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L-Cysteine Metabolism and the Effects on Mycelium growth of *Streptomyces albidoflavus* SMF301 in Submerged Culture

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Myceliuml growth and spore formation of *Streptomyces albidoflavus* SMF301 in submerged culture were compared with the metabolism of cysteine. Cysteine added to the culture was metabolized by cysteine desulfhydrase (EC 4.4.1.1.) to produce ammonium ions, hydrogen sulfide, and pyruvate. The redox potential of the culture broth was lowered immediately as the result of the metabolism of cysteine, which caused a lag period of mycelium growth. However enhanced activities of pyruvate dehydrogenase and α-ketoglutarate dehydrogenase were confirmed in the culture containing cysteine, indicating that pyruvate was utilized to support further mycelium growth.

The onset of antibiotic synthesis (physiological differentiation) in various actinomycetes always accompanies with spore formation (morphological differentiation), both are induced by the shift-down of some components in culture medium (1, 4-6). Although high sporulation is detrimental for the over production of antibiotic in some case, the relationships between the antibiotic synthesis and sporulation in actinomycetes have been interested (12, 17). The mechanisms involved in the differentiation processes have been studied by using solid cultures (7-10, 42). However, some species of *Streptomyces* can sporulate in submerged culture (1, 5, 11, 14, 18, 21, 22, 30, 31), which would provide some advantages over solid culture in the elucidation of the relationships between environmental changes and spore formation, and in quantitative analyses of the processes.

We isolated a strain (SMF301) of *Streptomyces albidoflavus* producing abundant spores both in submerged culture (submerged spore) and on solid culture (aerial spore) (20, 35, 36, 40). The chracteristics of the both forms of spores were compared to each other in the cellular contents of C, H, N, P, and metal ions (K, Na, Ca, and Mg). L-Glutamic acid, D-alanine, and D-glycine, all known to be cell wall components, were the major amino acids in both types of spores. However the cysteine content in submerged spores was higher than that in aerial spores. The major fatty acid in aerial spores was n-C_{18} (61.74%), whereas in submerged spores it was n-C_{16} (33.68%). The resistance of aerial spores to lysozyme digestion, mild acid treatment, heating and desiccation was higher than that of submerged spores, but the submerged spores were more resistant to sonication (26).

Kinetic parameters calculated from batch and chemostat cultures showed that the optimum dilution rate for mycelium growth was 0.1 h^{-1} and that for submerged spore formation was 0.05 h^{-}. The spore formation concurred with the endogenous consumption of mycelium. About 1.0×10^{7} spores were formed from 1 g of mycelium, and the turnover rate of biomass was calculated to be 0.029 h^{-1} (37). We have evaluated the optimum culture condition for the submerged spore formation, that was significantly stimulated by the limitation of cysteine but repressed by the addition of cysteine (37). In this work, the effects of cysteine on mycelium growth and submerged spore formation are discussed in relation to the cysteine metabolism.

MATERIALS AND METHODS

Microorganism and Media

The microorganism used in this study was *Streptomyces albidoflavus* SMF301. Rich medium consisted of 1% glucose, 0.2% peptone, 0.1% yeast extract, 0.1% beef extract, and 1.8% agar for solid culture. Chemically defi-

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ned medium was formulated with 2% glucose, 0.1% NH_4Cl, 0.13% KH_2PO_4, 0.09% Na_2HPO_4, 0.06% $MgSO_4 \cdot 7H_2O$, 0.0001% $FeSO_4 \cdot 7H_2O$, 0.0001% $MnCl_2 \cdot 4H_2O$, 0.0001% $CaCl_2 \cdot 2H_2O$, and 0.0001% $ZnSO_4 \cdot 7H_2O$. The initial pH of the medium was adjusted to 7.2 before steam sterilization, where phosphate and magnesium salts were sterilized separately and added aseptically.

