

Communications to the editor

ISOLATION OF A NEW SULFUR-CONTAINING BASIC SUBSTANCE FROM A *THERMOACTINOMYCES* SPECIES

Sir:

We have already reported the discovery of new alkaloids such as pyrindicin, NA-337 A *etc.* from *Streptomyces* sp.^{1,2,3)}

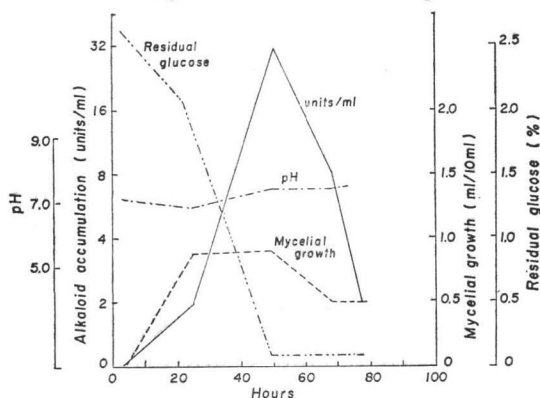
In the present communication, we wish to report the isolation of a new substance, TM-64, which is positive against DRAGENDORFF's reagent and which contains sulfur, produced by a thermophilic actinomycete, *Thermoactinomyces* strain No. TM-64.

Thermoactinomyces strain No. TM-64 was cultured for 24 hours at 45°C in a medium (100 ml/500 ml shake flask) consisting of 2.0 % glucose, 0.5 % peptone, 0.3 % baker's dry yeast, 0.5 % NaCl, 0.3 % CaCO₃ and 0.5 % meat extract (pH 7.0). One per cent inoculum was transferred to a 30-liter jar fermentor containing 20 liters medium consisting of 1.0 % glucose, 2.0 % starch, 0.5 % yeast extract, 0.5 % peptone and 0.4 % CaCO₃ (pH 7.0) and run at 45°C for 48~72 hours under 200 r.p.m. agitation and 10 liters/min aeration. The time course of the fermentation is shown in Fig. 1.

The culture filtrate (20 liters, 32 units*¹⁾)

Fig. 1. Time course of TM-64 production.

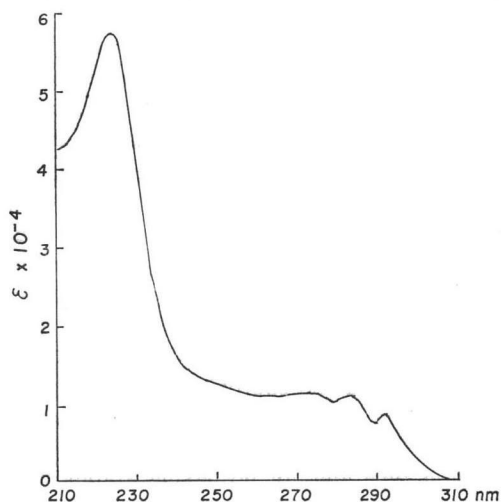
Culture conditions were as follows: agitation 200 rpm, temp. 45°C and aeration 10 liters/min. Mycelial growth was shown by packed wet-cell volume (ml/10 ml cultured broth) when it was centrifuged for 10 min. at 3000 rpm.



was adjusted to pH 10 with conc. ammonia and extracted with 4 liters butyl acetate. The solvent layer was extracted with 0.1N HCl (800 ml); the aqueous layer was adjusted to pH 10 and extracted three times with CHCl₃ (each 200 ml). The solvent layer was extracted with 0.1N HCl (60 ml), then the acid layer was adjusted to pH 10 and extracted three times with ethyl ether (each 100 ml). The solvent layer was dried over anhydrous Na₂SO₄, and evaporated to obtain 300 mg of pale yellow granular crystals. These crystals were recrystallized from benzene to obtain pure TM-64.

