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Production of 3-Hydroxypropionic Acid from Acrylic Acid by Newly Isolated *Rhodococcus erythropolis* LG12

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A novel microorganism, designated as LG12, was isolated from soil based on its ability to use acrylic acid as the sole carbon source. An electron microscopic analysis of its morphological characteristics and phylogenetic classification by 16S rRNA homology showed that the LG12 strain belongs to *Rhodococcus erythropolis*. *R. erythropolis* LG12 was able to metabolize a high concentration of acrylic acid (up to 40 g/l). In addition, *R. erythropolis* LG12 exhibited the highest acrylic acid-degrading activity among the tested microorganisms, including *R. rhodochrous*, *R. equi*, *R. rubber*, *Candida rugosa*, and *Bacillus cereus*. The effect of the culture conditions of *R. erythropolis* LG12 on the production of 3-hydroxypropionic acid (3HP) from acrylic acid was also examined. To enhance the production of 3HP, acrylic acid-assimilating activity was induced by adding 1 mM acrylic acid to the culture medium when the cell density reached an OD_{600} of 5. Further cultivation of *R. erythropolis* LG12 with 40 g/l of acrylic acid resulted in the production of 17.5 g/l of 3HP with a molar conversion yield of 44% and productivity of 0.22 g/l/h at 30°C after 72 h.

Keywords: 3-Hydroxypropionic acid, acrylic acid, *Rhodococcus erythropolis* LG12, resting cell reaction

3-Hydroxypropionic acid (3HP) is used as an intermediate for the synthesis of many commercially valuable chemicals, including 1,3-propanediol, malonic acid, acrylic acid, acrylonitrile, and acrylamide, all of which are used in large quantities for the production of adhesives, polymers, plastic packing, fibers, cleaning agents, and resins [9]. Nonetheless, despite its role as a key intermediate for many chemicals, commercially available chemical processes for the production of 3HP have not yet been described. Currently, 3HP is processed based on the hydration of acrylic acid or the reaction of ethylene chlorohydrin with sodium cyanide followed by the hydrolysis of the synthesized β-propiolactone [14]. However, the ammonia generated during this process is potentially explosive, as the reaction is strongly exothermic. Thus, as in the case of 1,3-propanediol production, biochemical processes using microorganisms can be an effective alternative to costly multistep chemical processes. Therefore, since 3HP is the key intermediate for autotrophic CO_2 fixation in *Chloroflexus aurantiacus* and various archaebacteria [2], and can be converted from many metabolic intermediates, such as glycerol, lactate, and β-alanine, several microorganisms have already been metabolically engineered for the production of 3HP from glucose. Yet, these strategies are still in the initial stages, as only small amounts of 3HP (approximately 0.2 g/l) have so far been produced from glucose [17]. In addition, various factors affecting the process availability, such as a high theoretical yield and the availability of enzymes and cofactors, need to be further considered for the successful development of a biological process. When compared with the process of synthesizing 3HP from glucose, the direct conversion of acrylic acid into 3HP using microorganisms has several benefits: acrylic acid as an inexpensive starting material, a simple conversion process, and relatively good conversion yield of acrylic acid into 3HP (50–90%) [12, 18]. As previously reported, several microorganisms, including *Candida rugosa* [12] and *Byssochlamys* sp. [18], are able to convert acrylic acid into 3HP. However, since acrylic acid severely inhibits microbial cell growth owing to its antibacterial impact across a wide spectrum of bacterial species [4, 8, 15, 16], it is important to find a microorganism that can support a high acrylic acid assimilation rate and resist a high acrylic acid concentration when developing a process that uses acrylic acid as the starting material. It has already been reported that *C. rugosa* can assimilate 20 g/l of acrylic acid within 4 days [12], whereas *Byssochlamys* sp. can degrade 70 g/l of acrylic acid within 11 days [18]. Thus, in the

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context of resistance to a high concentration of acrylic acid, these strains would seem to be suitable for a process using acrylic acid to produce fine chemicals. However, the use of these strains for the degradation of acrylic acid has various disadvantages, including a relatively long degradation time of acrylic acid, complex culture conditions for cell growth, a long culture time, and difficulties related to the preparation of recombinant strains. In contrast, the Gram-positive bacterium *Rhodococcus* sp., belonging to nocardioform actinomycetes, provides certain solutions to the abovementioned problems, as it is tolerant of acrylic acid, easy to cultivate, and already has a developed recombinant DNA technology. In a previous study, a high concentration of acrylic acid (180 g/l) was accumulated in the medium as a dead-end product during the conversion process of acrylonitrile using *Rhodococcus rhodochrous* immobilized in polymer beads [21].

Accordingly, this study reports on the isolation of an acrylic acid-assimilating bacterium, *Rhodococcus erythropolis* LG12, from soil. The culture characteristics for the high-yield conversion of acrylic acid into 3HP are also investigated.

