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Effect of Initial pH and L-Arginine on the Composition of Fatty Acids of *Streptomyces viridochromogenes*

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Mycelia of *Streptomyces viridochromogenes* grown under different pH were analysed for the fatty acid composition. The low relative proportion of 12-methyltetradecanoic acid and the high relative proportion of palmitic acid were characterisrtic for the young culture under slight acidic pH that caused delay of the aerial mycelium formation. The addition of L-arginine to the culture medium enabled an arginine auxotroph with bald phenotype to have the fatty acid composition similar to that of the wild type and to develop aerial mycelium. The ratio of 12-methyltetradecanoic acid to palmitic acid might be used as a parameter to explain the optimum growth in the respect of membrane fluidity.

Key words: *Streptomyces viridochromogenes*, fatty acids, pH, L-arginine

Fatty acid composition has been used as an important characteristic in the chemotaxonomy of streptomycetes. The major fatty acids in the genus Streptomyces were saturated iso and anteiso fatty acids(8,15), The patterns of fatty acid profiles were essentially same regardless of various species or the culture stages(15). However, the relative proportion of each fatty acid could be altered by growth temperature as were described in other bacteria (11,19) and yeasts(17).

In the conventional temperature range, from 35℃ to 26℃, the proportion of anteiso and unsaturated fatty acids increased while that of iso fatty acids decreased(18). Developmental mutants also showed altered content of anteiso fatty acids, especially 12-methyltetradecanoic acid(ai-15:0)(3,4,16).

In addition, the involvement of amino acids in culture broth has affected the fatty acid composition. Both L-threonine and L-isoleucine were thought to be degraded via 2-keto-3-methylvalerate and 2-methylbutyrate which had a function as a primer for the biosynthesis of anteiso fatty acids(20,21).

In bacteria the anteiso fatty acids have a similar function to that of unsaturated fatty acids, because anteiso fatty acids confer the lower phase transition temperature to the lipids(6). Then, it should be noted that the increasing relative proportion of of ai-15:0, for example, is related to the more fluid membrane at lower environmental temperature(6,14).

In this paper, we would like to analyse the change of fatty acid composition according to the initial pH of medium to show that a change in membrane fluidity would also occur to adapt to the changes in external pH.

Materials and Methods

Bacterial strains and growth conditions

S. viridochromogenes KCTC 9009 wild type and a mutant strain BR-2 with bald phenotype were maintained on the half-strength yeast extract malt extract medium (YEME) consisting of 0.2%(W/V) yeast extract, 0.5% malt extract and 0.2% glucose(16). Spores from agar surface were inoculated into 250 ml Erlenmeyer flasks containing 50 ml of YEME, supplemented with each amino acid as indicated in results, and incubated at 30℃ in a rotary shaker at 150 rpm. The initial pH was adjusted with 1 N NaOH or HCl before autoclaving.

The minimal medium(MM) was used to test amino acid requirement of the mutant strain(1). Each amino acid was added to the final concentration of 10 mM. Since the strain BR-2 was proven to be an arginine auxotroph, the spores of this strain were prepared from minimal medium containing L-arginine.

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Table 1. Fatty acid composition of the wild type(Wt) and mutant strain BR-2 of *Streptomyces viridochromogenes* grown for 7 days in the yeast extract malt extract medium supplemented with 10 mM amino acid

Fatty acid*(%)	LL-Ornithine		DL-Citrulline		L-Arginine	
	Wt	BR-2	Wt	BR-2	Wt	BR-2
i-14:0	2.9	2.8	2.5	3.0	2.8	4.0
14:0	2.2	0.6	2.2	0.6	1.6	1.7
i-15:0	10.5	14.1	10.5	10.7	14.5	6.1
ai-15:0	15.8	22.5	15.1	26.0	14.0	16.6
15:0	3.1	2.3	3.0	1.8	3.9	5.4
16:1	2.7	1.8	2.5	1.6	1.6	1.5
i-16:0	17.9	20.5	17.4	19.6	19.4	21.5
16:1	7.5	2.4	7.6	3.0	6.2	5.0
16:0	13.8	6.2	15.3	5.2	14.4	16.6
UI	3.2	3.2	3.0	3.0	2.8	2.1
UI	4.4	2.8	3.8	3.4	2.4	2.6
i-17:0	3.7	6.1	4.4	5.8	5.6	2.7
ai-17:0	8.7	12.6	8.9	16.3	8.9	10.6
UI	2.3	1.2	2.6	tr	0.8	2.0
17:0	1.2	0.7	1.2	0.7	1.2	2.9
ai-15:0/16:0	1.1	3.6	1.0	4.9	1.0	1.0

* i, iso acid; ai, anteiso acid; UI, uidentified acid; tr, trace.
The frist number denotes number of carbon atoms, the second number of double bonds.

