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Short communication

# Stimulation of growth and pyruvate oxidation in *Streptococcus faecalis* by asparagusic acid and its derivatives

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Plant growth inhibitors I, II, III, VII, VIII, which occur in asparagus, promoted growth and pyruvate oxidation in *Streptococcus faecalis* 10Cl. Activities were compared with those obtained with α-lipoic acid and several structurally related synthetic sulfur containing compounds.

In previous papers, we elucidated the structures of asparagusic acid (I), dihydroasparagusic acid (II), and S-acetyldihydroasparagusic acid (III) (*1*) and reported their inhibitory effect on the growth of higher plants (*2*). The structural features of these compounds and the presence of (I), its reduced form (II), and its monoacetyl derivative (III) in the same natural sources are reminiscent of α-lipoic acid (IV) and its derivatives (V, VI), which have been isolated from and identified in various plants (*3*) and animals (*4*). These latter are the essential co-factors for keto-acid oxidations (*5*). The similarity of both types of compounds led us to examine whether α-lipoic acid is replaceable with asparagusic acid in *Streptococcus faecalis* 10Cl, the growth of which has been shown to be stimulated by α-lipoic acid (*6*, *7*).

We report herein the stimulation of growth and pyruvate oxidation in *S. faecalis* 10Cl by whole acidic fractions, including asparagusic acid obtained from MeOH extracts of asparagus shoots. Results were compared with those obtained using α-lipoic acid and the chemical analogues of I to find the essential functional groups for the stimulatory activities.

The stock culture of *S. faecalis* 10Cl was carried out in stabs made by adding 20 g of agar to the broth (yeast extract, 5 g; bactopeptone, 10 g; glucose, 10 g; sodium acetate 10 g; water, 1 liter). The inoculum was grown in 5 ml of broth without agar. After incubation for 18 hr at 37°C, the culture was centrifuged, washed three times with 10 ml of normal saline solution, then was suspended in 500 times the volume of the saline as the inoculum.

The bioassay of the samples listed in the table was as follows. A given amount of each sample was placed in a tube and 2.5 ml of distilled water was added. Each



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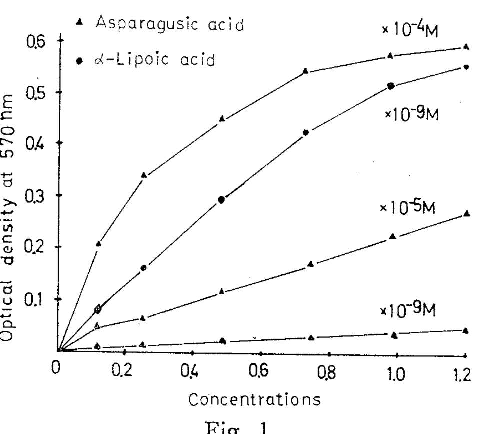

Fig. 1.

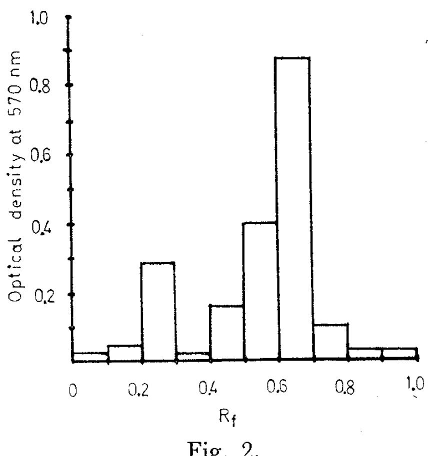

Fig. 2.

Fig. 1. *The influence of asparagusic acid and α-lipoic acid on growth in* Streptococcus faecalis *10C1.* Optical density was determined after 9 hr of incubation. The concentration of α-lipoic acid is shown with that of L-α-lipoic acid.

Fig. 2. *Effects of acidic ether fractions from the asparagus shoot extract on the growth of* Streptococcus faecalis *10C1.* Each acidic fraction (*I*, *II*) was separated with preparative TLC (silica gel, toluene : ethyl formate : formic acid=5 : 4 : 1). After developing, the dried silica gel was extracted with methylene chloride. Five μg of the separated acidic fraction was added to the assay mixture (5 ml), which was incubated for 12 hr.

tube was, after shaking, autoclaved 3 min at 120°C. Two and half ml of autoclaved Kamihara's medium (*8*) and one drop of the suspension of *S. faecalis* 10Cl were added to each tube. After incubation for 9–12 hr at 37°C, the growth of the bacteria was determined by the extinction at 570 nm. d,l-α-Lipoic acid was obtained from Tokyo Kasei Co. Ltd., and asparagusic acid was synthesized according to the improved Jansen's procedure (*9*).

