biotin (Li et al. 2001).

To achieve high yields of pyruvate from glucose is very important in the commercialization of pyruvate production by fermentative methods. Under normal culture conditions, the pyruvate yield of *T. glabrata* IFO 0005 is 0.41 g/g (Yonehara and Yomoto 1987a); this value is not high enough to realize cost-effective fermentative production of pyruvate compared with the chemical method. Therefore, *T. glabrata* IFO 0005 was mutagenized to give different additional genetic characteristics, such as L-arginine auxotrophy (Miyata et al. 1988b), L-valine and L-isoleucine auxotrophy (Miyata et al. 1989a), aminooxyacetic acid resistance (Miyata et al. 1989b), and 2-deoxyglucose resistance (Miyata and Yonehara 1990). Yields of 0.52–0.54 g/g could be achieved by mutants having one or more of these genetic characteristics (Table 2). As ethanol is a main by-product when producing pyruvate in yeast, a *Torulopsis* sp. mutant strain with lower PDC activity was selected, by which means a pyruvate yield of 0.58 g/g was achieved (Miyata et al. 1990).

Other yeasts, such as thiamine auxotrophs of *Candida lipolytica* (Uchio et al. 1976), *Debaryomyces hansenii* (Yanai et al. 1994), and *Saccharomyces cerevisiae* (Yonehara and Yomoto, 1987b), also accumulate pyruvate under conditions of thiamine limitation. The pyruvate yield, 0.37–0.44 g/g, was not as good as in the case of *T. glabrata*. However, these strains can use inorganic ammonium as a sole nitrogen source, while *T. glabrata* IFO 0005 and its derivatives cannot (Table 2). Halophilic *Torulopsis etchellsii* accumulates pyruvate from glucose in a medium supplementedwith 80 g/l salts, but the yield (0.02 g/g) is rather low (Kiuchi et al. 1987a, b).

For *T. glabrata*, the difficulty of achieving high yield and high concentration of pyruvate simultaneously seems to be a drawback. For example, in a report by Miyata and Yonehara (1996), 67.8 g/l pyruvate was achieved at 63 h in a fed-batch culture, but the yield from glucose was only 0.49 g/g. In another report (Hua et al. 1999), pyruvate yield of higher than 0.8 g/g was achieved in a typical pyruvate production phase. However, the overall yield, 0.53 g/g, was still not high despite applying a thiamine-addition strategy to a fed-batch culture. In order to overcome this problem, Li et al. (2001) selected an excellent pyruvate producer from *T. glabrata* WSH-IP12 (Li et al. 2000) that could use ammonium chloride as a sole nitrogen source. In this case, both high pyruvate concentration (69 g/l) and high pyruvate yield (0.62 g/g) were achieved in a batch culture.

Direct fermentative production of pyruvate by prokaryotic microorganisms

As shown in Table 2, glucose is the most commonly used substrate for pyruvate production in yeast. However, the category of substrates assimilated by prokaryotic microorganisms to produce pyruvate seems to be wider than in yeast. For example, as shown in Table 3, many microorganisms use gluconate, 1,2-propanediol or propinoic acid as a sole carbon source to accumulate pyruvate. Although these prokaryotic microorganisms (except *E. coli*) have no special genetic characteristics with respect to pyruvate accumulation, the pyruvate yield was also comparable (0.3–0.4 g/g). Excepting *E. coli*, the highest pyruvate concentration produced by a prokaryot-

Table 2 An overview of literature on the fermentative production of pyruvate by yeasts

