MICROBIAL CONVERSION OF COMPACTIN (ML-236B) TO ML-236A

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Approximately 1,600 fungal strains were tested for ability to convert compactin (ML-236B) to ML-236A and *Emericella unguis* IFO 8087 was found to be the most active. *E. unguis* converted ML-236B to ML-236A with a yield of over 90%.

Compactin (ML-236B) (Fig. 1), a metabolite of *Penicillium citrinum*, is a specific inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-controlling enzyme in cholesterol biosynthesis[1,2]. This compound has been shown to be highly effective in lowering plasma cholesterol levels in both animals and humans[3]. Along with ML-236B, a minor metabolite, ML-236A, is also produced by *P. citrinum*[1]. Although ML-236A is far less active in the inhibition of HMG-CoA reductase than ML-236B, a variety of ML-236B-related compounds may be chemically synthesized from ML-236A. Thus, ML-236A is a useful starting material for the semi-synthetic compactin analogs.

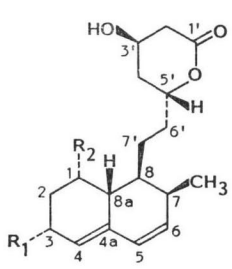
ML-236A can be derived from ML-236B by alkaline hydrolysis with a yield of less than 60%. The present study deals with microbial conversion of ML-236B to ML-236A, in which approximately 1,600 strains of fungi were tested for their ability to catalyze this reaction. As the result, 59 strains were found to be effective in catalyzing this hydrolytic reaction, of which *Emericella unguis* showed the most potent activity.

Materials and Methods

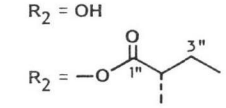
Microbial Strains

All fungal strains (1,600 strains) employed in the present study were obtained from Institute for

Fig. 1. Structures of the lactone forms of compactin (ML-236B) related compounds.
Numbers in the parenthes represent relative activity to inhibit HMG-CoA reductase.

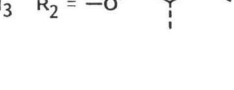


ML-236A (2) R_1 = H R_2 = OH

ML-236B (Compactin) (50) R_1 = H R_2 = 

ML-236C (10) R_1 = H R_2 = H

Monacolin J (4) R_1 = CH_3 R_2 = OH

Monacolin K (Mevinolin) (100) R_1 = CH_3 R_2 = 

Monacolin L (15) R_1 = CH_3 R_2 = H

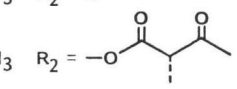
Monacolin X (20) R_1 = CH_3 R_2 = 

Table 1. Conversion of compactin (ML-236B) to ML-236A by several fungal strains. Experimental details are seen in the text.

Conversion of ML-236B to ML-236A (%)	No. of strain	Strains
61~70	2	*Dichotomyces cejpii* IFO 9929, *Diheterospora chlamydosporia* IFO 9249
71~80	1	*Humicola fuscoatra* IFO 9530
81~90	1	*Mortierella isabellina* IFO 7884
>90	1	*Emericella unguis* IFO 8087

Fermentation, Osaka (IFO).

Materials

ML-236A, ML-236B, monacolins J, K and X (lactone forms) (Fig. 1) were isolated as described previously[1,4~6]. These compounds were converted to respective acid forms (sodium salts) by saponification prior to use in conversion experiments.

Growth and HPLC Analysis

Fungal strains were aerobically grown at 25°C for 2~3 days in a medium consisting of glucose 1%, peptone (Daigo Eiyo) 0.2%, meat extract (Kyokuto Seiyaku) 0.1%, yeast extract (Difco) 0.1% and corn steep liquor 0.3% and after 3 days ML-236B (or related compounds) 0.05% was added to cultures. Growth was further continued. Aliquots were withdrawn at different time intervals and 1 ml portions of the culture filtrate were extracted twice at pH 3 with ethyl acetate (2 ml). The solvent layer was dried over Na_2SO_4 and evaporated to dryness. The resulting residue was dissolved in methanol (2 ml) and analyzed by HPLC on Silica ODS using 0.1% H_3PO_4 - acetonitrile (55: 45) as eluent. Under these conditions, ML-236B and ML-236A appeared in elution volumes of 22 and 6.5 ml, respectively. On HPLC these compounds were assayed by measuring the absorbance at 237 nm.

Experiments with Acetone - dried Mycelia

Emericella unguis IFO 8087 were grown at 25°C for 2 days as described above. The mycelia were collected by filtration, washed throughly with cold acetone (−30°C) and dried *in vacuo*. The resultant mycelial powder was used in some conversion experiments.

Results

Active Strains

Approximately 1,600 fungal strains were tested for their ability to convert ML-236B to ML-236A and 59 strains (42 genera, 53 species) were found to be active in the hydrolysis of ML-236B. *Dichotomyces cejpii* IFO 9929, *Diheterospora chlamydosporia* IFO 9249, *Humicola fuscoatra* IFO 9530, *Mortierella isabellina* IFO 7884 and *Emericella unguis* IFO 8087 carried out this conversion more efficiently than other strains (Table 1). Of the active strains, *E. unguis* IFO 8087 was the most active, which converted ML-236B to ML-236A with a yield of over 90% after incubation of 12 hours.