MATERIALS AND METHODS

Strain Isolation

Samples collected from wastewater treatment facilities, industrial chemical complexes, rice paddy fields, and soil were dried at room temperature for 24 h. After resuspending the dried samples in autoclaved distilled water, the supernatants were seeded into 50-ml baffled flasks containing 20 ml of a defined liquid medium containing 10 mM acrylic acid (Sigma Aldrich, U.S.A.) as the carbon source and cultured in a shaking incubator at 200 rpm and 30°C for 3 days. Thereafter, 1 ml of the culture broth was used to inoculate 20 ml of the same fresh liquid medium and cultured under the same conditions as mentioned above. This procedure was repeated three times, and then the culture was spread on an LB solid agar (containing per liter: tryptone 10 g, yeast extract 5 g, NaCl 5 g, agar 17 g) and incubated at 30°C. Finally, a novel strain utilizing acrylic acid as its carbon source was isolated and named LG12.

The defined liquid medium contained (per liter) $(NH4)_2SO_4$ 3.33 g, K_2HPO_4 6.4 g, $MgSO_4$ 2.78 g, and 10 ml of a trace metal solution which contained (per liter) $FeSO_4 \cdot 7H2O$ 200 mg, $ZnCl_2$ 100 mg, $MnSO_4 \cdot 5H_2O$ 30 mg, $Na_2B_4O_7 \cdot 10H_2O$ 100 mg, $CoCl_2 \cdot 6H_2O$ 20 mg, $CuSO_4 \cdot 5H_2O$ 10 mg, $NiCl \cdot 6H_2O$ 10 mg, $Na_2MoO_4 \cdot 2H_2O10$ mg, $CaCl_2 \cdot 2H_2O$ 10 mg, and 10 ml of a vitamin solution which contained (per liter) thiamine·HCl 4 mg, riboflavin 2 mg, D-pantothenic acid 4 mg, pyridoxine·HCl 4 mg, p-aminobenzoic acid 4 mg, nicotinic acid 4 mg, inocitol 20 mg, and biotin (0.02% solution) 100 µl.

Scanning Electron Microscopy (SEM)

The strain specimen was prepared according to a previous report with a slight modification [7]. The sample was fixed in a 2% glutaraldehyde solution in a 0.1 M cacodylated buffer (pH 7.2) for 2 h and washed with the same buffer. The sample was then dehydrated in 30% ethanol for 30 min and 70% ethanol for a further 1 h. Finally, the sample was left for 24 h in a 100% ethanol solution. The specimen was examined using a model S-510 SEM system (Hitachi, Japan).

16S rRNA Nucleotide Sequence Analysis

The chromosome of the LG12 strain was isolated using a Wizard Genomic DNA Purification Kit (Promega, U.S.A.). A PCR was then used to amplify the 16S rRNA gene from the isolated chromosome as the template using the primers HK12: 5-GAGTTTGATCCTGGC-TCAG-3 and HK13: 5-TACCTTGTTACGACTT-3 [10]. The PCR was performed using a Peltier Thermal Cycler PTC-200 (MJ Research, U.S.A.). *E. coli* XL1-Blue (*recA1, endA1, gyrA96, thi, hsdR17, suppE44, relA1, l⁻, lac⁻, F' [proAB lacI^q lacZΔM15, Tn10 (Tet)^r]*) (Stratagene Cloning Systems, U.S.A.) was used as the cloning host in the genetic manipulation. The resulting 16S rRNA PCR product was then inserted into a pGEM-T easy vector (Promega, U.S.A.), and the nucleotide sequence of the 16S rRNA gene determined with an automatic sequencer (ABI Prism 3700, U.S.A.) using the dideoxy method. Sequence similarity searches were performed using the BLAST 2.0 program [3], and the phylogenetic tree of the nucleotide sequence was analyzed using the Clustal X 1.83 program [20]. The accession numbers for the sequences of the 16S rRNA nucleotide used as references are shown in Table 1.

GenBank Accession Number

The complete sequence of the 16S rRNA nucleotide of *R. erythropolis* LG12 has been deposited in the GenBank database under Accession No. EU852376.

