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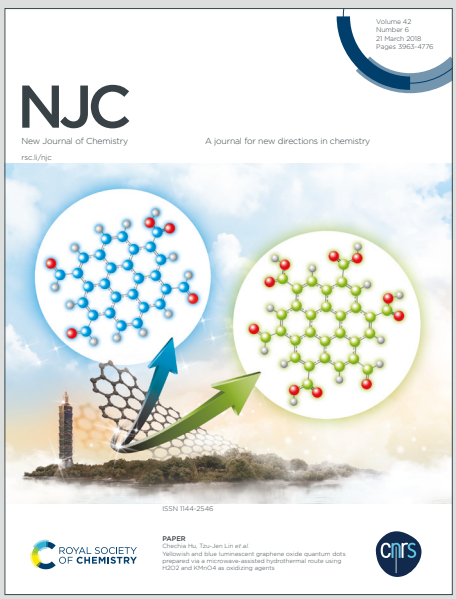

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ARTICLE

Dual-biocatalytic L-lactate production from gaseous CO_2 and acetaldehyde

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L-Lactate has attracted considerable attention as a precursor for biodegradable plastics, poly L-lactic acid (PLA). It is expected that producing biodegradable plastic precursors from CO_2 and bio-based materials will lead to sustainable plastic use, recovery, and recycling. In this study, a new method for the production of L-lactate via pyruvate as an intermediate from acetaldehyde and gaseous CO_2 in the presence of thiamine pyrophosphate (TPP) and nicotinamide adenine dinucleotide reduced form (NADH) was developed by dual-biocatalytic system of pyruvate decarboxylase (PDC; Enzyme Commission numbers (EC) 4.1.1.1) from *Lactobacillus* YK1 and L-lactate dehydrogenase (LDH; EC 1.1.1.27) from chicken heart without the toxic raw material such as a hydrogen cyanide. By using dual-biocatalytic system of PDC and LDH, 0.35% of the acetaldehyde was successfully converted to L-lactate after 5 h incubation.

Introduction

Pyruvate is an important chemical compound in biochemistry and is the output of the metabolism of glucose known as glycolysis.[1] In addition, pyruvate is a useful substance[2-4] that can be converted into precursors for various biodegradable polymers such as L-lactate, L-malate[5-7] and L-alanine,[8-11] as shown in Figure 1.

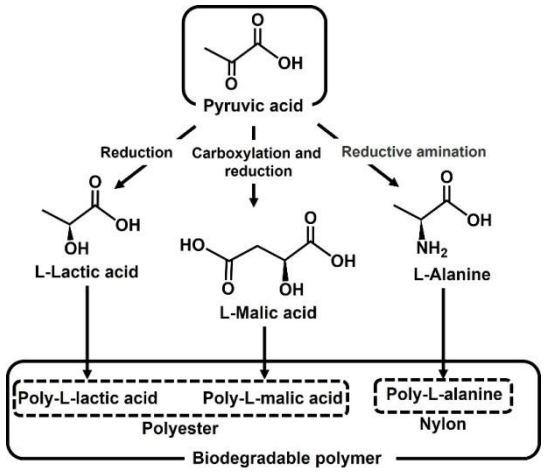

Fig. 1. Biodegradable plastics using pyruvic acid as a precursor.

In particular, among these materials, poly L-lactic acid (PLA) has long been considered a promising biodegradable polymer.[12] L-Lactic acid, the precursor of PLA, is produced by fermentation and chemical methods. Over 70 % of L-lactic acid is produced industrially by fermentation of carbohydrates with *Lactobacillus bacteria*.[13] However, the disposal of fermentation residues after the reaction is a major problem for environmental impact in the production of L-lactic acid using fermentation methods. In contrast, lactic acid is also produced through various chemical processes.[14] Lactic acid is chemically synthesized from a petrochemical source. The reaction step in lactic acid production using petrochemical feedstocks involves the oxidation of ethylene to form acetaldehyde in the presence of palladium(II) chloride. Acetaldehyde in the liquid phase is converted to lactonitrile by reacting with hydrogen cyanide in the presence of a base at high pressure. Lactonitrile is hydrolysed using sulfuric acid to produce a racemic lactic acid.[15] This reaction system requires the use of highly toxic hydrogen cyanide as a feedstock and produces ammonium chloride as a by-product.[16] Furthermore, this chemical process cannot differentiate between the synthesis of L- acid and D-lactic acid. Therefore, a new chemical process to synthesise only L-lactic acid without the highly toxic hydrogen cyanide and petrochemical feedstocks is desired. To solve these problems, a biocatalytic process is proposed that can synthesise pyruvic acid from acetaldehyde and CO_2 instead of hydrogen cyanide and further convert it to L-lactic acid. Here, utilisation of Pyruvate decarboxylase (PDC; EC 4.1.1.1) as a biocatalyst for producing pyruvate from acetaldehyde and CO_2 is suggested. In general, PDC catalyses the decarboxylation of pyruvate into acetaldehyde and CO_2 in the presence of thiamine pyrophosphate (TPP) as a co-enzyme and magnesium ion as shown in Figure 2.[17-23]