Experiments with Dried Mycelia of *E. unguis*

When 10 mg of the dried mycelia was suspended in 1 ml of 0.2 M potassium phosphate buffer (pH 7.0) containing 0.5 mg of ML-236B and incubated at 25°C, 20% of ML-236B added was converted to ML-236A after 2 hours and approximately 90% after 24 hours (Fig. 2).

Hydrolysis of ML-236B to ML-236A was most active at pH 8 when assayed at 25°C for 1 hour (Fig. 3). Optimum temperature for conversion was around 30°C (Fig. 4).

Fig. 2. Time course of the conversion of ML-236B to ML-236A by *Emericella unguis*.

Experimental conditions are described in the text and Materials and Methods.

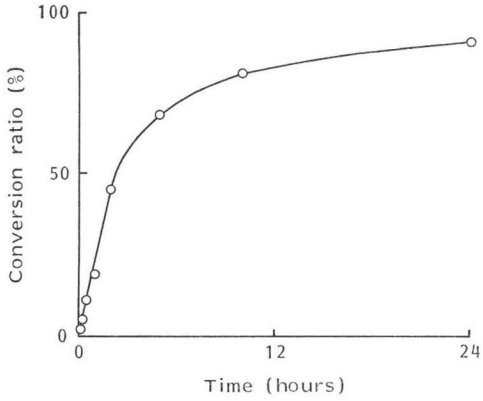


Fig. 3. Effects of pH on the conversion of ML-236B to ML-236A by *Emericella unguis*.

Experimental conditions were the same as in Fig. 2, except that pH values of the buffers used were varied as indicated and the mixtures were incubated for 1 hour.

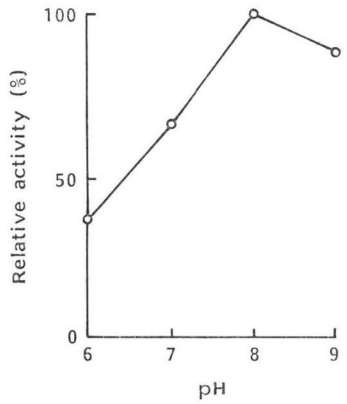


Table 2. Conversion of ML-236B related compounds by *Emericella unguis*.

Experimental conditions were the same as in Fig. 2, except that ML-236B was replaced by monacolins K and X as indicated. Mixtures were incubated at 29°C for 1 hour.

Compound	Relative activity toward (%)
ML-236B	100
Monacolin K	86.5
Monacolin X	93.4

Fig. 4. Effects of temperature on the conversion of ML-236B to ML-236A by *Emericella unguis*.

Experimental conditions were the same as in Fig. 2, except that reaction mixtures were incubated at different temperature for 1 hour.

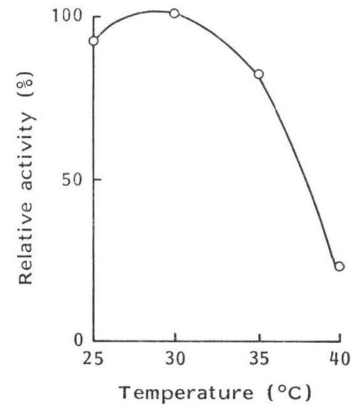
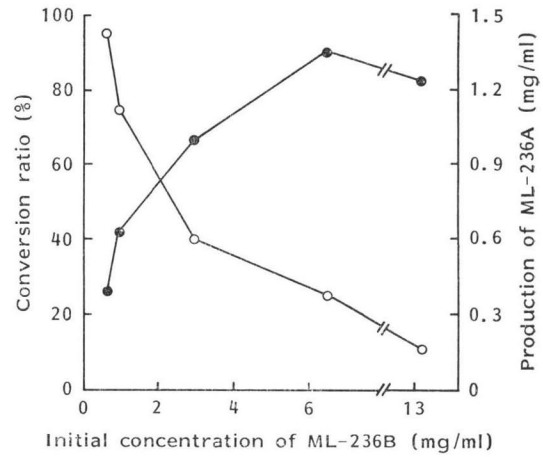


Fig. 5. Conversion of different concentrations of ML-236B to ML-236A by *Emericella unguis*.

Experimental conditions were the same as in Fig. 2, except that concentration of ML-236B added was varied as indicated and the mixtures were incubated for 12 hours.

○ Conversion ratio, ● production of ML-236A.



In the experiments shown in Fig. 5, increasing concentrations of ML-236B were incubated for 1 hour with dried mycelia of *E. unguis*. Thus, dried mycelia effectively catalyzed the hydrolytic conversion of up to 6.5 mg/ml of added ML-236B.

Of the ML-236B related compounds, mona-

colins K and X were also effectively converted to monacolin J (Table 2).

Discussion

In the present study, 360 strains of yeasts were also assayed for their ability to hydrolyze ML-236B to ML-236A. However, no strains were active in the transformation (data not shown). On the other hand, several fungi were found to be highly effective in ML-236B hydrolysis.

E. unguis IFO 8087, the most active strain, converted ML-236B to ML-236A with a yield of over than 90% (Fig. 2) and was active in the presence of approximately 1 mg of ML-236B (Fig. 5). The results suggest that the microbial conversion is an effective technique in the conversion of ML-236B to ML-236A.

Acknowledgment

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