RELATIONSHIP BETWEEN CELL MORPHOLOGY AND THE TYPES OF DIAMINOPIMELIC ACID IN *KITASATOSPORIA SETALBA*

YŌKO TAKAHASHI, YUZURU IWAI AND SATOSHI ŌMURA

*The Kitasato Institute and Kitasato University,
5-9-1, Shirokane, Minato-ku, Tokyo 108, Japan*

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The relationship between cell morphology and the type of 2,6-diaminopimelic acid (A_2pm) in *Kitasatosporia setalba* was studied in a submerged culture. When a strain of *K. setalba* was cultivated in a liquid culture with shaking, it produced two kinds of cells, spore-like cells and filamentous mycelia, which are morphologically distinct from each other. The former contained LL- A_2pm , and the latter *meso*- A_2pm . A time course study in a submerged culture inoculated with the spore-like cells demonstrated that each type of A_2pm detected in *K. setalba* is clearly associated with one of the two forms of cell.

A soil isolate, strain KM-6054, produces the new antibiotic setamycin which is active against trichomonads and gram-positive bacteria (1). The morphological and cultural characteristics of the strain resemble those of strains of the genus *Streptomyces* when grown on agar media. However, strain KM-6054 could not be classified in any of the known genera of the order *Actinomycetales*, because the cell wall type (2), which is one of the most important criteria in the classification of genera of actinomycetes, was a new one containing both LL- and *meso*-2, 6-diaminopimelic acid (A_2pm) as well as glycine and galactose. The strain was fully characterized and determined to belong to a new genus, for which the name *Kitasatosporia* has been proposed and the type species of *Kitasatosporia* is *Kitasatosporia setalba* ŌMURA, TAKAHASHI, IWAI and TANAKA (3).

We reported that different isomers of A_2pm were detected separately in aerial and in vegetative mycelia of *K. setalba* grown on an agar medium: the former contains LL- A_2pm , while the latter contains *meso*- A_2pm as the main component (4). In a further study with a submerged culture of *K. setalba*, spore-like cells and filamentous mycelia, were observed, which are morphologically distinguishable from each other. It was of interest to determine whether the cell types contain different two kinds of A_2pm . In fact LL- A_2pm is contained in the spore-like cells, while mainly *meso*- A_2pm is contained in the filamentous mycelia.

MATERIALS AND METHODS

Actinomycete strains. *K. setalba* was isolated from a soil sample collected at Setagaya-ku, Tokyo. Strain KM-6054 (ATCC 33774, IFO 14216), the type strain of *K. setalba*, and two reference strains, *Streptomyces albus* KA-1023 and *Nocardia asteroides* KB-76 were used. The cultures were maintained on inorganic salt-starch agar.

Separation of spore-like cells and filamentous mycelia. A loopful of the aerial mycelia of strain KM-6054 was transferred into 100 ml of Y-D medium consisting of 1% yeast extract (Difco) and 1% glucose in a 500-ml Sakaguchi flask and incubated on a reciprocal shaker at 27° for 3 days. The culture was filtered through a Toyo Roshi No. 1 filter. The residue was washed with water to give filamentous mycelia. Spore-like cells were obtained by centrifugation ($2,940 \times g$, 20 min) of the filtrate. Purity of the cell preparations was confirmed by microscopic observation.

Whole-cell preparations. The whole mycelia were harvested by centrifugation ($2,940 \times g$, 20 min) of the submerged culture in Y-D medium, and thoroughly washed with water. The washed mycelia were used as a whole-cell preparation after treatment with ethanol and drying at room temperature.

Time-course study in a submerged culture. The spore-like cells, obtained by filtration under sterile conditions, were inoculated into 100 ml of Y-D medium in a 500-ml Sakaguchi flask and incubated at 27° on a shaker. Portions of the culture were taken at intervals, and the whole mycelia were analyzed for the contents of two kinds of A_2pm , and their growth and morphology were observed by photo- and electron-microscopy. Growth was expressed in terms of dry weight of cells prepared by drying the washed mycelia at 60°.

Analysis of A_2pm . Ten mg of dried cells was hydrolyzed in 6 N HCl at 100° for 18 hr, and then analyzed for A_2pm by thin-layer chromatography on cellulose (Avicel SF-2020, Funakoshi Yakuhin Co.), developed with methanol-pyridine-10 N HCl-H₂O (32: 4: 1: 7) and detected with ninhydrin. Quantitative analysis of A_2pm was carried out with a TLC scanner (Model CS-920, Shimadzu) at 410 nm.

Photo- and electron-microscopy. The morphology of the strain was observed with a light microscope (Vanox, Olympus) and a scanning electron microscope (Model S-430, Hitachi). The samples were prepared from the washed cells stained with Pfeiffer's solution for light microscopy and with osmium-vapor fixation for scanning electron microscopy.

RESULTS

Since it is noteworthy that *K. setalba* contains two kinds of A_2pm as constituents of the cell wall, we studied the relationship between morphology and the A_2pm type in the cells grown in liquid media. Figure 1 is a photomicrograph of

Fig. 1. Photomicrograph of *K. setalba* grown in a submerged culture in Y-D medium for 3 days.

The bar marker represents 10 μ m.

Fig. 2. Thin-layer chromatogram of A₂pm in whole cells (A), spore-like cells (B) and filamentous mycelia (C) of *K. setalba* grown in a submerged culture.

