STUDIES ON THE α -GLUCOSIDE HYDROLASE INHIBITOR, ADIPOSIN

II. TAXONOMIC STUDIES ON THE PRODUCING MICROORGANISM

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Streptomyces sp. TM-521 which produced α -glucoside hydrolase inhibitors, adiposins¹⁾, was isolated from a soil sample collected in Hoya City, Tokyo. The strain belonged to the "gray color" series type of the ISP-classified *Streptomyces*. From the taxonomic studies, the strain TM-521 resembled with *Streptomyces calvus*²⁾ in the morphological and physiological properties. This strain was accordingly identified as a strain of *Streptomyces calvus*.

In the course of our screening program searching for α -glucoside hydrolase inhibitors, a strain numbered TM-521 was found to produce several α -glucoside hydrolase inhibitors. This paper deals with the taxonomic studies on the strain TM-521.

Taxonomic Studies of Streptomyces sp. TM-521

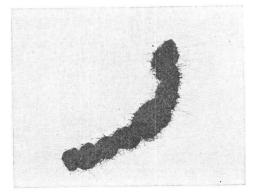
The strain TM-521 was isolated from a soil sample obtained in Hoya City, Tokyo. It grows well on various standard media including the ISP media that are recommended by SHIRLING and GOTTLIEB³⁾ for the description of *Streptomyces* cultures. The aerial mycelia on oat meal agar, yeast extract-malt extract agar and glucose asparagine agar form abundant spores. Microscopic examination of the culture grown on oat meal agar revealed abundant aerial mycelia and spore chains with primitive spirals (Fig. 1). Sporangium, flagellated spore, sclerotium, cormium or true verticil was not observed. A mature spore chain contained about 10 spores on the average. An electron micrograph of the spore shows oval to spherical $(0.7 \sim 1.0 \times 1.0 \sim 1.4 \mu)$ with a hairy surface (Fig. 2).

The cultural characteristics of the strain TM-521 on various media are shown in Table 1. The agar plates were incubated at 27° C or 37° C, and the results were recorded after 21 days of incubation.

Fig. 1. Aerial mycelium of strain No. TM-521 (on oat meal agar, $\times 180$).



Fig. 2. Electron micrograph of spores of strain No. TM-521 (on oat meal agar, \times 4,800).



Medium	Growth	Aerial mycelium	Soluble pigment	Note
Sucrose Сzарек's agar (27°С)	Moderate, white to white yellow, penetrating into medium	Scanty, white	None	
Glucose Czapek's agar (27°C)	Small colony of white yellow	None	None	
Glycerol CZAPEK's agar (27°C)	White yellow to brownish yellow, partly brownish gray	White to white yellow	None	5
Starch CZAPEK's agar (27°C)	White yellow to yellow	Powdery, white	Pale yellow	Starch hydrolysis
Glucose asparagine agar (27°C)	Good, gray white to white yellow, later becoming brownish yellow	White to gray white, partly gray or white	None	
Glycerol asparagine agar (27°C)	Good, gray white to white yellow	White	None	
Salt starch agar (27°C)	Good, white yellow to brownish yellow	Gray white to milk white, later white with pale brown	None	Starch hydrolysis
Nutrient agar (27°C)	Weak, white yellow	None	None	
Yeast extract - malt extract agar (27°C)	Good, white yellow to brownish yellow	White, later white with light brown	None	
Oat meal agar (27°C)	Good, gray white to milk white, later becoming gray yellow to brownish yellow	White to milk white, partly yellowish white with gray	None	
Tyrosine agar (27°C)	Good, white yellow to brownish yellow, later becoming brown	White to milk white, partly gray white	Weak, milk yellow	
Blood agar (27°C)	Weak, moist brownish gray	None	None	Haemolysis
Skimmed milk (37°C)	Weak, white to pale yellow ring around surface	None	None	Weak coagulation and no peptonization
Gelatin stab (27°C)	Weak, white yellow ring around surface	None	None	Weak liquefaction

Table 1. Morphological properties of Streptomyces sp. TM-521.

The physiological properties are summarized in Table 2.

Carbon source utilization was tested by the method of PRIDHAM *et al*⁴⁾. This strain, as shown in Table 3, fairly to fully utilizes D-glucose, D-xylose, L-arabinose, starch, lactose, D-fructose, D-mannitol, sucrose and raffinose, but does not utilize inositol, L-rhamnose, D-mannose, D-galactose or cellulose.

Comparison with *Streptomyces* Strains Similar to *Streptomyces* sp. TM-521 and the Identification of the Strain

These characteristics of the strain TM-521 are closely related with those of the "gray color" series of the ISP-classified *Streptomyces*⁵⁻⁸⁾. Among the known species of this series, *Streptomyces calvus* is most similar to the strain TM-521 as judged from various characteristics, especially those of the form

Tests	Results
Nitrate reduction	Negative
Production of H ₂ S	Negative
Tyrosinase reaction	Negative
Liquefaction of serum	Negative
Haemolysis	Positive
Liquefaction of gelatin	Weakly positive
Milk peptonization	Negative
Milk coagulation	Weakly positive
Hydrolysis of starch	Positive
Cellulose decomposition	Negative
Growth temperature	20~45°C
Optimal temperature	27~35°C

Table 2. Physiological properties of Streptomyces Table 3. Utilization of carbohydrates by Streptomyces sp. TM-521.

Arabinose	+	Galactose	-
Xylose	+	Sucrose	土
Rhamnose	-	Lactose	±
Inositol	-	Mannose	-
Mannitol	±	Raffinose	±
Fructose	土	Starch	+
Glucose	+	Cellulose	_

+: Positive utilization

 \pm : Doubtful utilization

-: Negative utilization

Table 4. A comparative studies of culture and physiological characteristics of Streptomyces sp. TM-521 and Streptomyces calvus.

	Streptomyces sp. TM-521	Streptomyces calvus
Sporophores	Primitive spirals	Short loose spirals of one to several turns
Spore surface at magnification	Hairy	Spiny to hairy
Color of colony	Belong to the "gray color" series	Belong to the "gray color" series
Color in medium	No pigment is found in medium in yeast - malt agar, oat meal agar, salt - starch agar or glycerol asparagine agar	No pigment is found in medium in yeast - malt agar, oat meal agar, salt - starch agar or glycerol asparagine agar
Gelatin liquefaction	Weak liquefaction	Partial liquefaction
Milk coagulation	Weak coagulation	Moderate coagulation
Milk peptonization	Negative	Positive, final pH 7.2
Cellulose decomposition	Negative	Negative
Starch hydrolysis	Hydrolysis	Slight hydrolysis

of spore bearing hyphae and surface of spore as shown in Table 4. This strain, however, differs from Streptomyces calvus in terms of the no production of peptone from skim milk, no utilization of inositol and L-rhamnose, and the strong hydrolysis of starch.

The strain, after all, may reasonably be concluded to belong to the species Streptomyces calvus, and was finally designated as Streptomyces calvus TM-521.

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