Comparison of Acrylic Acid-Degrading Activities of Various Microorganisms Using Resting Cell Reaction in Buffer

To compare the acrylic acid-degrading activity of the isolated *R. erythropolis* LG12 with that of other microorganisms, the degradation of acrylic acid was examined using resting cells in a 50 mM Tris-HCl buffer (pH 7.0). Each microorganism was first cultured in a baffled flask containing 200 ml of a YEPD medium [containing (per liter) 10 g of yeast extract, 20 g of bactopeptone, and 20 g of glucose] in a shaking incubator at 200 rpm and 30°C for 24 h. The cell pellets harvested by centrifugation at 6,000 ×*g* for 5 min were then resuspended in a 50 mM Tris-HCl buffer (pH 7) to make a cell concentration of 20 g/l. A reaction mixture containing 10 g/l of acrylic acid, 20 g/l of glucose, and 20 g/l of resting cells was then adjusted to a final volume of 20 ml. The resting cell reaction was carried out in a shaking incubator at 200 rpm and 30°C for 24 h. Thereafter, the cell pellets were removed by centrifugation at 10,000 ×*g* for 10 min and the organic acid concentration of the supernatant was analyzed using high-performance liquid chromatography (HPLC). Plus, to examine the effect of acrylic acid induction on the degradation of acrylic acid, resting cells of *R. erythropolis* LG12 were prepared using the same procedure as described above, except for the addition of 1 mM acrylic acid to the culture medium when the cell density reached an OD_{600} of 5, in order to activate the cells for more acrylic acid assimilation. Furthermore, the effect of temperature, pH, and neutralizers on the production of 3HP when using resting cells of *R. erythropolis* LG12 was examined in appropriate buffers, including 50 mM sodium acetate (pH 4, 5, and 6) and Tris-HCl (pH 7, 8, and 9), according to the reaction pH.

Flask Culture of *R. erythropolis* LG12 for Production of 3HP

R. erythropolis LG12 was cultured in baffled flasks containing 200 ml of the YEPD medium in a shaking incubator at 200 rpm and 30°C. When the cell density reached an OD_{600} of 5, 1 mM acrylic acid was added to the medium to activate the cells for the efficient

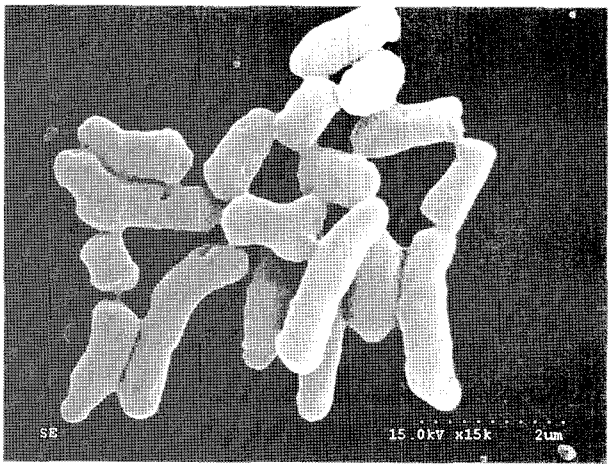

Fig. 1. Scanning electron microscope photo of *Rhodococcus erythropolis* LG12 isolated from soil.

assimilation of acrylic acid. When the cell density reached an OD_{600} of 15, an appropriate amount of calcium acrylate was added to the culture medium to produce 3HP.

Analytical Methods

The HPLC (Shimadzu, Japan) was carried out using a reverse phase C18 Capcel PAK column (Shiseido, Japan, 250 mm×4.6 mm) and the acrylic acid was analyzed at UV 210 nm (mobile phase, water/trifluoroacetic acid=100/0.1; flow rate, 1 ml/min). Meanwhile, an Aminex87H (Bio-rad, U.S.A.) ion-exchange column and RI detector (Shimadzu, Japan) were used to analyze the organic acids (mobile phase, 0.02 N sulfuric acid; flow rate, 0.6 ml/min).

The GC-mass spectrometry (GC-MS) was carried out using a Hewlett-Packard 5890 series gas chromatograph equipped with a capillary column (HP-5MS of Agilent Tech, U.S.A.; I.D. 0.25 mm, length, 30 m; film thickness, 0.25 μm). The temperature program was 50°C for 5 min, 10°C/min, and 320°C for 10 min. Helium was used as the carrier gas with a flow rate of 3 ml/min. The 3HP was analyzed using an HP 5971A mass selective detector.

