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NOTE

Production of Elaiophylin by the Strain MCY-846 in a Submerged Culture

LEE, SANG-YONG[1], SANG-CHUL HA, YOUNG-SOO HONG, SOON-DUCK HONG[1], AND JUNG-JOON LEE*

Natural Products Biosynthesis R.U., Korea Research Institute of Bioscience and Biotechnology (KRIBB), P.O. Box 115, Yusong, Taejon 305-600, Korea
[1]Department of Microbiology, Kyungpook National University, Taegu 702-701, Korea

Streptomyces sp. MCY-846 selected by *in vitro* cytotoxicity assay produced elaiophylin. Individual characteristics of the strains such as spore morphology, and physiological characteristics indicated that the strain is resembled to *Streptomyces hygroscopicus*. The time course of cell growth and antibiotic production was observed in the medium containing 0.5% trehalose and 0.5% soybean meal as carbon and nitrogen sources, respectively. The optimum production of elaiophylin was tested with different combinations of carbon and nitrogen sources and reached a maxima of 470 μg/ml in the PC-II medium.

Among the microbial genera, the actinomycetes continue to be the most important group because of their production of a wide variety of biologically active metabolites (5), particularly anticancer agents such as doxorubicin, aclacinomycin, mitomycin, bleomycin, actinomycin D etc.

In the course of screening for antitumor antibiotics from soil microbes, a strain, MCY-846 was isolated for its strong cytotoxicity against gastric cancer cell line, SNU-1 and liver cancer cell line, SNU-354. The active component of this strain was isolated by activity guided fractionation of acetone extract of the mycelium and was further purified by MPLC, and preparative HPLC. The structure of the active compound was identified as elaiophylin (7) (Fig. 1). Elaiophylin was previously isolated from cultures of *Streptomyces melanosporus* (1), *Streptomyces violaceoniger* (3), and *Streptomyces hygroscopicus* var. *azalomyceticus* (6). Those strains reported previously either produced elaiophylin alone or produced elaiophylin together with niphimycin (3) or salinomycin (12). Therefore, we investigated the identity of the strain and optimum culture conditions for the production of elaiophylin.

The growth of the strain on ISP media was good in general and the color of the aerial mycelium was gray on ISP 4 medium or white on ISP 6 medium. The spores of the isolate had a spiral form with a rugose surface and contains LL-diaminopimelic acid (2). According to our analysis of the physiological characteristics and the utilization of carbohydrates (9), the strain is closely related to *Streptomyces hygroscopicus* in spite of minor differences such as H_2S production, and utilization of L-histidine, and of DL-α-amino-n-butyric acid (Table 1). We therefore designated this strain *S. hygroscopicus* MCY-846 based on the culture characteristics, profile of sugar utilization, and morphological characteristics of the spores.

A preliminary study for antibiotic production by the strain MCY-846 was carried out using GSS medium (2% soluble starch, 1% glucose, 0.1% beef extract, 0.4% yeast extract, 0.2% NaCl, 0.005% K_2HPO_4, 0.2% $CaCO_3$, 2.5% soybean meal) as a basic medium. The strain was cultured on a rotary shaker at 200 rpm at 28°C for 7 days. Optimum culture conditions were investigated by determining the effect of various carbon and nitrogen sources, initial pH, temperature and aeration rate on the production of antibiotic.

For comparison of elaiophylin production using several different media, we tested the following media; GP (4% glycerol, 0.5% peptone, 0.2% glucose, 0.2% potato starch, 2.5% soybean meal, 0.5% yeast extract, 0.5% NaCl, 0.2% $CaCO_3$), SP (2.5% soytone, 2% glucose, 1% soluble starch, 0.1% beef extract, 0.4% yeast extract, 0.2% NaCl, 0.005% K_2HPO_4), Fish medium (1% soluble starch, 2% glucose, 0.1% beef extract, 0.4% yeast extract, 0.2% NaCl, 0.025% K_2HPO_4, 0.2% $CaCO_3$, 1% soybean meal, 1% fish meal), SGM (1% glucose, 1.5% soluble starch, 3% soybean meal, 0.3% NaCl, 0.1% K_2HPO_4,

*Corresponding author
Phone: 82-42-860-4360. Fax: 82-42-860-4595.
E-mail: jjlee@kribb4680.kribb.re.kr
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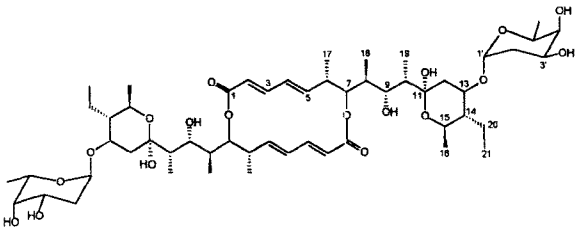

Fig. 1. Structure of elaiophylin.