Table 2. The effects of glycerol and mannitol on the growth of *Streptomyces viridochromogenes* wild type(Wt) and the mutant strain BR-2

Medium	Wt		BR-2	
	SM	AM	SM	AM
YEME	++	++	++	-
YEME+1% glycerol	++	++	++	-
YEME+1% mannitol	++	++	++	-
MM+0.1% arginine	+	+	+	+
MM+1% glycerol+0.1% asparagine	++	++	-	-
MM+1% glucose+0.1% arginine	++	++	++	-
MM+1% glycerol+0.1% arginine	++	++	++	++
MM+1% mannitol+0.1% arginine	++	++	++	++

Analysis

Lipids from wet mycelium were extracted by "Folch" extraction(2). Fatty acids in lipids were transesterified by 0.5 M sodium methoxide in anhydrous methanol(2). The gas chromatography of the methyl esters of fatty acids were carried out(16). The peaks were identified by mass spectrometry and comparing the retention time with the commercial standard mixture(Supelco).

D-Glucose in culture medium was determined using Sigma kit. Mycelial dry weight was measured, after washed mycelium were dried overnight at 105°C.

Results and Discussion

In our previous report we suggested that the fatty acid composition should affect the rise of aerial mycelium from substrate mycelium of *S. viridochromogenes* (16). To invest the possible stimulating factors for the aerial mycelium formation of the bald strain BR-2, we added each of 20 amino acids to YEME to the final concentration of 10 mM. Only L-arginine could restore the the ability to develop aerial mycelium of the mutant strain. Thus, we analyzed the fatty acid composition of the strain BR-2 grown with shaking in the presense of L-arginine. As shown in Table 1, the fatty acid composition of the mutant strain BR-2 was changed to that of wild type strain, more specifically, the increase of palmitic acid(16:0) content with the concomitant decrease of ai-15:0, resulting almost equal ratio of ai-15:0/16:0 to that observed in the wild type. Nonetheless, the precursors of L-arginine biosynthesis, LL-ornithine and DL-citrulline, had not affected the fatty acid composition of the mutant strain, whereas the fatty acid profiles of the wild type strain grown on media supplemented with 3 different amino acids were essentially same.

It has been known that arginine auxotrophic mutants of streptomycetes have lost their ability to develop aerial mycelium(9,13,22). Therefore, we examined the amino acid requirement of the strain BR-2 on the minimal medium and found that this strain also was an arginine auxotroph. LL-Ornithine, DL-citrulline, and arginosuccinate could not support the growth of the strain BR-2 on the minimal medium. Therefore, the arginine catabolism associated with the fatty acid biosynthesis would be related to increase of the content of 16:0 and decrease of ai-15:0(7).

Meanwhile, the ability to form aerial mycelium on the minimal medium was dependent on the carbon sources in the minimal medium. The strain BR-2 grown without aerial mycelium in glucose medium restored the ability of aerial mycelium formation in media replacing glucose by either glycerol or mannitol, as like the wild type (Table 2). The suppressive effect of glucose on aerial mycelium formation could be explained by the production of organic acids during the early growth from glucose, because 10 mM of citric acid added into YEME suppressed the aerial mycelium formation of wild type which displayed the bald colonies characteristic for the mutant strain BR-2. The production of acidic substances was indirectly confirmed during early-logarithmic growth phase of the wild type grown in YEME, a sharp drop of the initial pH 7.0 to about 5.4(Table 3). After the early acidic phase, the pH of the medium increased up to pH 8.3 and was maintained during prolonged cul-