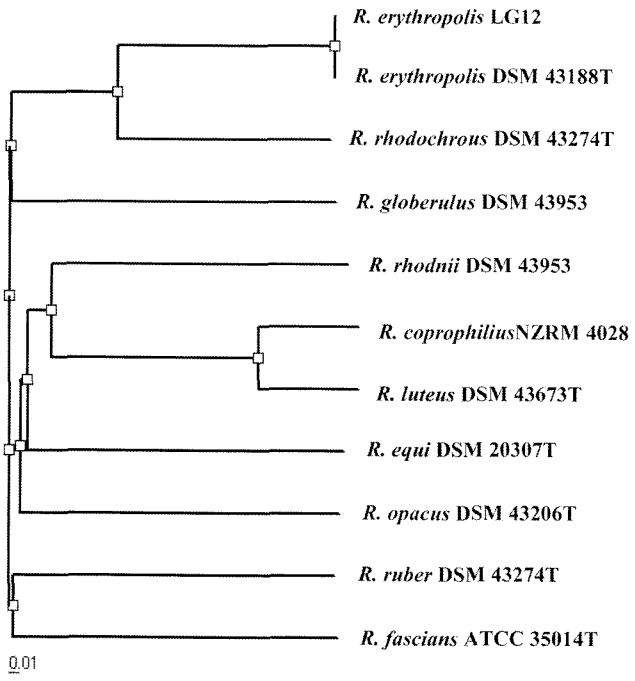

Fig. 2. Phylogenetic tree of *R. erythropolis* LG12 with representative *Rhodococcus* sp.
The tree is based on the 16S rRNA nucleotide sequence of *R. erythropolis* LG12 in the context of a homology search. Scale bar represents 1 base substitution per 100 bases.

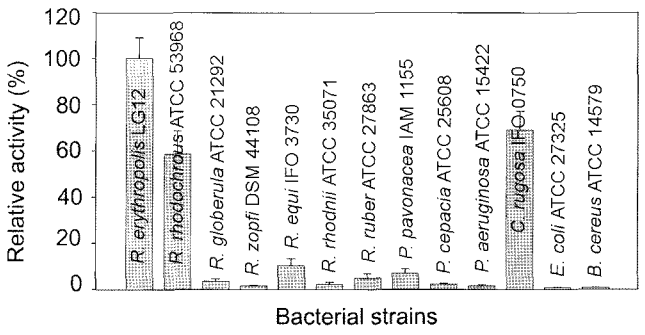

Fig. 3. Comparison of acrylic acid degradation activities of *R. erythropolis* LG12 and various microorganisms.
All the bacterial strains used in this experiment are represented in their corresponding bar.

Results

Identification of Isolated LG12 Strain

The LG12 strain was identified using electron microscopy and molecular biological methods. The LG12 strain was

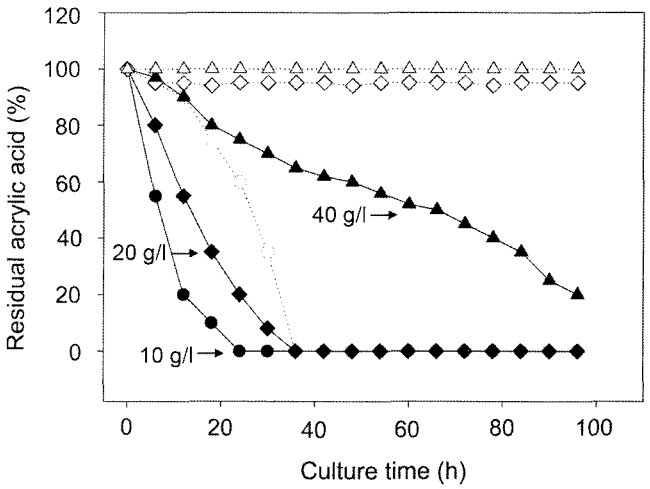

Fig. 4. Acrylic acid degrading activity of *R. erythropolis* LG12 in YEPD medium containing various amounts of acrylic acid.
Closed symbols indicate reaction with acrylic acid-induced *R. erythropolis* LG12, and open symbols indicate reaction with *R. erythropolis* LG12 without acrylic acid induction (●○; 10 g/l, ◆◇; 20g/l; △▲; 40g/l of acrylic acid).

Table 1. 16S ribosomal RNA sequence similarity between species of genus *Rhodococcus* and *Rhodococcus erythropolis* LG12.

Species	Accession number	Similarity (%)
R. erythropolis LG12	EU852376	100
R. erythropolis DSM43188T	X80618	100
R. rhodnii DSM43959	X80623	96
R .opacus DSM43206T	X80631	97
R. globerulus DSM43953	X80620	90
R. ruber DSM43338T	X80625	95
R. rhodochrous DSM43274T	X80624	95
R. equi DSM 20307T	X80614	95
R. luteus DSM 43673	X80620	96
R. fascians ATCC 35014T	X81932	96
R. coprophilus NZRM 4028	AJ306402	96

first spread on an LB plate and incubated at 30°C for 4 days. The morphological characteristics of the cells were then observed using a scanning electron microscope (Fig. 1). As shown in Fig. 1, the LG12 strain grew into a flock and had a smooth rod shape, which are characteristics generally observed for *Rhodococcus* sp., as previously reported [13]. The 16S rRNA gene amplified from the genomic DNA of the LG12 strain was sequenced and a homology search of the 16S rRNA gene sequence was carried out using the GenBank database. In addition, the 16S rRNA gene sequence for the LG12 strain was aligned with those for other *Rhodococcus* strains using the Clustal X program. Phylogenetic and sequence similarity analyses showed that the LG12 strain had a homology of more than 99% with *R. erythropolis* (Fig. 2; Table 1). Therefore, the results of the electron microscopic analysis and phylogenetic classification suggested that the isolated LG12 strain belongs to *R. erythropolis*.