Strains	Phenotype[a]	Carbon source	Nitrogen source	Time (h)	Pyruvate (g/l)	Yield (g/g)	Reactor	Reference
Candida lipolytica AJ 4546		Acetamide	Corn steep liquid	72	1.5	0.15	Flask	Uchio et al. 1974a
Candida maltosa		Propionic acid	$(NH_4)_2SO_4$, NH_4NO_3	72	2.2	0.22	Flask	Uchio et al. 1974b
Candida lipolytica AJ 14353	B_1^- Met^-	Glucose	NH_4NO_3	72	43.6	0.44	Flask	Uchio et al. 1976
Debaryomyces coudertii IFO 1381		Citrus peel	$(NH_4)_2SO_4$, NH_4NO_3	48	11.5		Flask	Moriguchi 1982
Debaryomyces hansenii Y-256	B_1^- Bio^-	Glucose	Peptone	96	42	0.42	Flask	Yanai et al. 1994
Saccharomyces cerevisiae IFO 0538	B_1^-	Glucose		48	36.9	0.37	Flask	Yonehara and Yomoto 1987b
Torulopsis glabrata IFO 0005	B_1^- Bio^- B_6^- NA^-	Glucose	Peptone	60	40.7	0.41	Flask	Yonehara and Yomoto 1987a
Torulopsis glabrata ACII-3	B_1^- Bio^- B_6^- NA^- pyruvate decarboxylase (PDC) activity decrease	Glucose	Polypepton	60	48.9	0.49	Flask	Miyata et al. 1988a
Torulopsis glabrata X-15	B_1^- Bio^- B_6^- NA^- Arg^-	Glucose	Polypepton	60	49.3	0.49	Flask	Miyata et al. 1988b
Torulopsis glabrata X-68	B_1^- Bio^- B_6^- NA^- Ile^- Val^-	Glucose	Polypepton	60	50	0.50	Flask	Miyata et al. 1989a
Torulopsis glabrata TR–2026	B_1^- Bio^- B_6^- NA^- AOA^r	Glucose	Polypepton	60	52.1	0.52	Flask	Miyata et al. 1989b
Torulopsis glabrata AOA-8	B_1^- Bio^- Arg^- AOA^r	Glucose	Peptone	60	27.3	0.54	Flask	Miyata et al. 1989c
Torulopsis glabrata P95–21	B_1^- Bio^- B_6^- NA^- 2-DG^r	Glucose	Peptone	60	52.1	0.52	Flask	Miyata and Yonehara 1990
Torulopsis glabrata ACII33	B_1^- Bio^- B_6^- NA^- PDC activity decrease	Glucose	Peptone	60	56.8	0.58	Flask	Miyata et al. 1990
Torulopsis glabrata IFO 0005	B_1^- Bio^- B_6^- NA^-	Glucose	Polypepton	59	57	0.57	Flask	Yonehara and Miyata 1994a
Torulopsis glabrata IFO 0005	B_1^- Bio^- B_6^- NA^-	Glucose	Soybean hydrolyzate $(NH_4)_2SO_4$	67	67.8	0.494	3 l Fermentor	Miyata and Yonehara 1996
Torulopsis glabrata ACII-3	B_1^- Bio^- B_6^- NA^- acetate-leaky	Glucose	Soybean hydrolyzate $(NH_4)_2SO_4$	47	60.3	0.678	3 l Fermentor	Miyata and Yonehara 1999
Torulopsis glabrata X-15	B_1^- Bio^- B_6^- NA^- Arg^-	Glucose	Soybean hydrolyzate $(NH_4)_2SO_4$	43	58.5	0.60	3 l Fermentor	Miyata and Yonehara 2000
Torulopsis glabrata IFO 0005	B_1^- Bio^- B_6^- NA^-	Glucose	Polypepton	42	42	0.45	5 l Fermentor	Hua and Shimizu 1999
Torulopsis glabrata IFO 0005	B_1^- Bio^- B_6^- NA^-	Glucose	Polypepton	40	25	0.53	5 l Fermentor	Hua et al. 1999
Torulopsis glabrata P120–5a	B_1^- Bio^- B_6^- NA^- pyruvate resistant	Glucose	Peptone	60	59.1	0.59	Flask	Miyata et al. 2000

Table 2 Continued

Strains	Phenotype[a]	Carbon source	Nitrogen source	Time (h)	Pyruvate (g/l)	Yield (g/g)	Reactor	Reference
Torulopsis glabrata WSH-IP 12	B_1^- Bio^- B_6^- NA^-	Glucose	Peptone, ammonium	55	57.3	0.50	5 l Fermentor	Li et al. 2000
Torulopsis glabrata WSH-IP 303	B_1^- Bio^- B_6^- NA^-	Glucose	NH_4Cl	56	69	0.62	5 l Fermentor	Li et al. 2001
Torulopsis etchellsii S-9	Halophilic	Glucose	Casein	240	5.1	0.10	1 l Fermentor	Kiuchi et al. 1987a
Torulopsis etchellsii S-9	Halophilic	Glucose	NH_4Cl, KNO_3	14 days	1.08	0.02	Flask	Kiuchi et al. 1987b

[a] B_1^- thiamine auxotroph, *Bio*$^-$ biotin auxotroph, B_6^- pyridoxine auxotroph, *NA*$^-$ nicotinic acid auxotroph, *Ile*$^-$ isoleucine auxotroph, *Val*$^-$ valine auxotroph, *Arg*$^-$ arginine auxotroph, *AOA*^{r} aminooxyacetic acid resistant, *2-DG*^{r} 2-deoxyglucose resistant, *Met*$^-$ methionine auxotroph

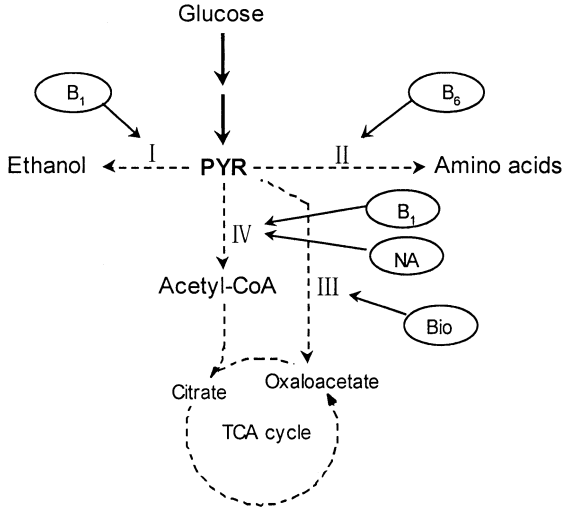

Fig. 1 Metabolism pathway of pyruvic acid in *Torulopsis glabrata* (Miyata and Yonehara 1996). *Broken lines* Weakened pathways, *I* Pyruvate decarboxylase (PDC), *II* transaminase (PT), *III* pyruvate carboxylase (PC), *IV* pyruvate dehydrogenase (PDH) complex, B_1 thiamine, B_6 pyridoxine, *NA* nicotinic acid, *Bio* biotin

ic microorganism was only 23 g/l (Uchio and Hirose 1975), which means that these microorganisms cannot be used industrially at present because the pyruvate concentration is too low to conduct a cost-effective downstream process.