As shown in Figure 2, PDC also catalyses the carboxylation of acetaldehyde and CO_2 to produce pyruvate in the presence of TPP and magnesium ion.[24]

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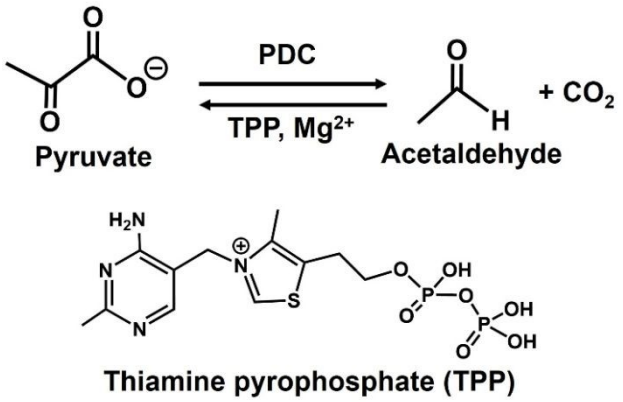

Fig. 2. PDC-catalysed decarboxylation of pyruvate into acetaldehyde and CO_2 and the reversed reaction in the presence of TPP and Mg^{2+}.

As shown in Figure 2, PDC also catalyses the carboxylation of acetaldehyde and CO_2 to produce pyruvate in the presence of TPP and magnesium ion.[24] L-Lactate dehydrogenase (LDH; EC 1.1.1.27) is an enzyme found in nearly all living cells and catalyses the conversion of pyruvate to L-lactate with NADH as a co-enzyme.[25-28] In other words, by using a dual biocatalytic system with PDC and LDH in the presence of TPP and NADH, L-lactate production from acetaldehyde and CO_2 via the intermediate pyruvate can be proposed as shown in Figure 3.

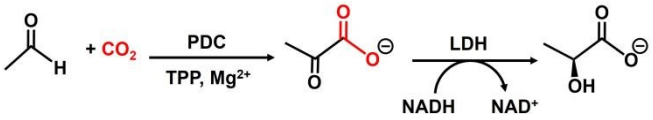

Fig. 3. L-Lactate production from acetaldehyde and CO_2 with dual-biocatalysis consisting of PDC and LDH in the presence of TPP and NADH.

By using the system shown in Figure 3, L-lactate production is achieved by bonding CO_2 to acetaldehyde as a carboxyl group without using petroleum-derived resources. In other words, this system is expected to lead to the establishment of carbon capture, utilization and storage (CCUS) technology, which captures CO_2, uses it as a raw material, and then stores it as acetaldehyde. L-lactate was successfully produced from acetaldehyde and bicarbonate by using PDC and LDH in the presence of TPP and NADH.[29,30] Furthermore, in order to develop this system into CCUS technology, a strategy is needed to use gaseous CO_2 directly as a feedstock.

In this work, the L-lactate production from acetaldehyde and gaseous CO_2 with the dual-biocatalytic system consisting of thermostable PDC from *Lactobacillus* YK1 and LDH from Chicken heart in the presence of TPP, NADH and magnesium ion was accomplished.

Experimental

Materials

PDC from *Lactobacillus* YK1 was purchased from Thermostable Enzyme Laboratory Co., Ltd. TPP was purchased from Sigma-Aldrich Co. LLC. LDH from chicken heart and NADH were purchased from Oriental Yeast Co., Ltd. Acetaldehyde, magnesium chloride hexahydrate, sodium bicarbonate, sodium carbonate, phosphoric acid, sodium dihydrogen phosphate dihydrate, disodium hydrogen phosphate dodecahydrate, 1-naphthol, 2,4-dinitrophenylhydrazine (DNPH), hydrochloric acid, aniline, methanol, acetonitrile, sodium hydroxide, and sodium pyruvate were purchased from Fujifilm Wako Pure Chemical Industries, Ltd. Acetoin and creatine were purchased from Tokyo Chemical Industry Co., Ltd.