Comparison of Acrylic Acid-Degrading Activity of Various Microorganisms

Acrylic acid is known to be toxic to many microorganisms, as it inhibits β-ketoacyl-CoA thioesterase that catalyzes the final step of fatty acid β-oxidation [19]. Thus, to produce 3HP at a higher titer, it is necessary to use a microorganism with a good resistance to acrylic acid and high acrylic acid assimilation activity as the host strain. The acrylic acid-degrading activities of various bacteria were examined using a resting cell reaction and compared with that of *R. erythropolis* LG12 (Fig. 3). As shown in Fig. 3, *R. erythropolis* LG12 exhibited a significantly higher acrylic acid-degrading activity than microorganisms belonging to other genera, such as *Pseudomonas*, *Candida*, *Escherichia*, and *Bacillus*, plus other species of the genus *Rhodococcus*. Whereas *R. erythropolis* LG12 was able to degrade the acrylic acid completely, most of the other microorganisms, with the exception of *C. rugosa*, were only able to degrade less than 10% of the acrylic acid in the resting cell reaction (Fig. 3).

Effect of Acrylic Acid Induction on Assimilation of Acrylic Acid by *R. erythropolis* LG12 in Resting Cell Reaction

The acrylic acid-degrading activity of *R. erythropolis* LG12 was also examined by varying the initial concentration of acrylic acid in the resting cell reaction buffer. To examine

Table 2. Production of 3HP from acrylic acid by flask cultures of *R. erythropolis* LG12.

Culture time (h)	Cell concentration (g/l)	3HP (g/l) (Productivity (g/l/h))[a]			Glucose consumed (g/l)		
		Acrylic acid added (g/l)					
		10	20	40	10	20	40
24	10.31 ± 2.54	5.13 ± 0.85 (0.21)	10.62 ± 1.43 (0.43)	5.12 ± 0.83 (0.22)	10.28 ± 0.56	13.28 ± 0.59	15.27 ± 2.08
48	10.67 ± 2.21	4.01 ± 1.13 (0.084)	7.95 ± 1.33 (0.16)	10.32 ± 1.36 (0.22)	11.21 ± 0.79	14.31 ± 0.78	17.20 ± 2.78
72	11.57 ± 2.63	4.03 ± 1.21 (0.056)	8.09 ± 1.23 (0.11)	17.49 ± 1.27 (0.22)	11.71 ± 0.72	14.30 ± 0.79	18.71 ± 2.21
96	11.51 ± 2.46	3.88 ± 1.07 (0.040)	7.88 ± 1.22 (0.082)	12.15 ± 2.17 (0.13)	12.00 ± 0.21	14.91 ± 0.21	19.50 ± 0.50
120	11.08 ± 1.27	-	-	6.33 ± 0.96 (0.053)	-	-	0

[a]Volumetric productivity was defined as the amount of 3HP converted from acrylic acid per liter per 1 h.

