TYPE OF DIAMINOPIMELIC ACID DIFFERENT IN AERIAL AND VEGETATIVE MYCELIA OF SETAMYCIN-PRODUCING ACTINOMYCETE KM-6054

Sir:

Strain KM-6054, an actinomycete strain isolated from a soil sample collected at Setagaya-ku, Tokyo, has been reported to produce a new antibiotic, setamycin, showing activity against some fungi, trichomonads and Gram-positive bacteria.[1] In the course of taxonomic study, we found that the mycelia of strain KM-6054 contains both LL-diaminopimelic acid (DAP) and its meso-isomer, although this strain may be thought to be a strain of genus *Streptomyces* from the morphology, cultural characteristics, *etc.* It was also found that when DAP analysis was carried out separately on aerial and vegetative mycelia grown on an agar medium, most of DAP contained in aerial mycelia is LL-isomer while that in vegetative mycelia is meso-isomer (Fig. 1). There is no report concerning actinomycete strains which exhibit the above characteristics. The present communication deals with taxonomic properties of strain KM-6054.

The procedures described by SHIRLING and GOTTLIEB[2] were followed for morphology and cultural and physiological characteristics. Strain KM-6054 is well developed on both synthetic and complex media. No fragmentation of the vegetative mycelium is observed (Plate 1). The aerial mycelium, morphology of which is classified in the section *Rectus-flexibilis*, has more than 20 spores per chain (Plate 2). The spores have smooth surfaces and are cylindrical in shape and $0.7 \times 0.8 \sim 1.6\ \mu$ in size. No sporangium and zoospore are observed. The colony of strain KM-6054 is leathery. Vegetative mass color shows yellow or brown. White aerial mycelia are abundantly formed on yeast extract - malt extract agar, oatmeal agar and inorganic salts - starch agar. The strain produces a small amount of yellow soluble pigment in inorganic salts - starch agar. The formation of melanoid pigment is not observed. The culture grows at 22 ~ 37°C.

The morphology described above and the gas-chromatogram pattern of cellular fatty acids by

Fig. 1. Paper chromatogram of the hydrolysates of (A) mycelia grown in a liquid medium and (B) aerial and (C) vegetative mycelia of strain KM-6054 grown on a solid medium.

The procedures described by LECHEVALIER and LECHEVALIER[4] were modified for DAP analysis. Paper chromatography was carried out by descending technique using a Toyo Roshi No. 51 paper, MeOH - pyridine - 10 N HCl - H_2O (64: 8: 2: 14) and ninhydrin.

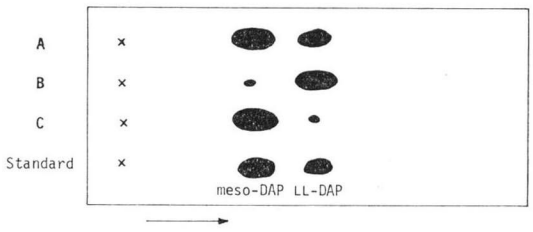


Plate 1. Vegetative mycelium of strain KM-6054. Inorganic salts - starch agar, 14 days at 28°C.

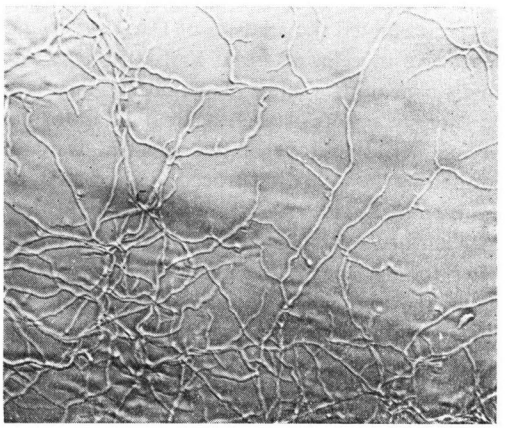
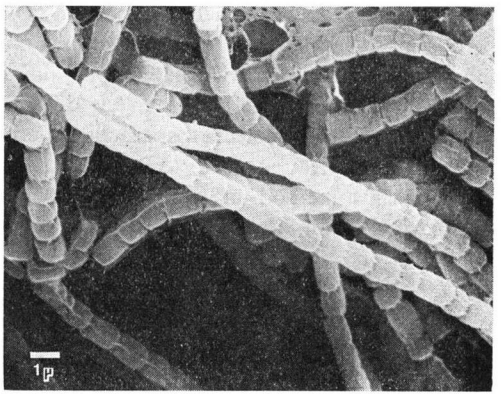