Reaction system

The reaction system for producing L-lactate using PDC and LDH as a catalyst is as follows. Screw tube bottles with a total volume of 20 or 30 mL were used as reaction vessels. First, 4.9 mL of a buffer solution containing the reaction reagents excluding PDC was added to a sample bottle, then was sealed, and CO_2 gas or air was introduced as the gas phase. Finally, 0.1 mL of buffer containing PDC was added to start the reaction. The reaction was carried out in a constant temperature shaking chamber at 30.5 °C and 80 rpm. The concentration of L-lactate produced was measured by an ion chromatograph system with electrical conductivity detector (Metrohm, Eco IC). Ion chromatographic separation was carried out using an ion exclusion column (Metrosep Organic Acids 250/7.8 Metrohm; column size: 7.8 × 250 mm; composed of 9 µm polystyrene-divinylbenzene copolymer with sulfonic acid groups). Experimental details for L-lactate quantification by ion chromatograph are explained in the supporting information. The concentration of L-lactate was determined using equation (S1) obtained from a calibration curve (Figure S1(b)) based on the chromatogram of the standard sample (Figure S1(a)). The concentration of acetoin produced was determined by UV-visible absorption spectroscopy using spectrometer (SHIMADZU, MultiSpec-1500) according to previous reported literature[31,32]. Experimental details for acetoin quantification by UV-visible absorption spectroscopy are explained in the supporting information. The concentration of acetoin was determined using equation (S2) obtained from a calibration curve (Figure S2(b)) based on the UV-visible absorption spectral changes of the standard sample (Figure S2(a)).

Results and discussion

L-Lactate production from acetaldehyde and bicarbonate with PDC and LDH

The production of L-lactate from acetaldehyde and bicarbonate using a dual catalyst system consisting of PDC and LDH was attempted. Figure 4 shows the time dependence of L-lactate production with the system of acetaldehyde (10 mM), sodium bicarbonate (0.1 M), magnesium chloride (5.0 mM), TPP (10 mM), NADH (1.0 mM), PDC (10 U) and LDH (10 U) in 5.0 mL of 0.2 M phosphate buffer (pH 6.6) during incubation. As shown in Figure 4, L-lactate produced with increasing incubation time. After 5 h incubation, 35 µM of L-lactate was produced and the conversion yield for acetaldehyde to L-lactate was estimated to be 0.35%.

Meanwhile, L-lactate production was investigated with only PDC, LDH, or in the absence of any biocatalyst.

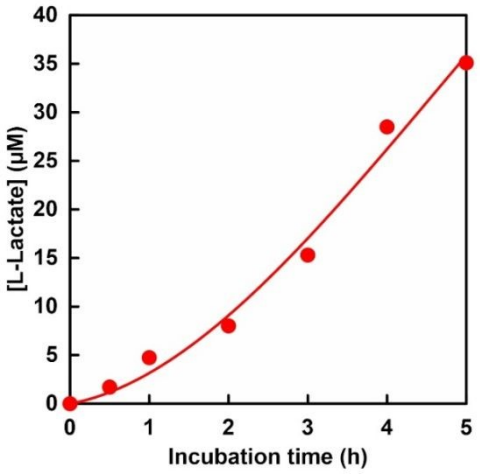

Fig. 4. Time dependence of L-lactate concentration in the phosphate buffer containing acetaldehyde, sodium bicarbonate, magnesium chloride, TPP, NADH, PDC and LDH during incubation.

Figure 5 shows the ion chromatograms sampled from the reaction solution of acetaldehyde (10 mM), sodium bicarbonate (0.1 M), magnesium chloride (5.0 mM), TPP (10 mM) and NADH (1.0 mM) in the presence or absence of biocatalysts before and after 5 h incubation.

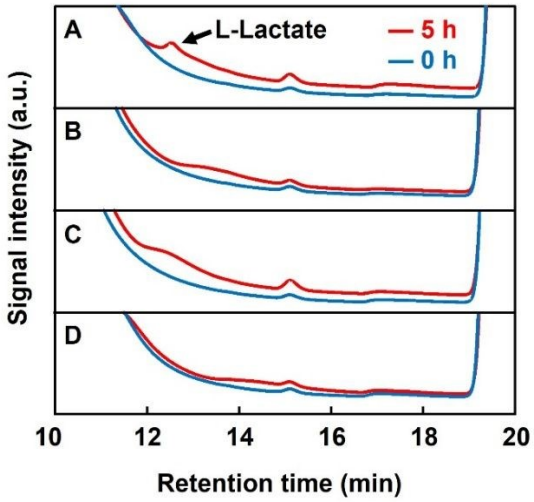

Fig. 5. Chart of an ion chromatogram sampled from the reaction solution of acetaldehyde, sodium bicarbonate, magnesium chloride, TPP and NADH in the presence or absence of biocatalysts before and after 5 h incubation. A: With PDC and LDH, B: Only PDC, C: Only LDH, D: Without any biocatalysts.

The peak around the retention time of 12.5 min was attributed to L-lactate in the ion chromatogram. In addition, the peak around the 15.0 min retention time was assigned to the carbonated species including bicarbonate and carbonate. The large peak before the 12 min retention time was assigned to phosphate, and the peak after 19 min was assigned to the system peak. As shown in Figure 5, L-lactate production was observed only in the presence of PDC and LDH (A). In addition, the slight increase in carbonate species concentration is predicted to be due to the supply of CO_2 from the gas phase to the reaction solution. The results showed that the fixation of bicarbonate to acetaldehyde proceeded catalysed by PDC to produce pyruvate as an intermediate, and then was reduced to L- lactate catalysed by LDH.

