UK-1, a Novel Cytotoxic Metabolite from *Streptomyces* sp. 517-02

IV. Antifungal Action of Methyl UK-1

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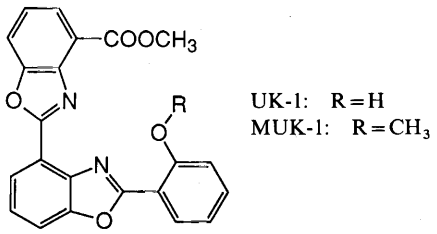
In the course of our screening program for new bioactive compounds, we have isolated a cytotoxic metabolite UK-1 from the acetone extracts of *Streptomyces* sp. 517-02[1]. UK-1 (Fig. 1) is structurally unique bis(benzoxazole) with the 2-position of one benzoxazole joining to the 4-position of the other[2,3]. We have previously reported that UK-1 did not show any antimicrobial activity, but its alkakine hydrolysate, demethyl UK-1, was active against some bacteria[4]. Interestingly, its methyl derivertive, methyl UK-1 (MUK-1, Fig. 1), was active against not only bacteria, but also fungi. This paper presents the antifungal action of MUK-1.

Fig. 1. UK-1 and MUK-1.



MUK-1 was obtained by methylation of UK-1 in anhydrous acetone with potassium carbonate and methyl iodide. Details of the methylation, ^{1}H and ^{13}C NMR data of MUK-1 were previously reported[2].

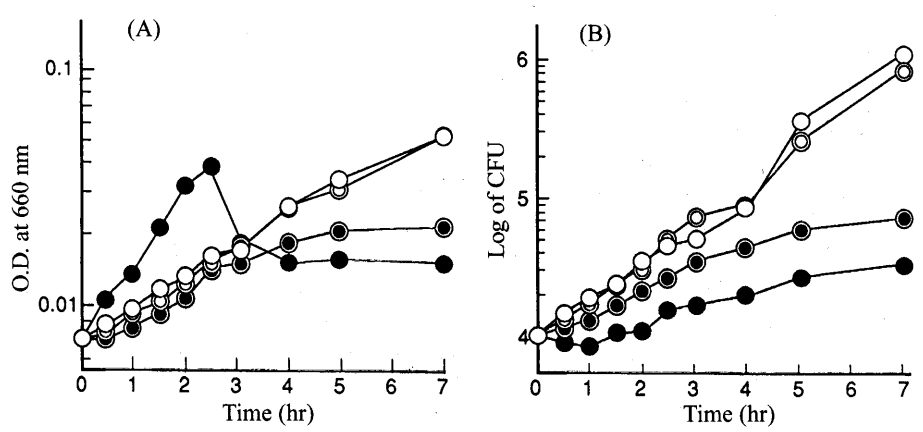
The MIC of MUK-1 was measured by the serial 2-fold agar dilution method in 3% nutrient agar at 30°C for bacteria and in Sabouraud dextrose agar at 25°C for yeasts and filamentous fungi. As shown in Table 1, MUK-1 completely inhibited the growth of not only bacteria but also yeasts and filamentous fungi (MIC ranges were approximately 1.56~3.13 μg/ml). MUK-1 showed no inhibition for the growth of *E. coli* IFO 3992

Table 1. Antimicrobial activities of MUK-1.

Organism	MIC (μg/ml)
Escherichia coli IFO 3992	>100
Pseudomonas aeruginosa IFO 3080	3.13
Proteus vulgaris IFO 3851	>100
Bacillus subtilis IFO 3007	3.13
Staphylococcus aureus NCTC 8530	3.13
Micrococcus luteus IFO 3333	1.56
Saccharomyces cerevisiae IFO 0203	25
Schizosaccharomyces pombe IFO 0342	50
Candida albicans IFO 1061	25
Rhodotorula rubra IFO 0001	3.13
Hansenula anomala IFO 0136	3.13
Torulospora delbrueckii DSM 70504	6.25
Aspergillus niger ATCC 6275	25
Rhizopus javanicus IFO 5441	3.13
Penicillium chrysogenum IFO 4626	1.56
Neurospora sitophila DSM 1130	1.56
Mucor mucedo IFO 7684	50

Fig. 2. Effect of MUK-1 on growth of *Rhodotorula rubra* IFO 0001 cells.

(A) Turbidity, (B) viability.
○: Control, ◎: MUK-1 at 0.78 μg/ml, ◉: MUK-1 at 3.13 μg/ml, ●: MUK-1 at 12.5 μg/ml.



and *Proteus vulgaris* IFO 3851, slight inhibition for the growth of *Schizosaccharomyces pombe* IFO 0342, *Mucor mucedo* IFO 7684 and some yeasts and filamentous fungi.

The effects of MUK-1 on the growth of *Rhodotorula rubra* IFO 0001 were examined. A 24-hours culture of *R. rubra* was diluted with Sabouraud dextrose broth to give approximately 10^4 cells/ml. After 1-hour incubation, known concentrations of MUK-1 were added to the cell suspensions, which were then incubated again with shaking at 25°C. Portions of the culture were withdrawn at intervals to measure O.D. at 660 nm and CFU/ml. Effects of MUK-1 on the growth inhibition measured in terms of turbidity and cell viability are shown in Fig. 2. When exponentially growing cells were exposed to MUK-1 at 3.13 μg/ml, the growth gradually decreased, and after 5-hours exposure, nearly stopped. Interestingly, the growth rate in tubidity rapidly increased for 2.5 hours after the addition of MUK-1 at 12.5 μg/ml, but such a

Fig. 3. Effects of MUK-1 on the morphological change of *Rhodotorula rubra* IFO 0001 cells.