In multi-vitamin auxotrophic strains of *T. glabrata*, pyruvate is considered to be produced by the impaired oxidative decarboxylation of pyruvate caused by a decrease in the activity of PDH in which thiamine acts as a cofactor. As thiamine is also a cofactor of transketolase, the operation of the pentose phosphate pathway in such a strain might also be affected because of the decrease of transketolase caused by thiamine deficiency. Considering that lipoic acid is also a cofactor of PDH, a screen by Yokota et al. (1994a) identified a lipoic acid auxotroph, *E. coli* W1485*lip2*, that accumulated 25.5 g/l pyruvate from 50 g/l glucose at 32 h. With *E. coli* W1485*lip2* as the parent strain, an F_1-ATPase-defective mutant strain, TBLA-1, was constructed by transduction of an F_1-ATPase-defective gene into *E. coli* W1485*lip2* (Yokota et al. 1994b). Although the growth level of strain TBLA-1 decreased to 67% of the parent strain due to the reduced energy metabolism, it produced more than 30 g/l pyruvate from 50 g/l glucose in a 24-h culture. The activities of both glucose consumption and pyruvate production per cell increased 1.9- and 2.8- fold compared to the parent strain, respectively, which indicates that the metabolism of glucose and the productivity of a certain metabolite via a central pathway can be manipulated by cell energy levels. In order to elucidate the physiological mechanisms of the enhanced pyruvate productivity in strain TBLA-1, Yokota et al. (1997) compared the differences in some key enzymes of the glycolytic pathway between the mutant and the parent strain. They found increases in the activities of the phosphotransferase system, phosphoglycerate kinase and pyruvate kinase, and thought these results have some relationship with the enhanced pyruvate productivity in strain TBLA-1.

That the pH of the medium is higher than 7.0 is another characteristic of pyruvate production by bacteria, which indicates that a coupled system between pyruvate production and enzymatic tryptophan production can be constructed. For example, Yokota and Takao (1984) and Takao et al. (1984) cultivated *Agaricus campestris* first in a medium containing 50 g/l glucose. When the pyruvate concentration reached 22–26 g/l, *Enterobacter aerogenes* with tryptophanase activity and indole as well as ammonium chloride were added to the fermentation broth to produce 15 g/l tryptophan after 12 h conversion. This coupled system was further improved by Yokota et al. (1989). A mutant, lipoic acid auxotroph of *E. aerogenes* with tryptophanase activity and pyruvate-producing ability, was used to produce tryptophan. Indole and ammonium chloride were added to the fermentation broth when the pyruvate concentration reached 17 g/l, after which 14 g/l tryptophan was produced. In addition, the bottleneck preventing further increase in the tryptophan concentration is the lower production of pyruvate by *E. aerogenes*. In order to achieve higher tryptophan productivity, genes encoding tryptophanase in *E. aero-*

Table 3 An overview of literature on the fermentative production of pyruvate by bacteria or mycetes

Strains	Characteristics	Carbon source[a]	Nitrogen source	Time (h)	Pyruvate (g/l)	Yield (g/g)	Reactor	Reference
Escherichia coli W1485*lip2*	Lipoic acid auxotrophy	Glucose	Polypepton	32	25.5	0.51	5 l Fermentor	Yokota et al. 1994a
Escherichia coli AJ 12631	Lipoic acid auxotrophy F_1-ATPase deficient	Glucose	Polypepton	40	29.2	0.58	Flask	Tomita and Yokota 1993
Escherichia coli TBLA-1	Lipoic acid auxotrophy F_1-ATPase deficient	Glucose	Polypepton	24	30	0.60	5 l Fermentor	Yokota et al. 1994b
Schizophyllum commune 550		Glucose	Polypepton	120	19	0.38	Flask	Takao and Tanida 1982
Nocardia lutea AJ9103		Propionic acid	Ammonium	72	2.5	0.25	Flask	Uchio et al. 1974b
Nocardia fumifera		Gluconate	$(NH_4)_2SO_4$	96	23	0.46	Flask	Uchio and Hirose 1975
Pseudomonas tabati		Gluconate	$(NH_4)_2SO_4$	96	13	0.26	Flask	Uchio and Hirose 1975
Pseudomonas stutzeri IFO12695		Gluconate	$(NH_4)_2SO_4$	144	12	0.24	Flask	Mori et al. 1987
Enterococcus casseliflavus A-12		Gluconate	Yeast extract	72	16	0.32	Flask	Yanase et al. 1992
Ralstonia eutrophus N9A-PHB$^-$02-HB$^-$1	Unable to utilize 3-hydroxybutyrate	Lactate	Ammonium		9.7	0.53	4 l Fermenter	Steinbüchel and Schlegel 1989
Corynebacterium sp.		1,2-Propanediol	Ammonium		8.1	0.41	Flask	Takao and Tanida 1977
Acinetobacter sp. 80-M	B_1^-	1,2-Propanediol	Polypeptone	96	11.6	0.58	Flask	Izumi et al. 1982
Pseudomonas sp. TB-135		1,2-Propanediol	NH_4NO_3	72	14	0.35	Flask	Shigene and Nakahara 1991