Effect of co-enzyme and metal ion cofactor in L-lactate production catalysed by PDC and LDH

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In the PDC-catalysed pyruvate decarboxylation reaction, the reaction proceeds with TPP functioning as a coenzyme at the active centre. Therefore, to clarify the role of TPP in the PDC-catalysed CO_2 fixation, the TPP concentration dependence of the L-lactate production reaction using PDC and LDH was investigated. The L-lactate production rate was calculated from the concentration after 5 h incubation. Figure 6 shows the relationship between TPP concentration and L-lactate production rate in the solution of acetaldehyde (10 mM), sodium bicarbonate (0.2 M), magnesium chloride (2.0 mM), TPP (0 - 50 mM), NADH (1.0 mM), PDC (20 U) and LDH (10 U) in 5.0 mL of 0.5 M phosphate buffer (pH 6.6). As shown in Figure 6, the L-lactate production rate increased with increasing TPP concentration up to 20 mM, whereas above 20 mM, L-lactate production rate decreased with increasing TPP concentration. The relationship between the L-lactate production rate and TPP concentration could be fitted to the Haldane equation (1) based on substrate inhibition.[33]

$$v = \frac{V_{max}[\mathrm{TPP}]}{[\mathrm{TPP}] + K_m + \frac{[\mathrm{TPP}]^2}{K_i}} \quad (1)$$

where v, V_{max}, K_m, and K_i are the L-lactate production rate, maximum reaction velocity, Michaelis constant, and substrate inhibition constant, respectively. By fitting with the Haldane equation, V_{max}, K_m, and K_i were calculated to be 0.12 μM min^{-1}, 9.2 mM, and 42 mM, respectively. This result indicates that L-lactate production is inhibited in the presence of excess TPP. In the pyruvate decarboxylation reaction catalysed by PDC, in contrast, no decrease in the reaction rate was observed even under conditions of excess TPP. These results suggest that the optimal range of TPP concentration is up to *ca*. 10 mM, where substrate activation prevails over inhibition.

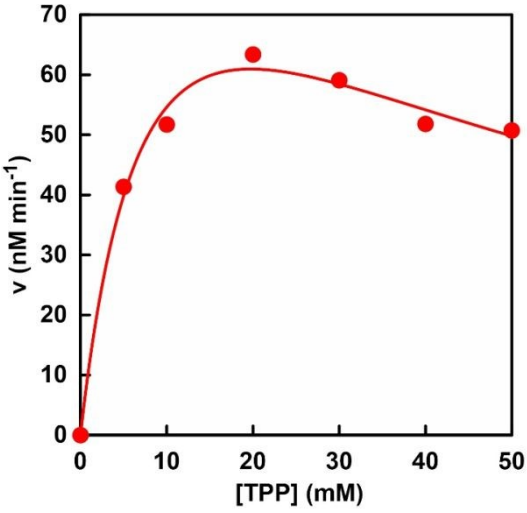

Fig. 6. Relationship between TPP concentration and L-lactate production rate (v) in the sample solution of acetaldehyde, sodium bicarbonate, magnesium chloride, TPP, NADH, PDC and LDH.

It has been reported that in the pyruvate decarboxylation reaction catalysed by PDC, magnesium ion is involved in the complexation of the catalytic active centre of PDC with TPP as shown in Figure 7. Figure 7 shows the complex formation mode

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of the amino acid residues constituting the catalytic active centre of PDC derived from *Saccharomyces uvarum* with TPP and magnesium ion.[34]

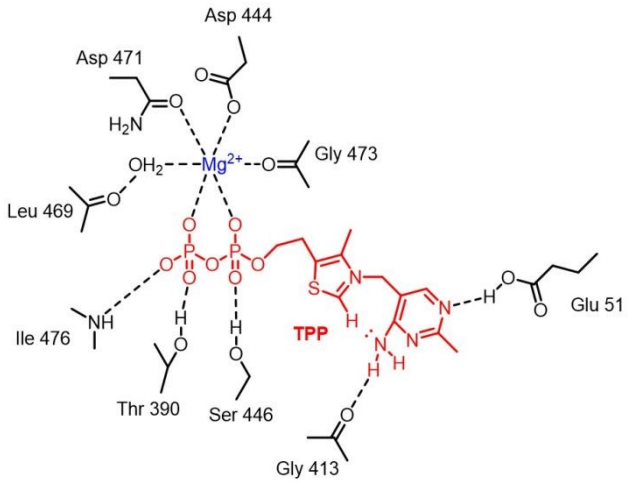

Fig. 7. Schematic representation of the complex formation of the amino acid residues constituting the catalytic active centre of PDC with TPP and magnesium ion. Aspartic acid (Asp), glutamic acid (Glu), glycine (Gly), isoleucine (Ile), leucine (Leu), serine (Ser), threonine (Thr).

