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Note

A Methanol-utilizing Actinomycete

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Abstract

An actinomycete, *Streptomyces* sp. No. 239, which was able to grow on methanol as its sole carbon and energy source was isolated from a soil sample. The isolate showed optimal growth at 37 to 40°C. The maximum growth yield of the actinomycete on methanol was 0.37 g of dry cell weight per g of methanol. Protein content of the isolate was 63.1 to 67.6%.

Many bacteria and yeasts of different genera are known to grow on methanol as their sole carbon and energy sourse.[1] However, no systematic attempt has been made to examine methanol utilization by actinomycetes, although the indications of primarily detection of actinomycetes[2] or of the growths on methanol in the presence of other organic carbon-sources[3,4] have been reported. In the course of our investigations on microbial utilization of methanol, an actinomycete which was able to grow on methanol as its sole carbon source was isolated. In this paper, the brief microbial characteristics, the cell growth on methanol and the cell composition of the isolate are described.

The actinomycete, strain No. 239, was isolated by enrichment cultures on methanol from a soil sample from New Guinea. The aerial mycelia of the actinomycete developed well on glucose-asparagine agar, bouillon agar, Czapek agar, and methanol-asparagine agar (1 g methanol, 0.05 g asparagine, 0.05 g K_2HPO_4 and 1.5 g agar in 100 ml tap water). On the above media, aerial mycelia were snow white to white, becoming yellow as the culture grew older. The mycelia were straight or blanched but did not form whorls or spirals (Fig. 1). Fragmentation of the vegetative mycelium was not observed. Oval shaped spores were formed in chains and the surfaces were smooth (Fig. 2). No brown pigmentation was observed in protein media. A soluble yellow pigment was produced only

Fig. 1. Photomicrograph of the strain No. 239. Cells were cultured on glucose-asparagine agar for a day at 37°C.

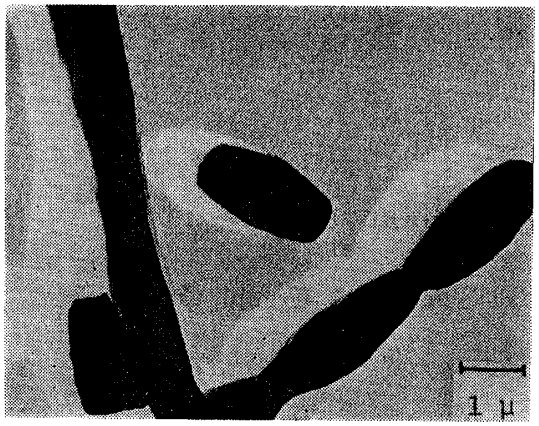

Fig. 2. Electron micrograph of the spores of strain No. 239.

in methanol-asparagine agar but no pigmentation was found in any other media. The organism was tentatively named as *Streptomyces* sp. No. 239 according to the descriptions of Waksman.[5]

For the investigation of methanol utilization of the *Streptomyces* sp. No. 239, medium consisting of 1.0 g methanol, 0.4 g NH_4Cl, 0.1 g K_2HPO_4, 0.1 g $NaH_2PO_4 \cdot 2H_2O$, 0.05 g $MgSO_4 \cdot 7H_2O$, 0.1 g asparagine, vitamins* and trace metal salts** in 100 ml tap water, pH 7.0, was mainly used. A hundred milliliters of the medium in a 500 ml-shake flask was inoculated with 4 ml of a cell suspension in the same medium, freshly prepared from a methanol-slant culture. Cultivation was carried out on a reciprocal shaker at 110 cycles per min. The cells in the cultured broth were collected by filtration and then the dry cell weight was measured.

Although the *Streptomyces* sp. No. 239 grew sufficiently well on medium containing only methanol, inorganic salts and vitamins, the growth rate was stimulated by the addition of an organic substance, such as yeast extract, meat extract, ammonium acetate or asparagine (in each 0.1% concentration) to the medium.

Figure 3 shows the effect of methanol concentration on growth. Higher growth rates were found at lower methanol concentrations. The maximum growth yields of the organism on methanol were 0.37, 0.35 and 0.18 g of dry cell weight per g methanol in 0.5, 1.0 and 2.0% methanol media, respectively.

The effect of temperature was studied by culturing the organism on 1% methanol medium. As shown in Fig. 4, temperatures of 37 to 40°C were found to give optimum growth.

The Kjeldahl nitrogen content of cells grown on 1% methanol medium at 37°C were found to be 10.1 to 10.5%. From the data, the crude protein was estimated to be 63.1 to 65.6%. The total nucleic acid content was found to be 9.8 to 10.3%. The amino acid profile of the actinomycete is shown in Table 1, in which those of a methanol-utilizing yeast, *Kloeckera* sp. No. 2201,[6] a methanol-utilizing bacterium, TM 20,[7] a waste cellulose-utilizing actinomycete, *Thermomonospora fusca*,[8] and FAO reference are presented for comparison. All of the essential amino acids for human diet are present except tryptophan, which can not be analyzed by the acid hydrolyzation used in the experiment. The cystine content is unusually high. The contents of isoleucine and valine are a little low compared

* Vitamins: Biotin 2 μg, Ca-pantothenate 400 μg, thiamine·HCl 400 μg, pyridoxine·HCl 400 μg, nicotinic acid 400 μg, *p*-aminobenzoic acid 200 μg, and riboflavin 200 μg in 1,000 ml medium.