[a] Classified according to the category of carbon source used

genes were cloned by Kawasaki et al. (1996) and used to construct a plasmid (pKT901EA) which was then transformed into pyruvate producer *E. coli* W1485*lip2*. In the recombinant strain, the tryptophan concentration was increased to 23.7 g/l in a medium supplemented with 50 g/l glucose.

Production of pyruvate by the resting cell method

Two fermentative methods, i.e. the direct fermentative method and the resting cell method, have been employed to produce pyruvate in the past 20 years of research. As described above, in the direct fermentative method, pyruvate was directly produced from certain carbon sources via the Embden-Meyerhof-Parnas (EMP) pathway if glucose was used as the substrate. However, in the resting cell method, pyruvate was produced by conversion of the substrate by cells separated from growth medium. Production of pyruvate by the resting cell method is different from the enzymatic method, which will be described in detail below. In the latter, pyruvate is synthesized from a substrate (such as lactate) by a single enzyme in microbial cells, while in the former, pyruvate is produced from a substrate (such as glucose) by a series of enzymes in microbial cells.

The microorganisms that have been used to produce pyruvate by the resting cell method are listed in Table 4. Some of these, such as *Acinetobacter* sp. and *Debaryomyces coudertii*, can also be used in direct fermentative methods. That the culture time can be reduced is the main advantage of the resting cell method. For example, although the pyruvate concentrations produced by *Acinetobacter* sp. and *Debaryomyces coudertii* using the resting cell method are lower than in the case of using direct fermentative methods, the cultivation time was decreased from 96 h to 24 h for *Acinetobacter* sp. and from 48 h to 10 h for *Debaryomyces coudertii*. However, in this process, cells must be cultivated, separated, and washed before being used in the biosynthesis of pyruvate; such a complicated operation seems to be a great drawback for the application of the resting cell method.

Table 4 An overview of literature on the fermentative production of pyruvate by resting cell method

Strains	Methods	Nutrient requested	Carbon source	Nitrogen source	Time (h)	Pyruvate (g/l)	Yield (g/g)	Reactor	Reference
Acinetobacter sp. 80-M	First cultivation, then pyruvate production	B_1^-	1,2-Propanediol		24	10	0.50	Flask	Izumi et al. 1982
Candida sp.	Cells grown at pH 4.5–5.5, pyruvate production at pH 3.5–4.5	B_1 is needed exclusively in the growth phase	Glucose	NH_4NO_3	180	40.6	0.41	5 l Fermentor	Besnainou et al. 1989, 1990
Debaryomyces coudertii	First cultivation, then pyruvate production	ATP and NAD^+ added as cofactors	Citrus peel extract		10	7.9	0	Flask	Moriguchi et al. 1984
Enterobacter aerogenes SM-18	First cultivation, then pyruvate production	Lipoic acid and $NaAsO_2$	Glucose		10	3.6	0.8	Flask	Yokota and Takao 1989
Xanthomonas campestris	After nitrogen was consumed, glucose was added to produce pyruvate	Yeast extract	Glycerol and glucose	$NaNO_3$	30	10.3	0.29	Flask	Behrens and Fiedler 1979
Yarrowia lipolytica 3–8	First cultivation, then pyruvate production		Glucose		96	75.4	0.754	Flask	Saeki 1997

Table 5 An overview of literature on the enzymatic synthesis of pyruvate by whole cells or enzymes

Strains	Enzyme	Substrate (h)	Time (g/l)	Pyruvate	Yield (%)	Reference
Acetobacter sp. ATCC 21409		D-Lactic acid		10	95	Cooper 1989
Bacteria	Formaldehyde dehydrogenase	Methylglyoxal	10	3.6		Tarama et al. 1988
Clostridium sporogenes	Pyruvate synthase	Acetyl phosphate		10		Lovitt et al. 1987; Dixon et al. 1989
Hansenula polymorpha *Pichia pastoris*	Glycolate oxidase catalase	L-Lactic acid	6	89.6	96	Anton et al. 1995, 1996 Eisenberg et al. 1997
Pediococcus homari IFO 12217		Glycerol	24	15	30	Yajime et al. 1986
Pseudomonas putida ATCC 17642	Tartrate dehydralase	L-Tartaric acid	35	27.5	92.3	Miyata et al. 1986
Rhodotorula gracilis	D-Amino acid oxidase Catalase			0.23 g $U^{-1}\cdot day^{-1}$		Buto et al. 1994

In addition, some resting cell methods do not require that the cells be separated from growth medium. In the patent of Behrens and Fiedler (1979), cells grew in a medium supplemented with glycerol and $NaNO_3$ as carbon and nitrogen source, respectively. When $NaNO_3$ was completely consumed, glucose was added to the medium to make cells accumulate pyruvate due to nitrogen deficiency. It is an interesting idea, but unfortunately the pyruvate concentration is not high. Another example was patented by Besnainou et al. (1989, 1990). Cells first grew under pH 4.5–5.5 for 90 h, then the pH was reduced to 3.5–4.5. Pyruvate production was promoted because the production of α-ketoglutarate was inhibited. However, the reactor operation is too complicated to scale-up and the cultivation time is too long to avoid contamination.