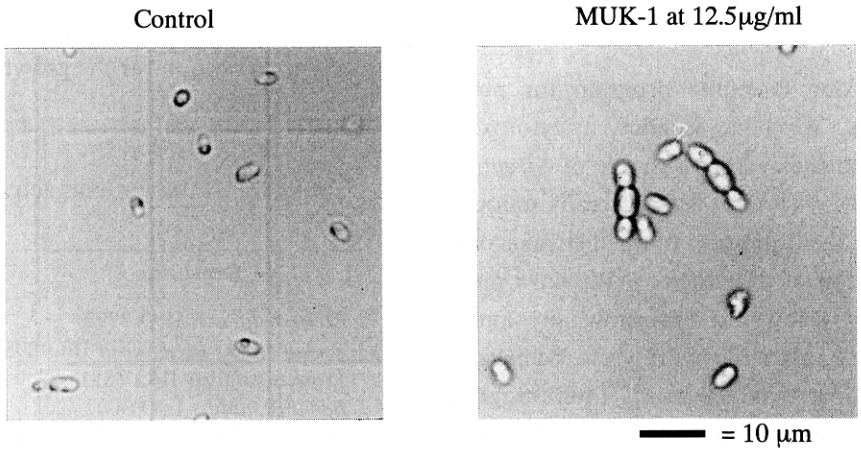
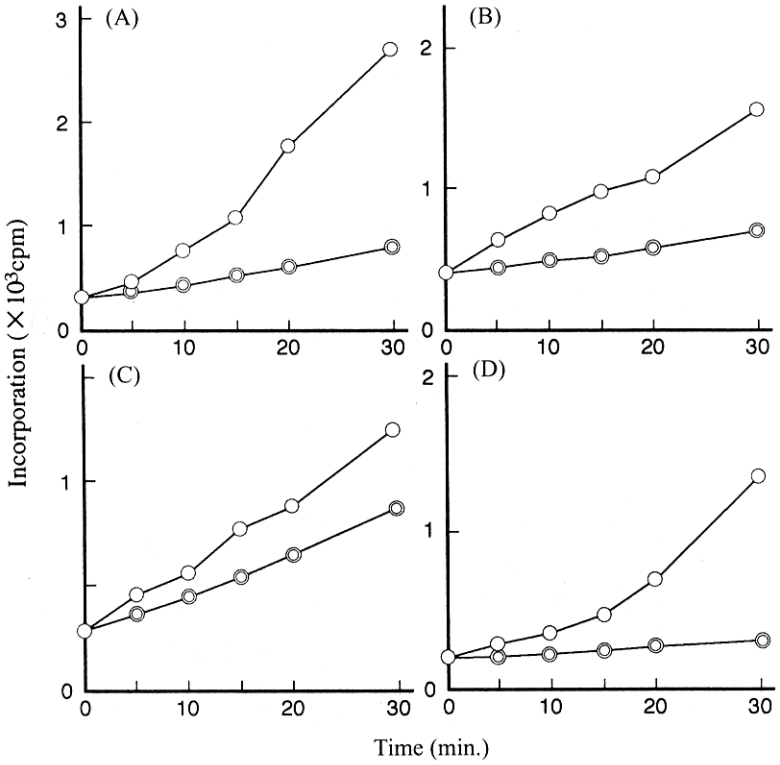


Fig. 4. Effect of MUK-1 on incorporation of radioactive macromolecular precursors into acid-insoluble fraction of *Rhodotorula rubra* IFO 0001 cells.

(A) ^{14}C-Adenine into RNA, (B) ^{14}C-adenine into DNA, (C) ^{14}C-phenylalanine, (D) ^{14}C-glucose.
○: Control, ◎: MUK-1 at 3.13 μg/ml.



rapid increase of the growth rate was not observed in viability. However, the turbidity decreased rapidly during 2.5~4 hours, and then was invariable. We observed that the cells were gradually swelling, but not changed in shape (data not shown). After 2-hours exposure to MUK-1 at 12.5 μg/ml, cells exposed were two times as large in size as control cells (Fig. 3). Moreover, we observed the swollen cells to be bursting when transferring them in distilled water (data not shown). It is supposed that MUK-1 at 12.5 μg/ml loosens the rigid cell wall of the yeast, therefore the cell swelling was observed. The decrease of turbidity during 2.5~4 hours might be due to an adaptation to the change in intracellular osmotic pressure.

The effects of MUK-1 on the incorporation of radioactive precursors into 5% trichloroacetic acid (TCA)-precipitative fraction in *R. rubra* cells are shown in Fig. 4. To determine incorporation of ^{14}C-adenine into DNA (alkaline resistant fraction), cells were incubated with 0.1 N NaOH for 30 minutes at 30°C before TCA precipitation. Incorporation into RNA fraction was determined as the difference of radioactivities in acid-insoluble fraction and in the alkaline resistant fraction. In the control cultures, the incorporation of radioactive adenine, L-phenylalanine and glucose started instantaneously, and the counts of each fraction increased almost linearly up to 60 minutes after the onset of incubation. MUK-1, at 3.13 μg/ml, weakly inhibited the incorporation of adenine and L-phenylalanine by about 50~70% compared with the control, while completely inhibiting the incorporation of glucose.

MUK-1, at 12.5 μg/ml within 2 hours, did not induce appreciable amounts of leakage of 260-nm absorbing materials and potassium ions from *R. rubra* cells, and did not affect their oxygen consumptive activity (data not shown). These may suggest that MUK-1 reduces biosynthetic activity of polysaccharides including those making up cell wall, and therefore loosens the wall to swell the cells. Further studies on the detail action of MUK-1 are currently in progress.

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