Therefore, it is also necessary to investigate the effect of magnesium ion on PDC-catalysed pyruvate carboxylation. Therefore, to clarify the role of magnesium ion in the PDC-catalysed CO_2 fixation, the magnesium ion concentration dependence of the L-lactate production reaction using PDC and LDH was investigated. Figure 8 shows the relationship between magnesium ion concentration and the concentration of L-lactate production after 5 h incubation in the solution of acetaldehyde (10 mM), sodium bicarbonate (0.1 M), magnesium chloride (0 - 5.0 mM), TPP (10 mM), NADH (1.0 mM), PDC (10 U) and LDH (10 U) in 5.0 mL of 0.2 M phosphate buffer (pH 6.6).

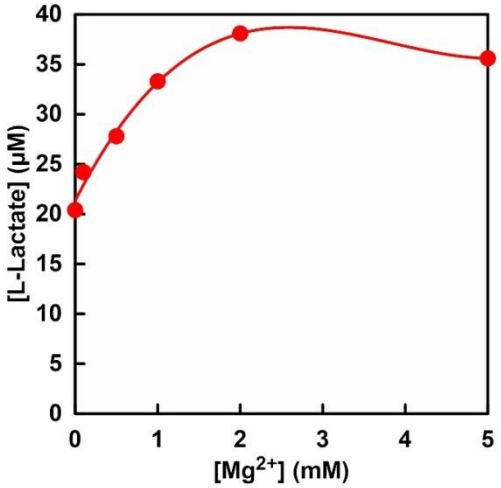

Fig. 8. Relationship between magnesium ion concentration and the concentration of L-lactate production after 5 h incubation in the sample solution of acetaldehyde, sodium bicarbonate, magnesium chloride, TPP, NADH, PDC and LDH.

As shown in Figure 8, the L-lactate production increased with increasing magnesium ion concentration in the range of 0 to 2.0 mM, whereas there was no significant change in the L-lactate production at magnesium ion concentrations above 2.0 mM. The addition of magnesium ion increased L-lactate production by up to approximately two times compared to the absence of magnesium ion. The reason why L-lactate production was promoted with the addition of magnesium ion is thought to be that, as in the pyruvate decarboxylation process, magnesium ion contributes to stabilizing the PDC-TPP complex, as shown in Figure 7, and this promotes the carboxylation of acetaldehyde. The fact that no change in L-lactate production at magnesium ion concentrations above 2.0 mM is likely due to the fact that all active sites on PDC are bound to magnesium ion.

pH dependence of L-lactate production from acetaldehyde and bicarbonate with PDC and LDH

In order to maximize the activity of each biocatalyst and improve the L-lactate production efficiency, the pH dependence of the L-lactate production catalysed by PDC and LDH was investigated. The production of L-lactate from acetaldehyde and bicarbonate using a dual catalyst system consisting of PDC and LDH was attempted under various pH conditions.

Figure 9 shows the time dependence of L-lactate production in the solution of acetaldehyde (10 mM), sodium bicarbonate (0.1 M), magnesium chloride (5.0 mM), TPP (10 mM), NADH (1.0 mM), PDC (10 U) and LDH (10 U) in 5.0 mL of buffer with various pH during incubation. As shown in Figure 9, L-lactate production was maximized under pH 6.6 condition. On the other hand, it was found that L-lactate production decreased with increasing pH. Furthermore, no L-lactate production was observed at pH 9.0 condition.

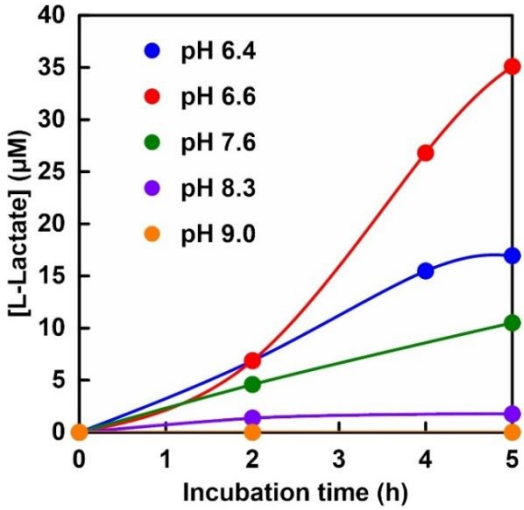

Fig. 9. Time dependence of L-lactate concentration in the buffer with various pH (6.4 -9.0) containing acetaldehyde, sodium bicarbonate, magnesium chloride, TPP, NADH, PDC and LDH during incubation.