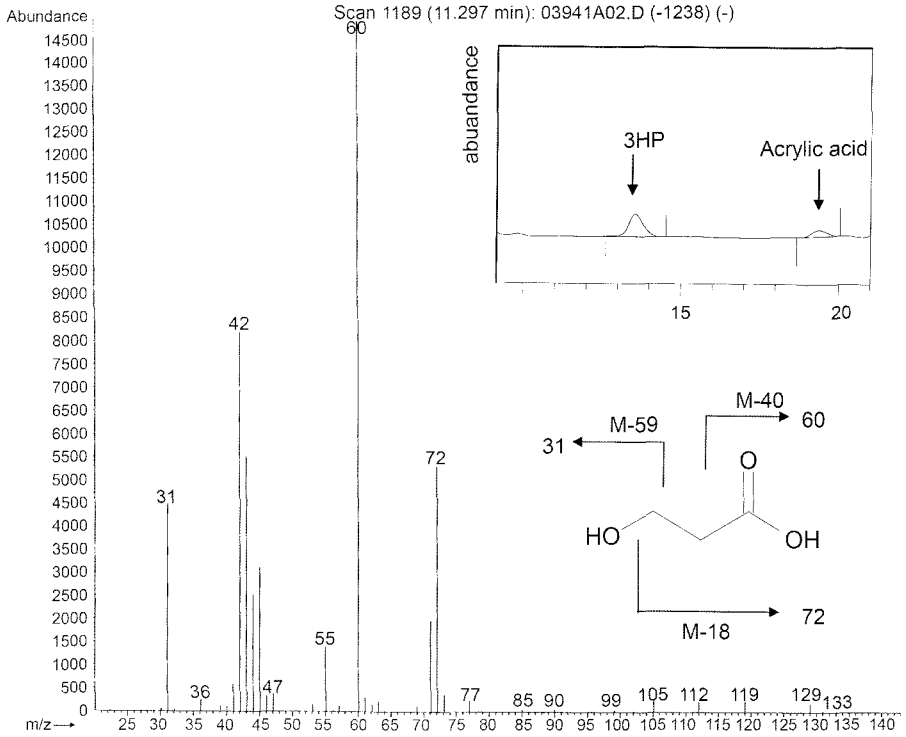

Fig. 5. Conversion of acrylic acid into 3HP by *R. erythropolis* LG12.
The production of 3HP from acrylic acid was identified by HPLC and GC-MS analyses. The proposed fragmentation mechanism of 3HP and its mass spectrum are also shown.

the effect of acrylic acid induction on the acrylic acid-degrading activity of *R. erythropolis* LG12, 1 mM acrylic acid was added to the medium when *R. erythropolis* LG12 grew to an OD_{600} of 5. When 10 g/l of acrylic acid was used for the reaction, *R. erythropolis* LG12 was able to degrade the acrylic acid very efficiently, regardless of acrylic acid induction (Fig. 4). However, without acrylic acid induction, *R. erythropolis* LG12 was not able to assimilate more than 20 g/l of acrylic acid. In contrast, with induction, *R. erythropolis* LG12 was able to degrade acrylic acid efficiently, resulting in 80% assimilation of 40 g/l of acrylic acid in 100 h (Fig. 4). Therefore, these results clearly showed that the adaptation of *R. erythropolis* LG12, based on adding small amounts of acrylic acid before the reaction, was important to achieve a good assimilation of a high concentration of acrylic acid.

Production of 3-Hydroxypropionic Acid Using Flask Cultures of *R. erythropolis* LG12

Flask cultures of *R. erythropolis* LG12 in a YEPD medium containing 10 g/l of acrylic acid were conducted for 48 h to evaluate the production of 3HP. The resulting 3HP was purified by HPLC for further analysis (Fig. 5). The purified fraction was freeze-dried, followed by acidification and extraction with ethyl acetate. The extracted fraction was confirmed based on a GC-MS analysis. The mass spectrum and proposed fragmentation mechanisms are presented in Fig. 5. The results clearly showed that *R. erythropolis* LG12 was able to convert acrylic acid into 3HP.

Flask cultures of *R. erythropolis* LG12 in YEPD media containing various amounts of acrylic acid were also performed for 120 h to evaluate the production of 3HP (Table 2). When 10 and 20 g/l of calcium acrylate were added to the medium, the concentrations of 3HP increased to 5.1 and 10.6 g/l, respectively, after 24 h and then reached a plateau with a slight decrease in the 3HP concentration during further cultivation for 72 h. Thus, a 3HP productivity of 0.21 g/l/h and 0.43 g/l/h and conversion yield of 51% and 53% were achieved after 24 h from 10 and 20 g/l of calcium acrylate, respectively. Meanwhile, when 40 g/l of calcium acrylate was added to the medium, 17.5 g/l of 3HP was synthesized with a productivity of 0.24 g/l/h and conversion yield of 50% after 72 h. However, the 3HP concentration decreased from 17.5 to 6.3 g/l when the glucose was depleted during further cultivation, which seemed to be due to the assimilation of 3HP by *R. erythropolis* LG12 as a carbon source.

Production of 3-Hydroxypropionic Acid by Resting Cell Reaction of *R. erythropolis* LG12

The 3HP production activity of *R. erythropolis* LG12 was examined under various conditions using a resting cell reaction. Based on a previous report [18], several factors affecting the conversion of acrylic acid into 3HP, such as