0.1% $MgSO_4 \cdot 7H_2O$, 0.007% $CuSO_4 \cdot 5H_2O$, 0.0001% $FeSO_4 \cdot 7H_2O$, 0.0008% $MnCl_2 \cdot 4H_2O$, 0.0002% $ZnSO_4 \cdot 7H_2O$), OMYM (2% oat meal, 0.2% malt extract, 0.2% yeast extract, 0.2% glucose, 0.0006% $CoSO_4 \cdot 7H_2O$, 0.0003% $ZnSO_4 \cdot 7H_2O$, 0.0003% $MnSO_4$, 0.003% $FeSO_4 \cdot 7H_2O$), PC-II (2.5% soluble starch, 1.5% soybean meal, 0.2% dry yeast, 0.4% $CaCO_3$), and YM (0.4% yeast extract, 1% malt extract, 0.4% glucose).

In order to measure titer, dry cell weight (DCW) and pH, 4 ml of culture broth was harvested by centrifugation and the supernatant was used for pH measurement. The mycelium was extracted with equal volumes of acetone. After filtration and concentration under vacuum, the residue was dissolved in 500 µl MeOH for cytotoxicity assay and the filter cake was dried at 70~80°C for 12 h for DCW measurement. The titer of elaiophylin was expressed as relative activity between samples, which was determined by the antibacterial activity against *Bacillus subtilis* and SRB (sulforhodamine B) method (11).

Table 1. Comparison of the strain MCY-846 and *Streptomyces hygroscopicus*.

	MCY-846	*S. hygroscopicus*
spore surface	rugose	rugose
spore chain morphology	spiral	spiral
color of spore mass	gray	gray
H_2S production	−	+
arbutin degradation	+	+
nitrate reduction	−	−
rifampicin (50 µg/ml) resistance	+	+
utilization of carbon source (1%, w/v)		
cellobiose	+	+
meso-inositol	+	+
D-mannitol	+	+
L-rhamnose	+	+
raffinose	+	+
D-fructose	+	+
adonitol	−	−
inulin	−	−
D-xylose	+	+
utilization of nitrogen source (1%, w/v)		
L-hydroxyproline	+	+
L-histidine	−	+
DL-α-amino-n-butyric acid	−	+

+, positive; −, negative.

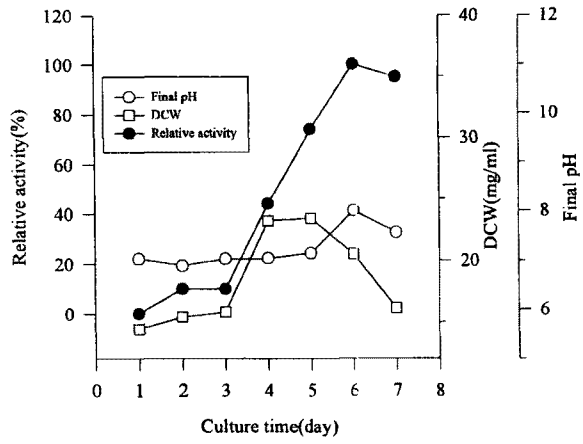

Fig. 2. Time course of the antibiotic production.
The strain MCY-846 was cultivated in GSS medium, pH 6.0 at 30°C, 200 rpm.

The time course of cell growth and antibiotic production in GSS medium is shown in Fig. 2. The maximum production of antibiotic was reached on day 6 at 30°C and at 200 rpm.

Selection of suitable nutrients for fermentation media is an important determinant in improving antibiotic production (10). Fermentation media are composed of carbon sources, nitrogen sources, inorganic salts, and buffering agents such as $CaCO_3$. Various carbon sources (19 kinds) which were fixed at a concentration of 1% were added to the medium containing soybean meal as a basic nitrogen source. Trehalose and arabinose gave rise to the best production of antibiotic, but sorbose did not. This means that the strain can hydrolyse trehalose (a non-reducing disaccharide) using an α-glucosidase, trehalase. However, antibiotic production was not related to any concentration of trehalose between 0.5% and 3% (Fig. 3).

