GROWTH INHIBITION OF YEAST BY COMPACTIN (ML-236B) ANALOGUES

RYUTARO IKEURA, SHIGEO MURAKAWA and AKIRA ENDO

Department of Agricultural and Biological Chemistry, Tokyo Noko University, 3-5-8 Saiwaicho, Fuchu-shi, Tokyo 183, Japan

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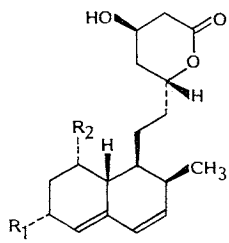
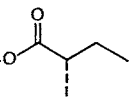
Compactin (ML-236B) (Fig. 1) is a specific potent inhibitor of 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase, the major rate-limiting enzyme in the biosynthesis of isoprenoid compounds, and has been widely used as a research tool for studying the regulation of cholesterol and other isoprenoid compounds[1~3]. Although compactin inhibits the growth of a variety of animal and plant cells[2,4,5], its antimicrobial activity has not been reported.

Fig. 1. Structures of the lactone forms of compactin-related compounds.

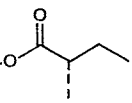


ML-236A (2) R_1 = H R_2 = OH

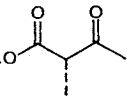
ML-236B (compactin) (50) R_1 = H R_2 = 

ML-236C (10) R_1 = 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 = 

Numbers in the parentheses represent relative activity to inhibit rat liver HMG-CoA reductase.

The present communication deals with sensitivity of the growth of yeast strains to compactin. Of approximately 300 strains tested, 4 strains, *Rhodotorula glutinis* H3-9-1, *Sporobolomyces salmonicolor* WF 188, *Aessosporon salmonicolor* IFO 1845 and *Citeromyces matritensis* IFO 0954, were found to be sensitive to compactin at 0.1~2.0 μg/ml.

Yeasts employed in the present study were 303 strains, which included 41 genera, 165 species. ML-236A, ML-236B, and monacolins K, L and X (lactone forms) (Fig. 1) were obtained as described previously[6]. *R,S*-Mevalonic acid lactone was obtained from Sigma. These compounds were converted to respective acid forms (sodium salts) by saponification prior to use.

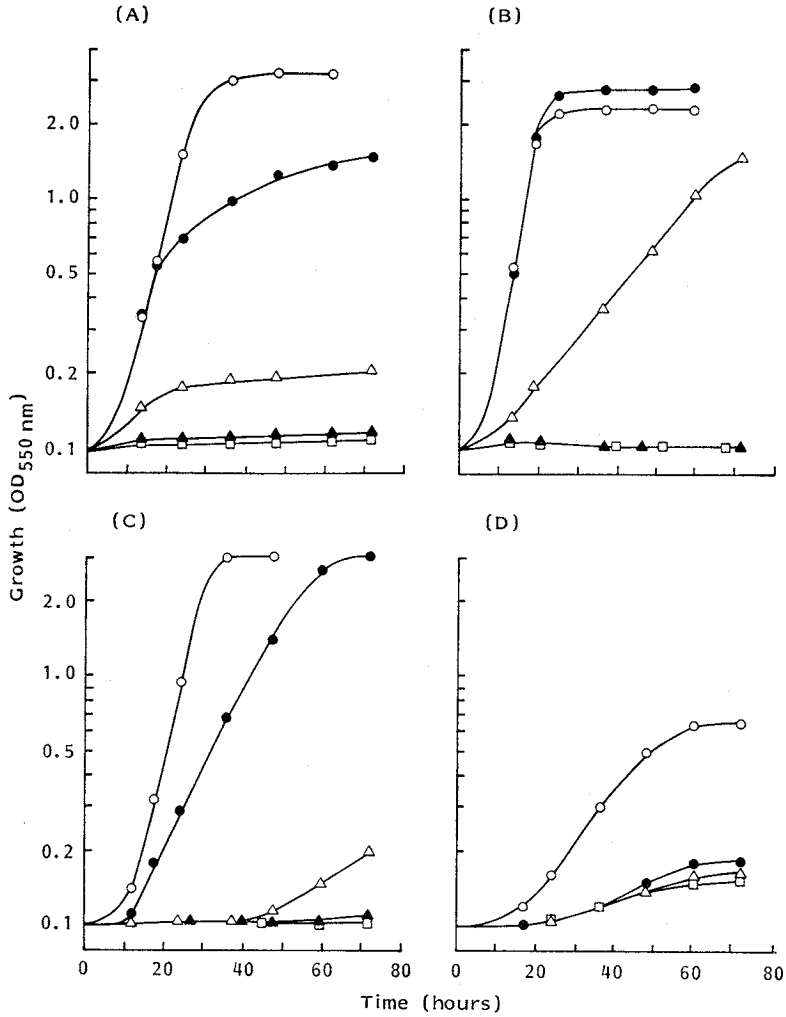
Yeast strains were inoculated by streaking on the medium containing yeast nitrogen base (Difco) 0.67%, glucose 0.5% and agar 1.5% (pH 5.3), and grown at 30°C. Where indicated, compactin (0~20 μg/ml) was supplemented to the medium. Growth was inspected after 4 days of cultivation. Of 303 strains tested, 43 strains (18 genera, 35 species), 21 strains (13 genera, 19 species) and 4 strains gave no detectable growth on the agar medium containing 20, 10 and 4 μg/ml of compactin, respectively, and the remaining 260 strains (34 genera, 135 species) were resistant to compactin at 20 μg/ml. The most sensitive 4 strains were *R. glutinis* H3-9-1, *S. salmonicolor* WF 188, *A. salmonicolor* IFO 1845 and *C. matritensis* IFO 0954. Under these conditions MICs were found to be 0.1 μg/ml for *R. glutinis*, 1.0 μg/ml for *S. salmonicolor*, 2.0 μg/ml for *A. salmonicolor* and 2.0 μg/ml for *C. matritensis*, respectively.