Production of pyruvate by whole cells or enzymes

Production of pyruvate by enzymatic methods is another emphasis in this field. The advantage of this method is thought to be the simple composition of reaction mixture and the high conversion rate of substrate, as well as the convenience of recovery. Table 5 summarizes the re-

search results on the enzymatic production of pyruvate. *Acetobacter* sp. can oxidize D-(–)-lactate to pyruvate with a high conversion rate (Cooper 1989). However, it is difficult to commercialize this process because D-(–)-lactate is more expensive than L-(+)-lactate. High substrate conversion rate can also be achieved by oxidizing L-lactate to pyruvate catalyzed by glycolate oxidase in *Hansenula polymorpha* (Anton et al. 1995). Hydrogen peroxide, which is produced during the process of oxidizing lactate to pyruvate, must be removed or transformed in due course, otherwise the pyruvate produced will be further oxidized to acetate. If this problem of further oxidation of pyruvate by hydrogen peroxide can be solved, this process has the potential to be commercialized because of the low price of lactate. In *Pichia pastoris*, catalase was used to remove hydrogen peroxide produced in the oxidation of lactate (Anton et al. 1996; Eisenberg et al. 1997). However, until now there is no information indicating that this process has been successfully industrialized.

Recovery of pyruvate

Free pyruvic acid cannot be crystallized from water systems and is unstable in alkaline conditions, which makes the recovery of free pyruvic acid from fermentation broth a bottleneck in the fermentative production of pyruvate. Ion-exchange is the method commonly used for the recovery of organic acids, but detailed process parameters for pyruvate recovery are difficult to find in the literature due to industrial secrecy (Matsuno et al. 1994). According to the characteristics of pyruvic acid, a weakly basic resin was chosen to separate pyruvate from fermentation broth (Fu 1999); however, the resin cannot be of OH^- type because of the alkali-instability of pyruvic acid. Therefore, Cl^- type resin was used in the experiments, but the low exchange capacity makes the resin easily saturated. After elution by HCl and neutralization by NaOH, the large amount of Cl^- will affect the purity of final product because the solubility of sodium pyruvate is also very high.

Thus, it seems that only a distillation operation can be used to obtain pyruvic acid with high purity. An alternative process, a solvent extraction plus distillation method, was developed by ourselves (unpublished data). We used an organic solvent to extract pyruvic acid from fermentation broth and purified it from the solvent by distillation. Although pyruvic acid with purity higher than 99% can be obtained, the high energy costs and low recovery rate cannot meet the demands of commercialization.

Production cost

Table 6 gives a cost comparison (raw materials) for the production of pyruvate by chemical, fermentation and enzymatic conversion methods. As far as the material cost is concerned, it is obvious that pyruvate production by fermentation is the most competitive method. However, considering that some by-products will be formed in the fermentation process, pyruvate production by enzymatic conversion of L-lactate is also competitive, not only because of the low cost and high conversion rate, but also because of the lower level of by-product formation as well as convenience of recovery.

Table 6 Cost comparison for the production of pyruvate by chemical, fermentation and enzymatic conversion methods[a]

Chemical method[b]				Fermentation method[c]				Enzymatic conversion method[d]			
Material	Price ($/ton)	Consumption (ton/ton pyruvate)	Cost ($/ton)	Material	Price ($/ton)	Consumption (ton/ton pyruvate)	Cost ($/ton)	Material	Price ($/ton)	Consumption (ton/ton pyruvate)	Cost ($/ton)
Tartaric acid	1,500	3.33	5,000	Glucose	450	1.9	855	Lactate	2,100	1.1	2,300
$KHSO_4$	730	5	3,650	NH_4Cl	150	0.1	15				
				K^+, Mg^{2+}	500	0.04	20				
				Vitamin			20				
				NaOH (30 w/v%)	50	1.5	75				
				H_2SO_4	50	0.6	30				
				Extraction solvent	600	0.4	240				
Total cost ($)			8,650				1,255				2,300

[a] The price of all raw materials was obtained from the latest data of the Chinese chemical market
[b] Assuming the yield of pyruvate from tartaric acid is 0.30 g/g according to Howard and Fraser (1932)
[c] Assuming the yield of pyruvate from glucose, pyruvate concentration, fermentation time, and recovery ratio are 0.55 g/g, 55 g/l, 60 h, and 70%, respectively, according to Li (2000)
[d] Assuming the yield of pyruvate from lactate is 0.96 g/g according to Eisenberg et al. (1997)

Biotechnological production of pyruvate: problems and perspectives

High concentration and high yield as well as high productivity are the objectives to realize a cost-effective biotechnological production of pyruvate. In order to further improve the competitiveness of fermentative production of pyruvate, research should be focused on the following two aspects (Li 2000). Firstly, the further degradation or transformation of pyruvate should be minimized or reduced to achieve high concentration and high yield; secondly, the metabolism of glucose to pyruvate should be accelerated to obtain high productivity. However, reduced TCA cycle flux, caused by the minimized pyruvate degradation to acytyl-CoA, will reduce the biomass concentration and consequently slow down glucose consumption. That is to say, for pyruvate production, it is very difficult to achieve high concentration and high yield as well as high productivity simultaneously.