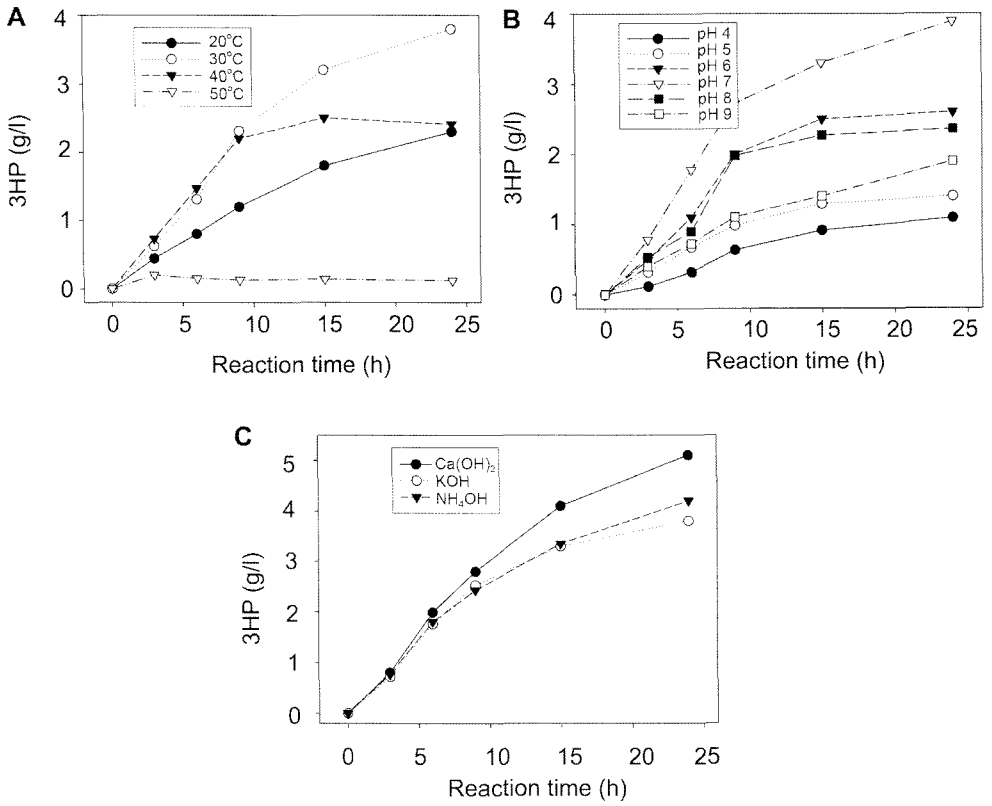

Fig. 6. Effect of reaction temperature, pH, and neutralizers on 3HP production from acrylic acid when using resting cell reactions of *R. erythropolis* LG12.
(**A**) Reaction temperature, (**B**) pH, and (**C**) neutralizers were changed as indicated.

the neutralizer, pH, and temperature, were examined. First, the effect of the reaction temperature was examined for 24 h in a potassium phosphate buffer (pH 7.0) (Fig. 6A). The highest concentration of 3HP was achieved (3.8 g/l) when the reaction was carried out at 30°C, whereas maintaining the temperature at 20°C and 40°C only resulted in half the 3HP concentration achieved at 30°C. Increasing the temperature to 50°C completely eliminated the cell activity converting acrylic acid into 3HP. The effect of the pH was investigated using the following 50 mM buffers: sodium acetate (pH 4, 5, and 6) and Tris-HCl (pH 7, 8, and 9) at 30°C for 24 h. pH 7.0 was identified as the most effective for 3HP production, with alkaline conditions being slightly more favorable for 3HP production than acidic conditions (Fig. 6B). Finally, $Ca(OH)_2$, NH_4OH, and KOH were tested as neutralizers for acrylic acid. This reaction was carried out in a Tris-HCl buffer (pH 7.0) at 30°C, and the highest concentration of 3HP was obtained (5.1 g/l) when using $Ca(OH)_2$ as the neutralizer (Fig. 6C).

DISCUSSION

At present, there are no commercially available chemical and biological processes for the production of 3-hydroxypropionic acid (3HP), an important C3 building block for various commodity and specialty chemicals. When compared with a chemical process using ethylene chlorohydrin and biochemical process consisting of the synthesis of 3HP from glucose, the direct conversion of acrylic acid into 3HP using a microorganism has several advantages, including inexpensive raw materials, a simple conversion process, and high conversion yield of acrylic acid into 3HP (50–90%). However, owing to the process development problems related to the molds that are presently known to convert acrylic acid into 3HP, such as *C. rugosa* [12] and *Byssochlamys* sp. [18], this study decided to screen and examine other microorganisms. As acrylic acid exhibits antimicrobial activity towards most microorganisms, it was important to screen bacteria showing good resistance to a high concentration of acrylic acid and a good assimilation rate of acrylic acid for the development of a microbial process using acrylic acid as the starting material. Thus, a new acrylic acid-degrading bacterium, *R. erythropolis* LG12, was isolated from soil, plus its growth characteristics and 3HP formation were examined in detail. Based on a phylogenetic analysis, strain LG12 was identified as a new species from the genus *Rhodococcus*. *R. erythropolis* LG12 also exhibited the highest acrylic acid-degrading activity among other acrylic acid-degrading bacterial strains (Fig. 3). The assimilation