Plate 2. Spore chains of strain KM-6054. Inorganic salts - starch agar, 14 days at 28°C.



OKAMI's method[3] may indicate that strain KM-6054 belongs to the genus *Streptomyces*. However, the cell-wall constituents of strain KM-6054 do not agree with those of *Streptomyces* strains, in which meso-DAP and galactose are not detected. From the results of the analysis by LECHEVALIER's method,[4] the mycelium hydrolysate was found to contain similar amounts of LL- and meso-DAP, and galactose. In general, the method of LECHEVALIER & LECHEVALIER[4] for analysis of cell wall DAP has been usefully applied to grouping of genera in *Actinomycetales*. However, there is no known genus in which this strain should be classified. Some strains belonging to genus *Micromonospora*[5] are known to contain both LL- and meso-DAP, but the content of the former is a little or a trace in comparison with the latter. Morphological characteristics of these strains are quite different from strain KM-6054. Further investigations are now in progress to determine the genus of the strain.

We attempted the analysis of DAP in aerial or vegetative mycelia of strain KM-6054 grown on an agar medium. After the strain was incubated on inorganic salts - starch agar at 27°C for two weeks, aerial and vegetative mycelia were separated by the following procedures and analyzed the type of DAP. Aerial mycelia grown on the agar surface were scraped off with a steel loop. After the surface of the agar was washed with water, the agar was melted on a water bath and filtered with cotton gauze. The residue was washed with hot water and air-dried at room temperature to give vegetative mycelia. Both aerial and vegetative mycelia (10 mg each of dry weight) were hydrolyzed in 6 N HCl at 100°C for 18 hours to analyze DAPs by paper chromatography. As shown in Fig. 1, it was found that there is a major amount of LL-DAP in the aerial mycelium, while there is mostly meso-DAP in the vegetative mycelium. Within our knowledge, this communication is the first example showing that different isomers of DAP were detected separately in aerial and in vegetative mycelia. In addition to LECHEVALIER's method in which a submerged culture of a strain is used for DAP analysis, chemical analyses of the cell wall constituents of aerial and vegetative mycelia grown on a solid medium may provide a useful indication in the taxonomic studies. These analyses are now being carried out on some known actinomycete strains, beside the further investigations on determining the genus of strain KM-6054.

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References

1) ŌMURA, S.; K. OTOGURO, T. NISHIKIORI, R. ŌIWA & Y. IWAI: Setamycin, a new antibiotic. J. Antibiotics 34: 1253~1256, 1981
2) SHIRLING, E. B. & D. GOTTLIEB: Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol. 16: 313~340, 1966
3) OKAMI, Y.; M. HAMADA & N. UEDA: Relationship between genera of actinomycetes with reference to gas chromatographic analysis. p. 457~475, Proc. 1st Int. Confer. Culture Collections of Microorganisms, H. IIZUKA and T. HASEGAWA (*ed.*), Univ. Tokyo Press, Tokyo, 1970
4) LECHEVALIER, M.P. & H. LECHEVALIER: Chemical composition as a criterion in the classification of aerobic actinomycetes. Int. J. Syst. Bacteriol. 20: 435~443, 1970
5) KAWAMOTO, I.; T. OKA & T. NARA: Cell wall composition of *Micromonospora olivoasterospora*, *Micromonospora sagamiensis*, and related organisms. J. Bact. 146: 527~534, 1981