The multi-vitamin auxotrophic strain *T. glabrata* has been used in the commercial production of pyruvate. However, since *T. glabrata* is not a commonly used strain in the fermentation industry, its physiological characteristics are not well known. For example, dissolved oxygen is a key factor affecting pyruvate accumulation, but the effect of dissolved oxygen on glycolytic activity under conditions of vitamin limitation has not been well studied. Since most NADH is produced in the EMP pathway, and the oxidative pathway of NADH will affect the energy level of cell, it is important to investigate the effect of intracellular NADH and ATP concentration on the production of pyruvate, based on the theoretical analysis contributed by Hua et al. (1999) and Hua and Shimizu (1999).

Fed-batch culture is widely used in the fermentative production of organic acids, amino acids, enzymes etc. However, to date no report has been published on the fed-batch fermentation of pyruvate. According to our results (Li et al. 2000), pyruvate production by *T. glabrata* WSH-IP12 was promoted by feeding glucose and nitrogen sources simultaneously. Pyruvate concentrations higher than 60 g/l will lead to product inhibition, so the detailed study of fed-batch operations, aimed at improving product inhibition in a given strain, is also interesting and attractive.

To summarise, pyruvate, which serves an important physiological role in sugar metabolism, has wide applications in many fields. Biotechnological production of pyruvate will facilitate its use due to low cost compared with chemical methods (Table 6). Although fermentative methods currently play a dominant role, enzymatic methods will be an alternative approach because of less pollution and high conversion rate, providing some of the technical problems mentioned above can be conquered in the future.

Acknowledgements The authors want to thank the Ministry of Education of China and Science & Technology Committee of Jiangsu Province of China for providing financial support. The authors also want to thank referees and Prof. A. Steinbüchel, for their constructive comments.

References

Anton DL, DiCosimo R, Witterholt VG (1995) Process for the preparation of pyruvic acid. WO patent 9500656

Anton DL, DiCosimo R, Witterholt VG (1996) Production of pyruvic acid using permeabilized transformants of *Hansenula polymorpha* and *Pichia pastoris* which express glycolate oxidase and catalase. US patent 5538875

Behrens U, Fiedler S (1979) Recovery of pyruvate. East German Patent 135213

Besnainou B, Giani D, Sahut C (1989) Process for the production of pyruvic acid by fermentation. EP patent 312453

Besnainou B, Giani D, Sahut C (1990) Method for producing pyruvic acid by fermentation. US patent 4918013

Borle A, Stanko RT (1996) Pyruvate reduces anoxic injury and free radical formation in perfused rat hepatocytes. J Appl Physiol 270:535–540

Buto S, Pollegioni L, D'Angiurl L, Pilone MS (1994) Evaluation of D-amino-acid oxidase from *Rhodotorula gracilis* for the production of alpha-keto acids. Biotechnol Bioeng 44:11–18

Cooper B (1989) Microbial manufacture of pyruvic acid from D-(–)-lactic acid. DE patent 3733157

DeBoer LWV, Bekx PA, Han L, Steinke L (1993) Pyruvate enhances recovery of rat hearts after ischemia and reperfusion by preventing free radical generation. J Appl Physiol 265:1571–1576

Dixon NM, James EW, Lovitt RW, Kell DB (1989) Electromicrobial transformation using the pyruvate synthase system of *Clostridium sporagenes*. Bioelectrochem Bioenerg 21:245–259

Eisenberg A, Seip JE, Gavagan JE, Payne MS, Anton DL, DiCosimo R (1997) Pyruvic acid production using methylotrophic yeast transformants as catalyst. J Mol Catal B Enzymatic 2:223–232

Fu W (1999) Recovery of pyruvate by ion-exchange process. Masters thesis, Wuxi University of Light Industry, Wuxi, China

Howard JW, Fraser WA (1932) Preparation of pyruvic acid. Org Synth Coll 1:475–480

Hua Q, Shimizu K (1999) Effect of dissolved oxygen concentration on the intracellular flux distribution for pyruvate fermentation. J Biotechnol 68:135–147

Hua Q, Yang C, Shimizu K (1999) Metabolic flux analysis for efficient pyruvate fermentation using vitamin-auxotrophic yeast of *Torulopsis glabrata*. J Biosci Bioeng 87:206–213

Izumi Y, Matsumura Y, Tani Y, Yamada H (1982) Pyruvic acid production from 1,2-propandediol by thiamine-requiring *Acinetobacter* sp. 80-M. Agric Biol Chem 46:2673–2679