In addition, in the dual-biocatalytic system, it is necessary to optimise the system by considering the optimal pH of PDC and LDH. Generally, the optimal pH for L-lactate production catalysed by LDH is neutral to weakly basic condition.[25-28] In addition, the reported pyruvate production based on the carboxylation of acetaldehyde with CO_2 catalysed by PDC proceeds under weakly basic conditions.[24,29] However, in our experiment, almost no L-lactate production under weakly basic

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conditions observed. One possible explanation is that the undesirable acetoin production described later proceeded under weakly basic conditions and was quantified as pyruvate. Based on these results, we concluded that a pH of around 6.6 is the optimal pH for PDC-catalysed pyruvate production in our experiments. In addition, PDC-catalysed pyruvate production is the rate-limiting step in dual-biocatalytic L-lactate production. Since LDH-catalysed L-lactate production proceeds even under conditions of pH 7.0 or lower, the optimal pH of PDC was applied to the dual-biocatalytic system.

Here, let us focus on the relationship between the L-lactate production concentration and the carbonate species abundance ratio in sample solution under various pH conditions. Figure 10 shows the relationship between pH and the concentration of L-lactate production after 5 h incubation in the solution of acetaldehyde (10 mM), sodium bicarbonate (0.1 M), magnesium chloride (5.0 mM), TPP (10 mM), NADH (1.0 mM), PDC (10 U) and LDH (10 U) in 5.0 mL of buffer during incubation.

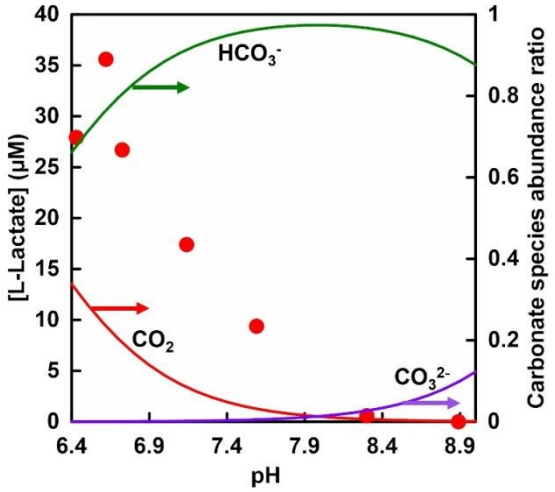

Fig. 10. Relationship between pH and the concentration of L-lactate production after 5 h incubation in the sample solution of acetaldehyde, sodium bicarbonate, magnesium chloride, TPP, NADH, PDC and LDH. Relationship between pH and the abundance of carbonate species.

Figure 10 also shows the abundance of carbonate species under various pH conditions. The abundance of CO_2, bicarbonate and carbonate in the solution at a wide range of pH were calculated using an equation suggested by Plummer and Busenberg.[35-37] As shown in Figure 10, a decrease in L-lactate production was observed with a decrease in CO_2 concentration in the solution. These results suggest that CO_2 in the sample solution is used as a raw material for the PDC-catalysed carboxylation of acetaldehyde.

Next, let us focus on the L-lactate production relative to the total concentration of carbonate species in the sample solution with pH 6.6.

Figure 11 shows the relationship between initial sodium bicarbonate concentration and the concentration of L-lactate production after 5 h incubation in the sample solution of acetaldehyde (10 mM), sodium bicarbonate (0 – 0.3 M), magnesium chloride (2.0 mM), TPP (10 mM), NADH (1.0 mM), PDC (10 U) and LDH (10 U) in the phosphate buffer (pH 6.6). Figure 11 also shows the total concentration change of carbonate species in the sample solutions. As shown in Figure 11, it was found that L-lactate production increased with increasing initial concentration of sodium bicarbonate. The CO_2 concentration in the gas phase can also be roughly calculated from the initial sodium bicarbonate concentration and the concentration of total carbonate species in the solution. These results suggest that the vapor-liquid equilibrium of CO_2 is important for L-lactate production.

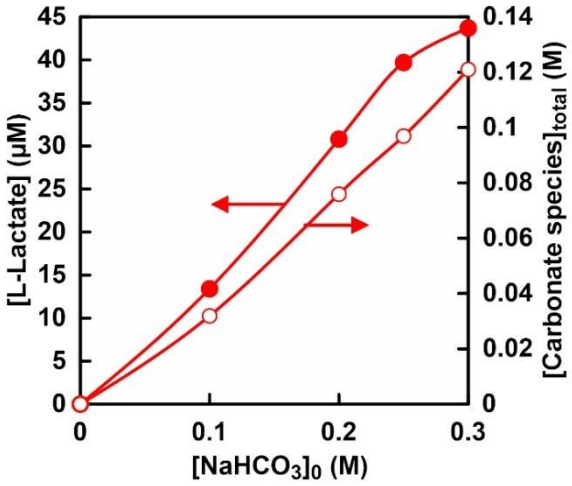

Fig. 11. Relationship between initial sodium bicarbonate concentration and the concentration of L-lactate production after 5 h incubation in the sample solution of acetaldehyde, sodium bicarbonate, magnesium chloride, TPP, NADH, PD and LDH in the phosphate buffer (pH 6.6). Open circle: Total concentration changes of carbonate species in the sample solutions.