** Trace metal salts: $FeCl_3 \cdot 6H_2O$ 1,000 μg, $ZnSO_4 \cdot 7H_2O$ 100 μg, $CuSO_4 \cdot 5H_2O$ 100 μg, $MnSO_4 \cdot 7H_2O$ 40 μg, and $MoO_3 \cdot H_2O$ 20 μg in 1,000 ml medium.

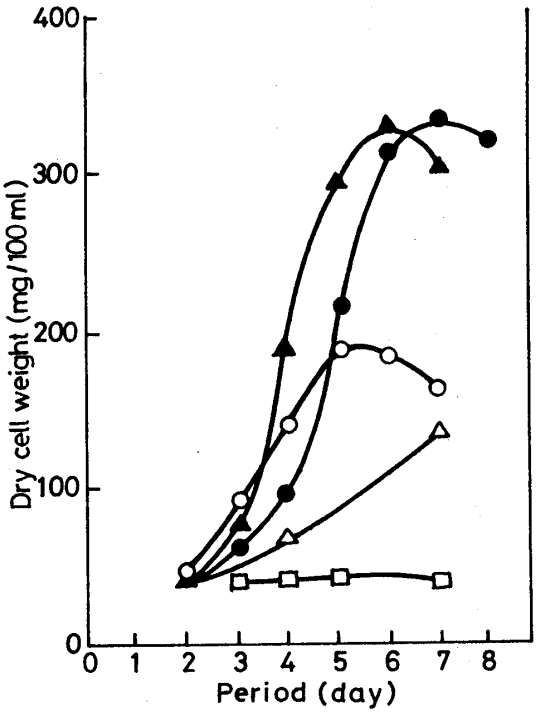

Fig. 3. Effect of methanol concentration on the growth of *Streptomyces* sp. No. 239.
The compositions of medium was described in the text. The cultivation was carried out at 37°C.
Methanol concentration:
-□-, 0%; -○-, 0.5%; -▲-, 1.0%; -●-, 2.0 %; -△-, 3.0%.

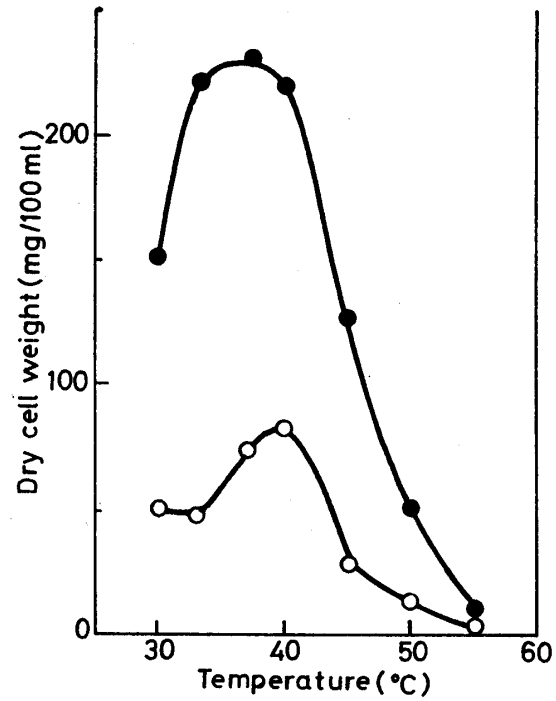

Fig. 4. Effect of temperature on the growth of *Streptomyces* sp. No. 239.
The cultivation was carried out under the standard conditions at various temperatures. The growth was measured after 3 (○) or 5 days (●) cultivations.

with the other microbial proteins.

Although a number of species of *Streptomyces* are well known antibiotic producers, no antibiotic activity on *Staphylococcus aureus* was detected in the cultured broth of the *Streptomyces* sp. No. 239.

Table 1. Amino acid profiles of *Streptomyces* sp. No. 239 and other microorganisms.

Amino acid	*Streptomyces* sp. No. 239	*Kloeckera* sp. No. 2201*[6]	TM 20[7]	*T. fusca*[8]	FAO reference
	Amino acid content (g/100 g protein)				
Lysine	5.53	3.4	5.30	3.63	4.2
Histidine	2.13	0.8	1.73	1.96	—
Arginine	7.55	2.4	7.10	5.62	—
Aspartic acid	8.84	4.7	8.47	6.74	—
Threonine	5.81	2.3	4.52	4.00	2.8
Serine	3.11	2.3	3.62	2.57	—
Glutamic acid	15.47	3.5	10.92	18.03	—
Proline	5.03	1.4	3.81	6.10	—
Glycine	5.56	2.4	5.55	4.42	—
Alanine	9.31	3.1	7.91	13.92	—
Cystine	9.75	trace	0.32	0.41	2.0
Valine	2.14	2.4	5.85	12.97	4.2
Methionine	1.28	0.4	1.81	2.06	2.2
Isoleucine	2.91	2.3	3.90	3.20	4.2
Leucine	7.27	3.2	6.96	6.10	4.8
Tyrosine	2.62	1.5	2.91	1.87	—
Phenylalanine	3.82	1.8	4.18	2.64	2.8
Tryptophan	—	—	—	—	1.4

The cells of *Streptomyces* sp. No. 239 were acid-hydrolyzed (in 6N HCl for 20 hr at 100°C) prior to analysis on a Hitachi auto-analyzer, type KLA-5. Separate assays for tryptophan and cystine were not conducted. The contents of *Streptomyces* sp. No. 239 were averages of the values of two run experiments.
* Amino acid g/100 g cells.

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