Strain Maintenance and Culture Conditions

The strain was maintained by transfer to slopes of rich medium each month and storage at 4°C. The spores developed on rich medium were suspended in glycerol-rich medium and kept in a deep-freezer at −70°C (44). The frozen spores suspension (about 10^9 spores, ml^{-1}) was thawed at ambient temperature. For seed culture, 1 m*l* of the spore suspension was inoculated into 50 m*l* of the chemically defined medium in 100 m*l* baffled flasks and incubated for 5 days at 28°C on a rotary shaking incubator (150 rpm). The seed culture was inoculated into 2 *l* of the chemically defined medium in a jar fermentor (Model KCF-5, Korea Fermentor Co). The culture temperature was controlled at 28°C, and pH was maintained at 7.0 by automatic addition of 1 N HCl or 1 N NaOH. Aeration (1.0 vvm) and agitation (300 rpm) were provided throughout the culture.

Analytical Methods

Mycelial growth was measured by determination of dry mycelia weight (DMW). Mycelium was harvested by centrifugation at 10,000×g for 10 min and then washed twice with physiological saline solution and once with distilled water. The washed mycelium was dried at 100°C for 12 h. The number of spores formed in submerged cultures was counted as reported previously (26).

The concentration of reducing sugar in the culture broth was determined by the dinitrosalicylic acid reagent (27). The concentration of ammonium ions was analyzed by a specific ion analyzer (Model EA940; Orion Research). The concentration of sulfide in the culture broth was measured by using the methylene blue method (39). Redox potential of the culture broth was determined with a redox potential meter and a platinum redox electrode (EA940 Ion Analyzer; Orion Research, USA).

For the determination of intracellular compounds and enzymes, mycelia were harvested by centrifugation (10,000×g for 10 min) and washed twice with Tris-HCl buffer (0.1 M, pH 7.6) and resuspended in the same buffer. The washed mycelia were disrupted by using a sonicator (100 W, 5 min; Lab-Line Ultratip Sabsonic System) in an ice bath. Mycelium free supernatant (mycelium extracts) after centrifugation (15,000×g for 15 min) was used for the determination of the intracellular concentrations of cysteine and pyruvate, and activities of intracellular enzymes. The concentration of pyruvate was analyzed by lactate dehydrogenase (43) and cysteine was estimated by the method of Gaitonde (13). Cysteine desulfhydrase (CDSH) was assayed by the method reported by Kredich *et al.* (25). One unit of CDSH was defined as the amount to produce 1 μmole of sulfide from cysteine per min. Pyruvate dehydrogenase complex (PDH) and α-ketoglutarate dehydrogenase complex (KGDH) were analyzed by the ferricyanide reduction method (34). One unit of the PDH and KGDH was defined as the amount which produced 2 μmole of ferrocyanide from ferricyanide per hour. The concentration of protein was determined by the method of Bradford (3).

Chemicals, Reagents and Reproducibility

Amino acids and lactate dehydrogenase were purchased from the Sigma Chemical Co.. All other chemicals were of reagent grade. Each experiment was repeated three times, and their mean values are given.

RESULTS AND DISCUSSION

Effect of Cysteine on Mycelium Growth and Spore Formation in Submerged Culture

The effects of individual amino acid as a sole nitrogen source on mycelium growth and submerged spore formation in a batch culture of *S. albidoflavus* SMF301 were evaluated. Consequently, it was found that mycelium growth was enhanced, but spore formation was significantly repressed in the culture containing cysteine as a sole nitrogen source (data are not shown).

10 mM of cysteine was added to the culture broth at different growth phases, and the changes in submerged spore formation were evaluated (Fig. 1). It was evident that submerged spore formation was more significantly repressed by the addition of cysteine at the earlier stage (0 to 24 h), but not by the later addition (48 to 72 h). The effects of concentrations of cysteine added to the culture broth at 24 h on the mycelium growth and spore formation were evaluated (Fig. 2A and 2B). It was clear that mycelium growth was enhanced by the higher concentration of cysteine added, but on the other hand, submerged spore formation was apparently repressed.