For the growth assay, these strains (~1×10^6 cells/ml) were inoculated to the liquid medium containing yeast nitrogen base 0.67% and glucose

Table 1. Inhibition by compactin-related compounds of the growth of *Rhodotorula glutinis* H3-9-1 and *Sporobolomyces salmonicolor* WF 188.

Compound	MIC (μg/ml) toward	
	R. glutinis H3-9-1	*S. salmonicolor* WF 188
ML-236A	5.0	50
ML-236B	0.1	1.0
Monacolin K	0.1	1.0
Monacolin L	3.3	6.0
Monacolin X	2.5	25

Fig. 2. Effect of mevalonate supplementation on the growth inhibition by compactin.



(A) *Rhodotorula glutinis* H3-9-1, (B) *Sporobolomyces salmonicolor* WF 188, (C) *Aessosporon salmonicolor* IFO 1845 and (D) *Citeromyces matritensis* IFO 0954.

The cells were cultured in the growth medium in the absence (○) or presence of 4 μg/ml of compactin supplemented with 0 mM (□), 1.0 mM (▲), 3.0 mM (△) and 10 mM (●) of *R,S*-mevalonate. Details are described in the text.

0.5%. Where indicated, compactin-related compounds (0~100 μg/ml) and/or mevalonate (0~10 mM) were supplemented to the medium. The cells were cultured with shaking at 30°C for 4 days, and growth was monitored by measuring OD at 550 nm. Table 1 shows the MICs of compactin-related compounds for *R. glutinis* H3-9-1 and *S. salmonicolor* WF 188. Of these compounds, ML-236B and monacolin K were most potent, and their MICs were 0.1 μg/ml for *R. glutinis*. ML-236A, and monacolins L and X showed activity 1/25~1/50 of those for ML-236B and monacolin K. *S. salmonicolor* was about 10 times more resistant to those compounds than *R. glutinis*.

It has been shown that the growth inhibition of a variety of animal cells by compactin could be overcome by the addition of small amount of mevalonate to the culture medium[2,4]. Fig. 2 shows the effect of mevalonate supplementation on the growth of compactin-sensitive strains. When 10 mM *R,S*-mevalonate was supplemented to the medium containing compactin, the growth of *R. glutinis* H3-9-1 was normal up to 20 hours

of cultivation. However, growth inhibition was observed after 20 hours (Fig. 2A). The supplementation of 10 mM mevalonate overcame the growth inhibition of *S. salmonicolor* WF 188 by compactin (Fig. 2B). In the presence of 10 mM mevalonate and compactin, the growth of *A. salmonicolor* IFO 1845 became normal after 10 hours (Fig. 2C). On the other hand, *C. matritensis* IFO 0954 showed no detectable growth restoration under the same conditions (Fig. 2D).

Mevalonate is a precursor of a variety of isoprenoid compounds, such as sterols, ubiquinones, dolichols, carotenoids and isopentenyl adenine[2,7]. Recently, several mutant strains have been isolated as the tools for the study of isoprenoid metabolism in yeasts[8,9]. The present results indicate that combinations of compactin and the sensitive strains might also be a useful system for studying mechanism and regulation of isoprenoid biosynthesis.

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