Selective production of L-lactate from acetaldehyde and bicarbonate using PDC and LDH

By using PDC and LDH, production of L-lactate from acetaldehyde and bicarbonate in the presence of TPP and NADH is accomplished. However, even after examining reaction conditions such as pH, the L-lactate production yield remains unfortunately low. Therefore, identification other products in addition to L-lactate from acetaldehyde and bicarbonate catalysed by PDC and LDH in the presence of TPP and NADH was attempted. The effect of the initial acetaldehyde concentration on the L-lactate production using PDC and LDH was investigated. Figure 12 shows the relationship between the initial acetaldehyde concentration and L-lactate production after 5 h incubation in the solution of acetaldehyde (0 - 50 mM), sodium bicarbonate (0.2 M), magnesium chloride (2.0 mM), TPP (20 mM), NADH (1.0 mM), PDC (20 U) and LDH (10 U) in 5.0 mL of 0.5 M phosphate buffer (pH 6.6).

Regarding L-lactate production, the L-lactate concentration increased with increasing acetaldehyde initial concentration less than 10 mM, whereas it decreased rapidly more than 10 mM of acetaldehyde initial concentration. This result indicates that the CO_2 fixation catalysed by PDC is affected by substrate inhibition, and suggests that increasing the acetaldehyde initial concentration is causing a process other than the CO_2 fixation to proceed. It has been reported that acetoin production with high concentrations of acetaldehyde condition in pyruvate decarboxylation catalysed by PDC.[38] The effect of the initial acetaldehyde concentration on the acetoin production using PDC and LDH also was investigated. Figure 12 also shows the relationship between the initial acetaldehyde concentration

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and acetoin production after 5 h incubation. As shown in Figure 12, the acetoin concentration increased with increasing acetaldehyde initial concentration.

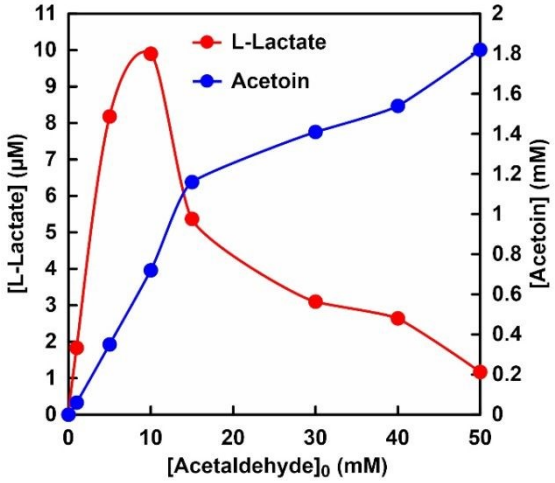

Fig. 12. Relationship among the initial acetaldehyde concentration, L-lactate and acetoin production after 5 h incubation in the presence of acetaldehyde, sodium bicarbonate, magnesium chloride, TPP, NADH, PDC and LDH.

In the carboxylation of acetaldehyde catalysed by PDC and LDH, the amount of acetoin produced was significantly greater than that of L-lactate. Therefore, let us consider the mechanism of carboxylation of acetaldehyde and acetoin production catalysed by PDC in the presence of TPP. Acetoin was synthesized from acetaldehyde using vitamin B1 and N-heterocyclic carbene as a catalyst.[39-41] Figure 13 shows suggested mechanism for pyruvate and acetoin production using PDC in the presence of TPP.

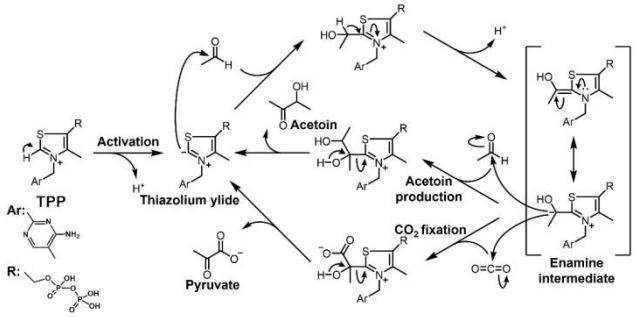

Fig. 13. Possible mechanism for pyruvate and acetoin production using PDC in the presence of TPP.

As shown in Figure 13, after the enamine intermediate is produced, it reacts with CO_2 to produce pyruvate, but under conditions of high acetaldehyde concentrations, it is predicted to react with acetaldehyde to produce acetoin. In other words, it is expected that increasing the CO_2 concentration in the reaction system suppresses acetoin production and promotes pyruvate production.