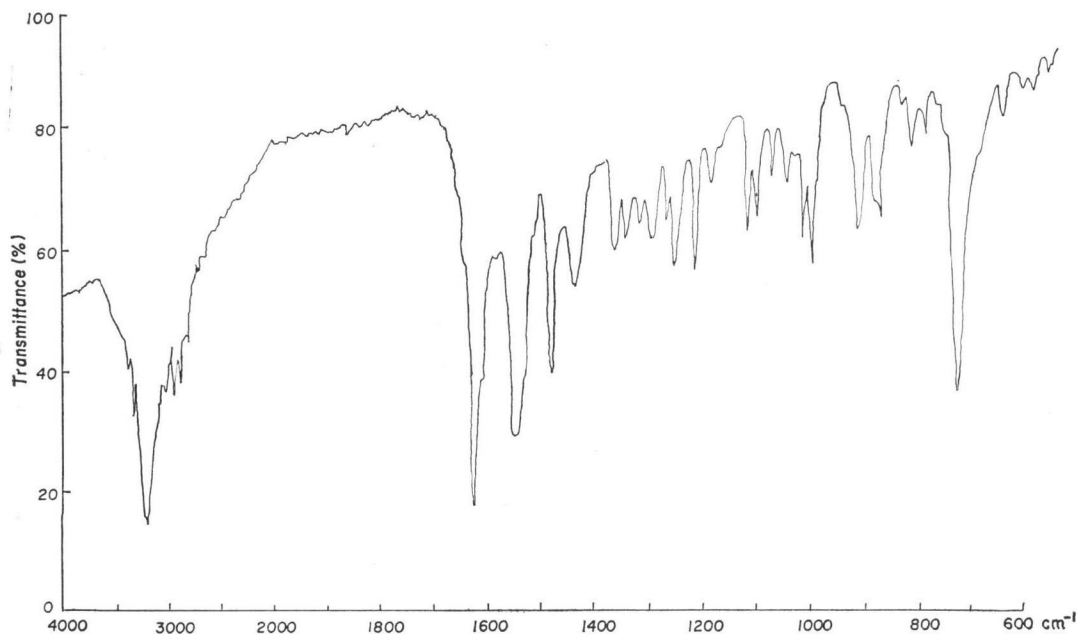
Physicochemical properties of TM-64 were as follows: m.p. 120~122°C, Anal. Found: C, 61.37; H, 5.89; N, 18.04; S, 9.70 %. Calcd. for C₁₆H₁₈N₄SO: C, 61.12; H, 5.77; N, 17.82; S, 10.19 %. Mass spectrum, Found, *m/e*: 314.1183 (M⁺). Calcd. for C₁₆H₁₈N₄SO: 314.1190, [α]_D²⁰ -6.0° (c 1, MeOH). UV_{MeOH}^{max} nm(ε): 223 (57150), 275 (10500), 282 (10500), 291 (8350) (Fig. 2). As shown in Fig. 3, its infrared (IR) spectrum indicated an absorption of amide at 1630 cm⁻¹. This substance gave positive reactions to DRAGENDORFF's, EHRLICH's, ninhydrin, MAYER's reagents and

Fig. 2. U.V. Spectrum of TM-64 (in MeOH)



* The assay of the alkaloid was followed the method previously reported.¹⁾ The titer (units) of alkaloid was indicated by maximum dilution number of the DRAGENDORFF's reagent positive solution.

Fig. 3. IR spectrum of TM-64 (KBr)



sulfur test but negative reactions to SAKAGUCHI, FeCl_3 and anthrone reagents. From the UV spectrum and positive reaction to EHRICH's reagent, the TM-64 substance was considered to have an indole skeleton. From a positive reaction for sulfur, it was also considered to have a residue containing this moiety. Its detailed structure is now under study.

Pharmacological activities of TM-64 were examined and a weak deteriorated reflex action of cornea was observed when 3% solution of TM-64 was dropped to eyes of guinea pigs.

The growth temperature of the strain No. TM-64 is above 37°C and the optimal growth temperature is 45°C . This strain grows well on oatmeal-yeast extract agar and nutrient agar, and forms a white powdery aerial mycelium. Morphologically, it bears one spore each on the substrate mycelium and aerial mycelium, and the sporophore is very short or the spore is directly attached to the mycelium. The cell wall is type III, with DAP in meso type and not possessing arabinose or galactose. Consequently, strain No. TM-64 is considered to belong to the genus *Thermoactinomyces*, and it has been deposited in the Fermentation Research Institute, Chiba, Japan

(FERM-P No. 3076).

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