Kawasaki K, Yokota A, Tomita F (1996) L-Tryptophan production by a pyruvic acid-producing *Escherichia coli* strain carrying the *Enterobacter aerogenes* tryptophanase gene. J Ferment Bioeng 82:604–606

Kiuchi M, Mori T, Tabei H, Kadoma M (1987a) Pyruvic acid production by *Torulopsis etchellsii*. JP patent 6214789

Kiuchi M, Mori T, Takami I, Monma M, Tabei H (1987b) Pyruvate production by a halophilic yeast *Torulopsis etchellsii* (in Japanese). J Jpn Soc Food Sci Technol 33 579–584

Li Y (2000) Microbial over-production of pyruvate and metabolic flux analysis. Ph.D dissertation, Wuxi University of Light Industry, Wuxi, China

Li Y, Chen J, Liang DF, Lun SY (2000) Effect of nitrogen source and nitrogen concentration on the production of pyruvate by *Torulopsis glabrata*. J Biotechnol 81:27–34

Li Y, Chen J, Lun SY, Rui XS (2001) Efficient pyruvate production by a multi-vitamin auxotroph of *Torulopsis glabrata*: key role and optimization of vitamin levels. Appl Microbiol Biotechnol 55:680–685

Lovitt RW, James EW, Kell DB, Morris JG (1987) Bioelectrochemical transformations catalyzed by *Clostridium sporogenes*. In: Moody GW, Baker PB (eds) Bioreactors and biotransformations. Elsevier, London, pp 263–276

Matsuno H, Goto M, Yonehara T (1994) Purification of pyruvic acid by weakly-basic anion exchange resin. JP patent 6345683