To investigate the effects of different nitrogen sources on antibiotic production, 14 nitrogen sources were examined using 0.5% trehalose as a sole carbon source. Among them soybean meal (0.5%) was the most effective for maximum antibiotic production (Fig. 4). On the other hand, no antibiotic production was observed using the inorganic nitrogen sources such as KNO_3 and $(NH_4)_2HPO_4$.

The effect of inorganic salts and trace metal elements on antibiotic production was evaluated. Zn, Cu, and Co were not related to antibiotic production but 0.3% $CaCl_2$ showed a positive effect on antibiotic production (data not shown). For maximum growth and antibiotic production 0.2% $CaCO_3$ was selected as a buffering agent.

From these results, the optimized medium for the production of antibiotic by *S. hygroscopicus* MCY-846 was

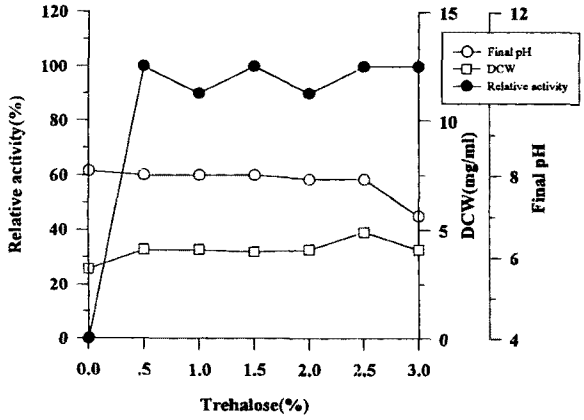

Fig. 3. Effect of concentration of trehalose on the production of elaiophylin.
The strain was cultivated at 30°C for 4 days in the medium containing 1% soybean meal, 0.2% NaCl, 0.005% K_2HPO_4, 0.2% $CaCO_3$, and indicated % of trehalose.

formulated as follows: 0.5% trehalose, 0.5% soybean meal, 0.3% $CaCl_2$, 0.2% $CaCO_3$. This compares with other elaiophylin-producing medium containing 2% soybean meal and 2% mannitol (4).

The metabolite pattern and yield of the different antibiotics are dependent on the culture medium as well as on culture conditions. To determine the effect of culture media on the production of elaiophylin the strain was cultured in nine different media. The production of antibiotic in media such as PC-II, optimized medium (TS) was superior to that of SP and OMYM media. Due to the transformation of elaiophylin through *O*-methylation of the hemiketal hydroxyl group of elaiophylin in a

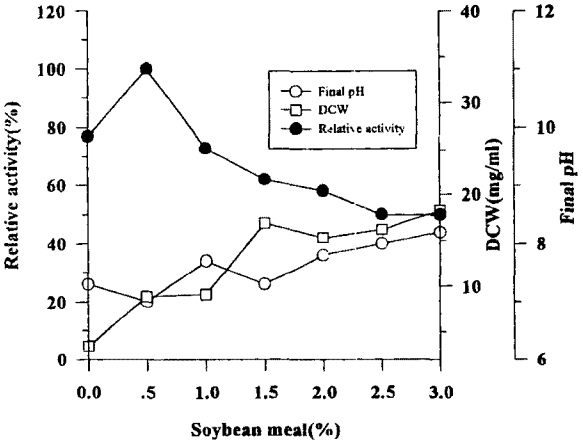

Fig. 4. Effect of concentration of soybean meal on the production of elaiophylin.
The strain was cultivated at 30°C for 4 days in the medium containing 0.5% trehalose, 0.2% NaCl, 0.005% K_2HPO_4, 0.2% $CaCO_3$, and indicated % of soybean meal.

methanol solution (8), the acetone and/or ethyl acetate extract was analyzed by HPLC (solvent; 80% acetonitrile) to measure the titer of elaiophylin. According to this analysis, elaiophylin reached to 470 μg/ml in the PC-II medium and 340 μg/ml in the GP but SP, GSS, and TS media gave 20~40 μg/ml production yield. This result supported the fact that metabolite production can be modulated by changing the composition of culture media.

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