For the assay with pyruvate oxidation, bacteria were incubated in the same basal medium as used in the growth test. Cells were collected by centrifugation after 10 hr of incubation and washed three times with 0.033 M potassium phosphate

Table 1 *Stimulatory effects of asparagusic acid and α-lipoic acid on the rate of pyruvate oxidation in* Streptococcus faecalis *10C1*

| Preincubation system | Rate of oxygen consumption [c] | | | |
|---|---|---|---|---|
| | No addition | Asparagusic acid [d] | α-Lipoic acid [e] | Ratio Asparagusic acid / α-Lipoic acid |
| Asparagusic acid [a] | 0 | $2.15\times10^{-2}$ | $3.87\times10^{-2}$ | 0.56 |
| α-Lipoic acid [b] | 0 | $2.67\times10^{-2}$ | $3.77\times10^{-2}$ | 0.70 |

[a] $2.42\times10^{-5}$ M

[b] $1.45\times10^{-9}$ M (racemic form)

[c] The rate of oxygen consumption was determined after 3 min of incubation and is expressed as the μmoles of $O_2$/min/mg of the dry weight of cells.

[d] $1.21\times10^{-6}$ M

[e] $2.42\times10^{-6}$ M (racemic form)

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Table 2 *Specificities of asparagusic acids and their analogues on growth and pyruvate oxidation in* Streptococcus faecalis *10C1*

| Compounds | | Optical density [a] (%) | Rate of oxygen consumption [b] |
|---|---|---|---|
| S–S (ring) >–COOH | | 100 | $2.15\times10^{-2}$ |
| HS–, HS– >–COOH | | 123 | $2.68\times10^{-2}$ |
| $H_3COCS$–, HS– >–COOH | | 74 | $2.09\times10^{-2}$ |
| O←S–S (ring) >–COOH | *Anti* | 6 | $0.28\times10^{-2}$ |
| | *Syn* | 6 | $0.27\times10^{-2}$ |
| S–S (ring) >–$COOCH_3$ | | 98 | $2.10\times10^{-2}$ |
| $H_3COCS$–, $H_3COCS$– >–COOH | | 0 | 0 |
| $(CH_3)_2C$<S, S> >–COOH | | 0 | 0 |
| S–S (ring) >–$CH_2OH$ | | 0 | 0 |
| HS–, HS– >–$CH_2OH$ | | 0 | 0 |

[a] Optical density after 10 hr of incubation with each compound at $1.21\times10^{-4}$ M.
[b] The rate of oxygen consumption was determined after 3 min of incubation under the conditions noted in Table 1 and is expressed as the $\mu$moles of $O_2$/min/mg of the dry weight of cells.

buffer (pH 7.0). Fifty-eight mg of the dried weight of the cells was suspended in 1 ml of the phosphate buffer. Five $\mu$l of the cell suspension was added to the assay mixture (*10*) in each vessel containing 30 $\mu$moles of glutathione, 40 $\mu$moles of magnesium sulfate, 1 $\mu$mole of adenosine, 120 $\mu$moles of sodium pyruvate, and 2 ml of water. The rate of pyruvate oxidation was determined with an oxygen electrode, Kyusui Chemical Institute Model SB-OA.

When bacteria were cultured in a medium containing $0.1–1.2\times10^{-9}$ to $0.1–1.2\times10^{-4}$ M of asparagusic acid, we observed a linear increase in growth (Fig. 1), as was the case with $\alpha$-lipoic acid (*7*). The activity of asparagusic acid was approximately $10^{-5}$-fold lower than that of $\alpha$-lipoic acid at the same concentration. But $10^{-4}$ M of asparagusic acid gave almost the same maximum growth achieved with $10^{-9}$ M of $\alpha$-lipoic acid under these conditions. Asparagusic acid ($1.21\times10^{-6}$ M) significantly stimulated pyruvate oxidation in two kinds of cells preincubated differently (Table 1). $\alpha$-Lipoic acid also promoted pyruvate oxidation under the same conditions; the ratio of the oxidation rate with asparagusic acid to that with

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S—S COOH I
HS SH COOH II
HS $SCOCH_3$ COOH III
S—S $(CH_2)_4COOH$ IV
HS SH $(CH_2)_4COOH$ V
HS $SCOCH_3$ $(CH_2)_4COOH$ VI
S—S→O H COOH VII
S—S→O H COOH VIII

Fig. 3. *Chemical structures of asparagusic acids and α-lipoic acids.*

α-lipoic acid (racemic form) was 56–70%. Pyruvate oxidation in bacteria was completely depressed by treatment with $NaN_3$.

Since the above results revealed that asparagusic acid stimulated growth and pyruvate oxidation in *S. faecalis* 10Cl, we next examined the whole acidic fractions and some sulfur containing derivatives; the results of which are shown in Fig. 2 and Table 2. As is evident from the figure, several acidic fractions showed activity in the bacteria. From the portions of Rf 0.65, 0.55, 0.75, respectively, asparagusic (I), dihydroasparagusic (II), and S-acetyldihydroasparagusic acids (III) were isolated (*1*), while the active principles of zones Rf 0.31 and 0.25 were respectively clarified as asparagusic acid-*anti* (VII), and -*syn* (VIII)-S-oxides (*11*).

Strong stimulation was observed in compounds I, II, III, and the methyl ester of I (Table 2). The S-oxides (VII, VIII) exhibited small but clear stimulation, while the diacetyl, thioketal, and alcohols of I and II had no effect. These observations suggest that the existence of both S-S linkage (or a group readily convertible to it) and a carboxyl group in a molecule is required for pyruvate oxidation.

Experiments on the role of asparagusic acid in higher plants, especially in asparagus itself, are now in progress.

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