Miyata R, Yonehara T (1990) Fermentative manufacture of pyruvic acid with *Torulopsis* species. JP patent 02308795
Miyata R, Yonehara T (1996) Improvement of fermentative production of pyruvate from glucose by *Torulopsis glabrata* IFO 0005. J Ferment Bioeng 82:475–479
Miyata R, Yonehara T (1999) Breeding of high-pyruvate-producing *Torulopsis glabrata* with acquired reduced pyruvate decarboxylase. J Biosci Bioeng 88:173–178
Miyata R, Yonehara T (2000) Breeding of high-pyruvate-producing *Torulopsis glabrata* and amino acid auxotrophic mutants. J Biosci Bioeng 90:137–141
Miyata R, Yonehara T, Yomoto K (1986) Manufacture of pyruvic acid from L-tartaric acid with *Pseudomonas putida*. JP patent 61216695
Miyata R, Yonehara T, Yomoto K (1988a) Manufacture of pyruvic acid with *Torulopsis* species. JP patent 63258586
Miyata R, Yonehara T, Yomoto K (1988b) Manufacture of pyruvic acid with *Torulopsis* species. JP patent 63258587
Miyata R, Tsutsui H, Yonehara T (1989a) Manufacture of pyruvic acid with *Torulopsis* species. JP patent 0155185
Miyata R, Tsutsui H, Yonehara T (1989b) Manufacture of pyruvic acid with aminooxyacetic acid-resistant *Torulopsis* species. JP patent 0155186
Miyata R, Yonehara T, Yotsumoto K, Tsutsui H (1989c) Preparing pyruvic acid by fermentation with *Torulopsis* species. WO patent 8901523
Miyata R, Yonehara T, Tsutsui H (1990) Method for producing pyruvic acid by fermentation. US patent 4971907
Miyata R, Igarashi H, Yonehara T (2000) Manufacture of pyruvic acid with (halogenated) pyruvic acid-resistant microorganisms. JP patent 2000078996
Mori N, Jitsunari K, Yoshida M, Kitamoto Y, Ichikawa Y (1987) Pyruvic acid production from gluconic acid by *Pseudomonas stutzeri* (in Japanese). Tottori Daigaku Nogakubu Kenkyu Hokoku 40:15–20
Moriguchi M (1982) Fermentative production of pyruvic acid from citrus peel extract by *Debaryomyces coudertii*. Agric Biol Chem 46:955–961
Moriguchi M, Shuto K, Hashimoto T (1984) Production of pyruvic acid from saccharified citrus peel extract by dried cells of *Debaryomyces coudertii*. J Ferment Technol 62:243–248
Roufs JB (1996) Pyruvate: does it amp endurance and burn more fat? Muscle Fitness 57:195–197
Saeki H (1997) Manufacture of pyruvic acid with *Yarrowia*. JP patent 09252790
Shigene T, Nakahara T (1991) Production of pyruvic acid from 1,2-propanediol by *Pseudomonas* sp. strain TB-135 which does not require thiamine. Biotechnol Lett 13:821–826
Stanko RT, Robertson RJ, Galbreath RW, Reilly JJ, Greenawalt KD Jr, Goss FL (1990) Enhanced leg exercise endurance with a high-carbohydrate diet and dihydroxyacetone and pyruvate. J Appl Physiol 69:1651–1656
Stanko RT, Tietze DL, Arch JE (1992) Body composition, energy utilization, and nitrogen metabolism with a 4.25-MJ/d low-energy diet supplemented with pyruvate. Am J Clin Nutr 56:630–635
Stanko RT, Reynolds HR, Hoyson R, Janosky JE, Wolf R (1994) Pyruvate supplementation of a low-cholesterol, low-fat diet: effects on plasma lipid concentrations and body composition in hyperlipidemic patients. Am J Clin Nutr 59:423–427
Steinbüchel A, Schlegel HG (1989) Excretion of pyruvate by mutants of *Ralstonia eutrophus* which are impaired in the accumulation of poly(β-hydroxybutyric acid)(PHB), under conditions permitting synthesis of PHB. Appl Microbiol Biotechnol 31:168–175
Takao S, Tanida M (1977) Pyruvic acid production from 1,2-propylene glycol by *Corynebacterium* sp. (in Japanese). Nippon Nogei Kogaku Kaishi 51:239–233
Takao S, Tanida M (1982) Pyruvic acid production by *Schizophyllum commune*. J Ferment Technol 60:277–280
Takao S, Yokota A, Tanida M (1984) Enzymatic production of tryptophan coupled to pyruvic acid fermentation. J Ferment Technol 62:329–334
Tarama S, Sogabe Y, Mitsuhida N (1988) Manufacture of pyruvic acid with enzymes or bacteria. JP patent 63133990
Tomita F, Yokota A (1993) Manufacture of pyruvic acid with *Escherichia* and pyruvic acid-producing mutant of *Escherichia*. JP patent 05137568
Yajime Y, Sakuratani N, Ando M, Inoue H (1986) Production of pyruvic acid. JP patent 61227789
Yanai T, Tsunekawa H, Okamura K, Okamoto R (1994). Manufacture of pyruvic acid with *Debaryomyces*. JP patent 0600091
Yanase H, Mori N, Masuda M, Kita K, Shimao M, Kato N (1992) Pyruvate production by *Enterococcus casseliflavus* A-12 from gluconate in an alkaline medium. J Ferment Bioeng 73: 287–291
Yokota A (1997) Breeding of useful microorganisms by mutation in energy metabolism (in Japanese). Nippon Nogei Kagaku Kaishi 71:9–14
Yokota A, Takao S (1984) Conversion of pyruvic acid fermentation to tryptophan production by the combination of pyruvic acid-producing microorganisms and *Enterobacter aerogenes* having high tryptophanase activity. Agric Biol Chem 48:2663–2668
Yokota A, Takao S (1989) Pyruvic acid production by lipoic acid auxotrophs of *Enterobacter aerogenes*. Agric Biol Chem 53:705–711
Yokota A, Tomita F (1995) Enhancement of fermentative production of pyruvic acid by a mutant defective in energy metabolism (in Japanese). Baiosaiensu to Indasutori 53:410–412
Yokota A, Oita S, Takao S (1989) Tryptophan production by a lipoic acid auxotroph of *Enterobacter aerogenes* having both pyruvic acid productivity and high tryptophanase activity. Agric Biol Chem 53:2037–2044
Yokota A, Shimizu H, Terasawa Y, Takaoka N (1994a) Pyruvic acid production by a lipoic acid auxotroph of *Escherichia coli* W1485. Appl Microbiol Biotechnol 41:638–643
Yokota A, Terasawa Y, Takaoka N, Shimizu H, Tomita F (1994b) Pyruvate production by an F_1-ATPase-defective mutant of *Escherichia coli*. Biosci Biotechnol Biochem 58:2164–2167
Yokota A, Henmi M, Takaoka N, Hayashi C, Takezawa Y, Fukumori Y, Tomita F (1997) Enhancement of glucose metabolism in a pyruvic acid-hyperproducing *Escherichia coli* mutant defective in F_1-ATPase activity. J Ferment Bioeng 83:132–138
Yonehara T, Miyata R (1994a) Fermentative production of pyruvate from glucose by *Torulopsis glabrata*. J Ferment Bioeng 78:155–159
Yonehara T, Miyata R (1994b) Fermentative production of pyruvic acid by yeast (in Japanese). Baiosaiensu to Indasutori 52: 567–570
Yonehara T, Yomoto K (1987a) Pyruvic acid manufacture by *Torulopsis*. JP patent 62275688
Yonehara T, Yomoto K (1987b) Microbial production of pyruvic acid and its enhancement by thiamine. JP patent 62201589
Yonehara T, Miyata R, Matsuno H, Goto M, Yahanda S (2000) Development of fermentative production of pyruvate by metabolic control – monograph (in Japanese). Seibutsu Kogaku Kaishi 78:56–62
Uchio R, Hirose Y (1975) Fermentative production of pyruvic acid. JP patent 7582284
Uchio R, Hirose Y, Node I (1974a) Fermentative production of pyruvic acid. JP patent 74132291
Uchio R, Maeyashiki I, Okada H (1974b) Fermentative production of pyruvic acid. JP patent 74102894
Uchio R, Kikuchi K, Enei H, Hirose Y (1976) Process for producing pyruvic acid by fermentation. US Patent 3993543