Changes in redox potential after the addition of cysteine were measured (Fig. 3). It was clear that the redox potential in the culture broth was more profoundly lowered by higher concentration of cysteine added. But the lowered redox potential was returned to the original state as culture time elapsed. The results suggested that cysteine caused a lag period for the mycelium growth, the reduction of the redox potential of the culture might account for the lag. Moreover cysteine or its metabolite

played an important role on spore formation.

The effects of various forms of inorganic sulphur compounds on mycelium growth and submerged spore formation were evaluated with the changes in redox potential of the culture (Table 1). Submerged spore formation was inhibited clearly by the addition of sodium sulfide, this effect was similar to those caused by the addition of cysteine. Moreover it was evident that the redox potential of the culture broth was also lowered by the addition of sodium sulfide.

Cysteine is an important amino acid providing sulfur to microbial biomass (41, 24), but the metabolic pathway of cysteine in *Streptomyces* spp. has been scarcely reported. Cystathione γ-lyase are the most responsible enzymes involved in the metabolism of cysteine, But cysteine desulfhydrase producing H_2S, NH_3, and pyruvate was a prevalent enzyme in microorganisms isolated

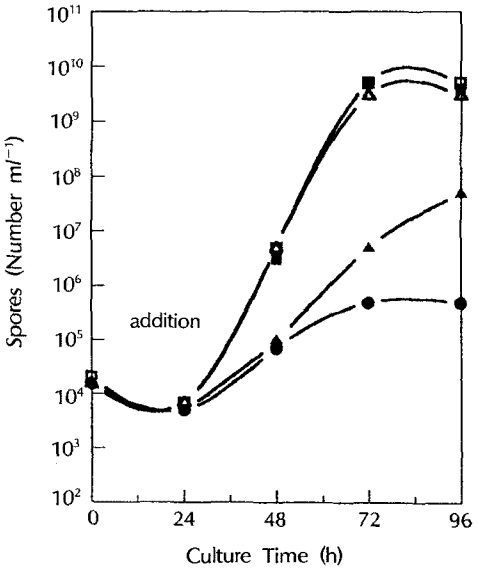

Fig 1. Effects of cysteine addition on spore formation in submerged culture of *Streptomyces albidoflavus* SMF301.
Cysteine was added to the culture at 0 (▲), 24 (●), 48 (△), 72 h (■) to give final concentration 10 mM. A culture without addition of cysteine (□) was done as a control.

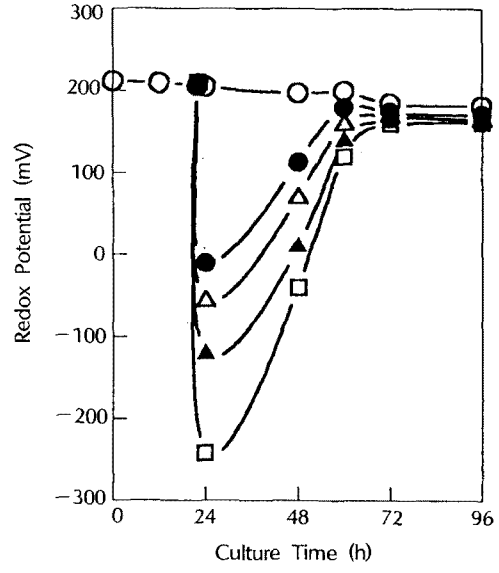

Fig 3. Effects of cysteine concentration on the changes of redox potential in submerged batch cultures *Streptomyces albidoflavus* SMF301.
Cysteine was added to the culture at 24 h to give final concentration of of 0 mM (○), 0.1 mM (●), 1 mM cysteine (△), 5 mM cysteine (▲), and 10 mM cysteine (□).