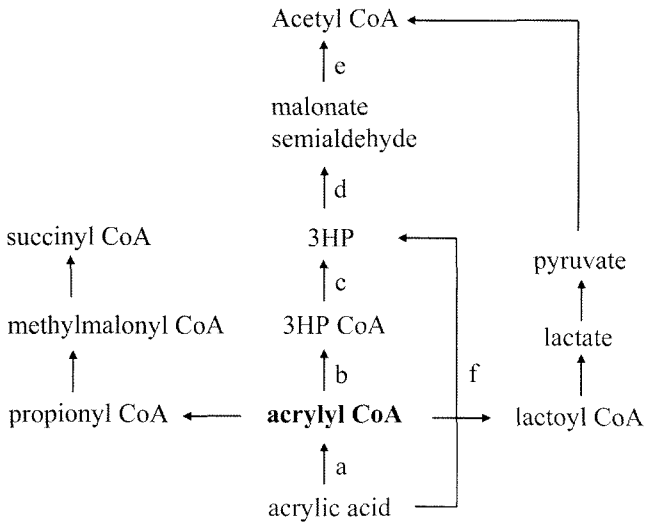

Fig. 7. Proposed metabolic pathway for acrylic acid assimilation. The enzymes were designated as follows: (**a**) acrylyl-CoA (propionyl-CoA) synthetase, (**b**) enoyl-CoA hydratase, (**c**) 3-hydroxyisobutyryl-CoA hydrolase, (**d**) 3-hydroxypropionate dehydrogenase, (**e**) malonate semialdehyde dehydrogenase, and (**f**) acrylase.

rate of acrylic acid dramatically increased by adding 1 mM acrylic acid during the early growth stage (Fig. 4). This may have been due to the induction of proteins involved in the metabolism of acrylic acid, such as propionyl-CoA synthetase (acrylyl-CoA synthetase), enoyl-CoA hydratase, and 3-hydroxyisobutyryl-CoA hydrolase (Fig. 7).

As summarized in Table 2, *R. erythropolis* LG12 showed a superior 3HP production ability (0.43 g/l/h) than previously reported (0.18 g/l/h, [18]; 0.25 g/l/h, [12]) after a short cultivation time, and higher resistance to acrylic acid in flask cultures. Maintaining the glucose concentration was also important to achieve a high 3HP productivity and prevent 3HP degradation, as the depletion of glucose and a low concentration of glucose around 8 g/l led to a reduction of the 3HP accumulated in the medium when adding 40 g/l of acrylic acid, and 10 and 20 g/l of acrylic acid, respectively, to the medium (Table 2). It is already well known that carbon catabolite repression (CCR) is a regulatory mechanism by which the gene expressions required for the utilization of secondary carbon sources are prevented by the presence of a preferred substrate, such as glucose, in bacteria. Thus, when considering that the presence of glucose did not completely prevent the degradation of 3HP in the medium, it would seem to be more important to develop mutant strains that are deficient in the metabolism of 3HP to improve the production of 3HP. Adjusting the pH of the culture medium to around 7 was also important to produce a high yield of 3HP. When free acrylic acid was directly added to the medium, the production of 3HP was significantly reduced, as the culture pH became as low as 4. As shown in the resting cell reaction of *R. erythropolis* LG12, lowering the pH to 4 significantly inhibited the conversion of acrylic acid into 3HP (Fig. 6). A pH that is too low or too high may lead to some thermodynamic constraints as regards the proton gradient required to maintain an electrochemical gradient across the cytoplasmic membrane and inactivate the enzymes involved in the metabolism of acrylic acid. As previously reported, $Ca(OH)_2$ was effective in neutralizing the effect of acrylic acid on the cells [18], thereby increasing the 3HP accumulation during the resting cell reaction (Fig. 6). The calcium ions may have neutralized the negative charges of the carboxylic anions by forming a salt bridge between two acrylic acid molecules. Notwithstanding, the concomitant acrylate dimer ($CH2CH2COO^{-}$-$Ca2^{+}$-$^{-}OOCH2CH2$) no longer inhibited the β-oxidation reaction in the cell, and this change seemed to increase the hydrophobicity of the acrylic acid molecule and facilitate the translocation of the acrylate dimer with a high concentration to the cytosol. However, the metabolic pathway for acrylic acid assimilation in microorganisms remains uncertain. On the basis of available reports [1, 2, 5, 6, 11, 17], a metabolic pathway is proposed in Fig. 7. Finally, since recombinant DNA technology tools are already well developed for *Rhodococcus* strains, the metabolic engineering of *Rhodococcus* to modify the metabolism of acrylic acid and assimilation of 3HP may be a good strategy to enhance the production of 3HP from acrylic acid, along with fermentation process development.

In conclusion, a new acrylic acid-degrading bacterium, *R. erythropolis* LG12, was isolated from soil and its characteristics were examined as regards producing 3HP from acrylic acid. As a result, *R. erythropolis* LG12 would appear to be a good candidate for the production of 3HP from acrylic acid, owing to its high acrylic acid resistance and high 3HP productivity.

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