L-Lactate production from acetaldehyde and gaseous CO_2 using PDC and LDH

Based on the obtained results, it is necessary to maximize the CO_2 concentration in the reaction system in order to achieve pyruvate production based on the carboxylation of acetaldehyde and conversion to L-lactate catalysed by PDC and LDH. Therefore, to improve the L-lactate production yield by introducing CO_2 gas into the gas phase in the reaction vessel was investigated. Figure 14 shows the time dependence of L-lactate production in the system of acetaldehyde (10 mM), sodium bicarbonate (0 - 0.2 M), magnesium chloride (2.0 mM), TPP (10 mM), NADH (1.0 mM), PDC (20 U) and LDH (10 U) in 5.0 mL of 0.5 M phosphate buffer (pH 6.6) under conditions CO_2- or air-filled in the gas phase (18.7 mL) during incubation. As shown in Figure 14, it was found that the production efficiency of L-lactate catalysed by PDC and LDH was improved by adding sodium bicarbonate to the reaction sample and filling the gas phase with gaseous CO_2. On the other hand, it also was found that the reaction proceeded simply by adding gaseous CO_2 to the gas phase without sodium bicarbonate in the sample solution, although the L-lactate production yield was low compared with those of the other conditions.

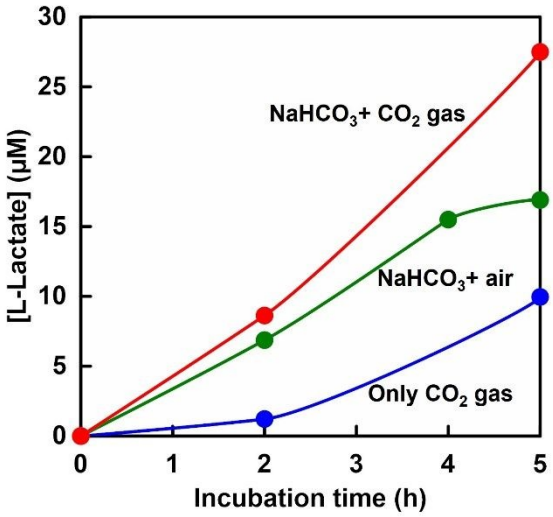

Fig. 14. Time dependence of L-lactate concentration in the phosphate buffer (pH 6.6) containing acetaldehyde, sodium bicarbonate, magnesium chloride, TPP, NADH, PDC and LDH under conditions CO_2- or air-filled in the gas phase during incubation. Red: CO_2 gas filled in the gas phase, Green: Air filled in the gas phase, Blue: CO_2 gas filled in the gas phase and absence of sodium bicarbonate.

Furthermore, the reaction temperature was investigated to improve the yield of dual-biocatalytic L-lactate production. When the reaction temperature was increased, acetaldehyde and CO_2 in the sample solution moved into the gas phase, causing the pressure inside the reaction vessel to rise, and L-lactate production was hardly observed. On the other hand, while lowering the temperature prevented acetaldehyde and CO_2 from moving to the gas phase in the sample solution, it did not lead to an increase in the reaction rate of L-lactate production. As a result, it was suggested that a reaction temperature of 30.5°C is optimal for dual-biocatalytic L-lactate production.

In conclusion, we were unable to find reaction conditions that accomplished a yield of 0.35% or higher in the production of L-lactate using a dual-biocatalyst composed of PDC from *Lactobacillus* YK1 and LDH from chicken heart. In this system, pyruvate production from acetaldehyde and CO_2 catalysed by PDC is an important factor in improving the yield of L-lactate production. To improve the yield of pyruvate production catalysed by PDC, suppression of acetoin production is essential, and currently, this cannot be controlled by reaction conditions. In the future, protein engineering techniques will be needed

that prioritize the carboxylation of pyruvate by CO_2 through mutations of the amino acid residues that constitute the catalytic activity of PDC.

Conclusion

In conclusion, by using a dual-biocatalyst system consisting of PDC and LDH, L-lactate production was achieved from acetaldehyde and CO_2 via pyruvate in the presence of TPP and NADH. The pH dependence of L-lactate production using a dual-biocatalyst system consisting of PDC and LDH was investigated, and it was found that the production amount reached a maximum at around pH 6.6 (conversion yield for acetaldehyde to L-lactate: 0.35%). This indicates that the carboxylation of acetaldehyde using a dual-biocatalyst system of PDC and LDH involves CO_2 in the sample solution. Furthermore, it was suggested that maintaining the gas-liquid equilibrium of CO_2 gas in the reaction system is important for improving the L-lactate production yield. As a future challenge, suppressing the simultaneous production of acetoin will lead to an improvement in the L-lactate production yield. Furthermore, by incorporating an NADH regeneration, such as a photocatalytic system, it will be possible to develop CCUS technology that utilizes biocatalysts and renewable energy.

Conflicts of interest

There are no conflicts to declare.

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Data Availability Statement

The authors confirm that the data supporting the findings of this manuscript are available within the article and its supplementary materials.