S. albus and *N. asteroides* were used as reference organisms. Thin-layer chromatography on cellulose was developed with methanol-pyridine-10 N HCl-H₂O (32:4:1:7) and detected with ninhydrin.

mycelia grown in a liquid culture with shaking at 27° for 3 days. *K. setalba* was found to develop filamentous mycelia and spore-like cells, which are morphologically distinguishable from each other and can be effectively separated by filtration with a Toyo Roshi No. 1 filter. The A₂pm analysis revealed that the spore-like cells contain only LL-A₂pm and the filamentous mycelia contain mostly *meso*-A₂pm (Fig. 2). *S. albus* and *N. asteroides*, the reference organisms, contained only LL-A₂pm and *meso*-A₂pm, respectively.

To confirm the above observations and to investigate morphological changes in *K. setalba* in submerged cultures, time course studies were carried out. As shown in Fig. 3, only LL-A₂pm was detected at 0 hr, because only spore-like cells containing LL-A₂pm were inoculated as seed culture. Mainly *meso*-A₂pm

Fig. 3. Time course of LL- and *meso*- A_{2pm} contents in the whole cells of *K. setalba* grown in a submerged culture.

Spore-like cells only were inoculated into 100 ml of Y-D medium in a 500-ml Sakaguchi flask and incubated at 27° with shaking.

Whole broth was withdrawn at intervals to determine the levels of LL- (●) and *meso*- A_{2pm} (○), and the dry weight of cells (■).

was detected during 5–11 hr of incubation. After 11 hr LL- A_{2pm} increased rapidly. Similar amounts of LL- and *meso*- A_{2pm} were detected after 50 hr of incubation.

Since the most significant changes in the levels of LL- and *meso*- A_{2pm} were observed during the first 24 hr (Fig. 3), the morphological changes were also observed during this period. Figures 4 and 5 show the photomicrographs and scanning electron micrographs of *K. setalba* grown in a liquid culture. The spore-like cells transferred as a seed culture began to elongate in one hr (Fig. 5-b). Then, the cells developed gradually to filamentous mycelia. During 5–11 hr, filamentous mycelia only and no spore-like cells were observed. Spore-like cells appeared again at 15 hr, and thereafter both the spore-like cells and filamentous mycelia were observed. Apparently the increase of *meso*- A_{2pm} content during 0–5, 19–24 and 41–43 hr is closely related to the synchronous elongation of the filamentous mycelia from the spore-like cells. From the information represented in Figs. 3–5, it is concluded that the A_{2pm} types are clearly related to the cell morphology.

DISCUSSION

In a submerged culture of *K. setalba*, both LL- and *meso*- A_{2pm} were detected in the whole mycelia, which included the two kinds of cells, spore-like cells and

Fig. 4. Photomicrograph of *K. setalba* grown in a submerged culture at 0 (a), 1 (b), 5 (c), 11 (d), 15 (e) and 24 hr (f).

The culture conditions are described in Fig. 3. The bar markers represent 10 μ m.

filamentous mycelia (Fig. 1). The former contained LL-A₂pm, while the latter contained *meso*-A₂pm (Fig. 2). The time course study indicates that the A₂pm type clearly corresponds to the cell morphology (Figs. 3–5): only LL-A₂pm was detected at 0 hr when spore-like cells were inoculated as a seed culture. When mainly *meso*-A₂pm was detected during 5–11 hr, only filamentous mycelia were observed. The LL-A₂pm content increased again after incubation for 11 hr, when spore-like cells were actively produced. It is of interest that the morphology in a submerged culture of *K. setalba* can be checked by analysis of the A₂pm type as shown in Fig. 3.

Comparing these results with the previous information that two kinds of A₂pm were detected separately in aerial and in vegetative mycelia (4), it was found that the cell morphology of *K. setalba* was apparently associated with the A₂pm type: the two kinds of short cells, aerial-mycelium spores and submerged spore-like cells, contained LL-A₂pm, whereas the two kinds of long cells, vegetative and

Fig. 5. Scanning electron photomicrograph of *K. setalba* grown in a submerged culture at 0 (a), 1 (b), 9 (c) and 15 hr (d).

The culture conditions are described in Fig. 3. The bar markers represent 1 μ m.

filamentous mycelia, contained *meso*-A₂pm.

Compared with the studies of spores grown on a solid culture, only a few descriptions of spores produced in a submerged culture of actinomycete strains have been reported (5). CARVAJAL (6), WILKIN and RHODES (7) and ZASLAVSKAYA and ZHUKOV (8) called these spores "submerged spores," "secondary mycelium spores" and "deep spores," respectively. Further it is known that some strains of the genus *Micromonospora* have different isomers of A₂pm as cell-wall components (9). However, the relationship between morphology and A₂pm type was not investigated until *K. setalba* was studied by the present authors. To our knowledge, our studies are the first investigations of the relationship in an actinomycete strain between the morphology and constituents of cells grown in submerged and on solid cultures.

It is not clear whether we can call the spore-like cell a true spore. But it appears to be so in the following regards. The spore-like cells resemble the aerial-mycelium spores in morphology, in the presence of LL-A₂pm and in resistance to

ultrasonication¹. Also, in the submerged cultures, the spore-like cells are more resistant to lysozyme¹ than the co-produced filamentous mycelia. To develop the studies further, we plan to investigate the constituent amino acids of the peptidoglycans in the four kinds of cells: the aerial and vegetative mycelia, the spore-like cells and the filamentous mycelia.

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¹ Data are not shown.