Table 3. Growth characteristics in the yeast extract malt extract medium of the wild type and mutant strain BR-2 of *Streptomyces viridochromogenes*

	pH			Dry weight[a] (mg/ml)	Residual glucose[a] (mg/ml)
	Initial	1 day	7 day		
Wild type	7.2	5.4	8.3	1.5	0
BR-2	7.1	4.7	4.7	0.3	0.8

[a], measured at seventh day

Table 4. Fatty acid composition of the wild type grown in the different initial pH

Fatty acid(%)	pH6.5		pH7.5	
	1 day	9 day	1 day	9 day
i-14:0	2.2	2.8	2.5	3.4
14:0	2.7	2.1	2.5	2.2
i-15:0	6.9	10.6	13.0	8.8
ai-15:0	7.2	17.1	16.5	15.0
15:0	4.5	3.2	4.5	2.8
16:1	0.5	3.4	0.9	3.6
i-16:1	12.2	15.7	9.8	17.1
16:1	8.8	7.5	10.8	8.7
16:0	39.8	13.0	22.4	12.4
UI	1.7	3.9	2.6	4.7
UI	0.8	4.6	1.5	4.9
i-17:0	3.5	3.6	3.8	3.2
ai-17:0	5.4	8.1	6.8	8.1
UI	1.1	4.4	0.7	4.1
17:0	2.5	tr	1.7	1.1
ai-15:0/16:0	0.2	1.3	0.7	1.2

Abbreviation, see Table 1.

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But the culture broth of the mutant strain was maintaining acidic environment throughtout the culture period. And a poor growth occurred due to a reduction of glucose consumption, remaining 40% of initial amount of glucose added to the medium(Table 3). The marked difference in pH changes during the growth between the wild type and mutant strain led us to determine whether the fatty acid composition was changed by initial growth pH. We assayed the fatty acids at first day(early-logarithmic phase) and at seventh day(stationary phase) (Table 4).

The mycelium grown for one day at initial pH 6.5 exhibited a lower content of isopentadecanoic acid(i-15:0) and ai-15:0 and the higher content of 16:0 comparing to those of old mycelium. Among fatty acids, ai-15:0 from pH 6.5 showed the most noticeable changes according to the culture time. It increased almost twice at nineth day. At higher pH of the medium, there was only minor changes in the composition of ai-15:0. The highest level of 16:0 of the lower pH medium at first day decreased to one third at nineth day, but this change was not characteristic of the lower pH medium. A less severe but similar decrease in the level of 16:0 was shown in the higher pH medium, too.

From this fatty acid analysis, 16:0 appeared to be a characteristic fatty acid for young culture regardless of initial pH and the increase of the level of ai-15:0 during growth was typical for the growth at lower initial pH. When growing on agar surface at pH 6.5, the wild type showed a delayed aerial mycelium formation for two days, after which the normal growth with white powdery surface could be seen.

Since cell membrane should be a first structure to cope with adverse environments, it is very important to cell to maintain proper membrane fluidity by varing the fatty acid composition in the membrane(5). It has been generally known that the changes in the content of unsaturation and fatty acid type were accompanied by changing growth temperature or salt concentration(6,11, 18,19).

Our present results, a lower proportion of ai-15:0 and a higher proportion of 16:0, suggested that the cell membrane of *S. viridochromogenes* in early growth phase at pH 6.5 had a lower fluidity compared with that of cells grown at pH 7.5, but more evidence for the phase transition of membrane would be obtained with direct measurement. A decreased level of ai-15:0 was reproted in *S. griseus* at higher growth temperature, 35 oC(18). Thus, streptomycetes seemed to show a common adaptation both to high temperature and acidic pH, to the latter, however, the adaptation occured temporarily in young mycelium.

The changed fatty acid composition affected the neomycin production(12), or potassium ion permeability(14). Therefore, proper level of the membrane fluidity, expressed in this paper as the ratio of ai-15:0/16:0, would be one of the determining factor for the optimum growth. The changes in this ratio would be caused by adding amino acids and controlling initial pH, which had affected the secondary growth of streptomycetes. How the fatty acid composition was actually related to the fluidity, however, was not studied in this paper. To verify this, the thermal behaviour of the intact membrane or lipid should be studied as was carried out for *S. hydrogenans* (14) or *Bacillus stearothermophilus*(10).

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