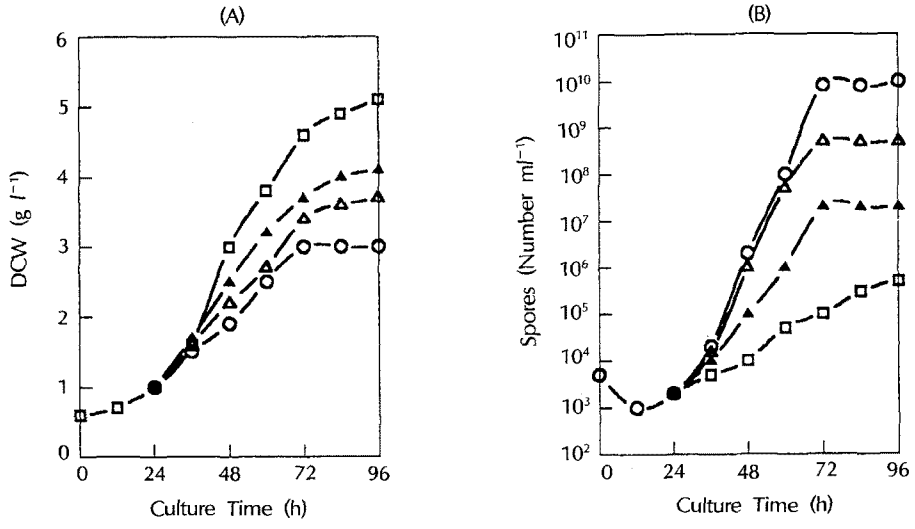

Fig 2. Effects of cysteine concentration on (A) mycelium growth and (B) spore formation in submerged culture of *Streptomyces albidoflavus* SMF301.
Cysteine was added to the culture at 24 h to give final concentration of 0 mM (○), 1 mM (△), 5 mM (▲), and 10 mM (□).

from soil, (28). Cysteine or its metabolites, especially sulfide, were thought as important regulatory compounds in both the bacterial and fungal systems (2, 23, 32, 33). Other free radical metabolites of cysteine were considered to interfere with the biosynthesis of leucine, threonine and valine, and also to inhibit respiratory chain activity (15, 16, 19).

Metabolism of Cysteine and Utilization of the Metabolites of Cysteine

In order to elucidate the metabolism of cysteine added to culture, the changes in the concentrations of cysteine and pyruvate were determined (Fig. 4A). The intracellular activity of cysteine desulfhydrase was analyzed also in relation to the rate of hydrogen sulfide production (Fig. 4B). The intracellular concentration of cysteine increased as the extracellular concentration of cysteine decreased. The intracellular concentration of pyruvate increased steadily with the decrease of the concentration of intracellular cysteine. Moreover, the activity of cysteine desulfhydrase increased immediately after the addition of cysteine and gave maximum values at 48 h, where the hydrogen sulfide production rate was simultaneously increased with the enzyme activity. Hydrogen sulfide production rate was retarded with the decreasing activity of cysteine desulfhydrase. The results indicated that cysteine was metabolized intracellularly by cysteine desulfhydrase to yield pyruvate, ammonium ions, and hydrogen sulfide.

Activities of pyruvate dehydrogenase (PDH) and α-ketoglutarate dehydrogenase (KGDH) were determined with mycelia grown with different concentrations of cysteine added at 24 h (Table 2). The activities of the pyruvate dehydrogenase (PDH) and α-ketoglutarate dehydrogenase (KGDH) were greatly activated when 10 mM of cysteine was added. The activity of PDH and

Table 1. Effect of addition of various sulfur compounds on redox potential, pH, the mycelium growth and submerged spores formation in submerged culture of *Streptomyces albidoflavus* SMF301.

Compounds[a] 10 mM	Redox potential[b] (mV)	pH[c]	DCW[c] (g l^{-1})	Spore[c] (Number ml^{-1})
Na_2SO_4	+122.7	7.33	4.49	8.1×10^8
Na_2SO_3	+ 33.9	7.26	4.65	9.0×10^8
$Na_2S_2O_3$	+ 70.2	7.25	4.06	8.8×10^8
Na_2S	−298.5	7.21	4.37	3.0×10^3
Cysteine	−210.0	7.11	5.11	8.5×10^4
No addition[d]	+183.5	7.28	4.60	8.2×10^8

[a] sulfur compounds were added at 24 h of the cultures [b] determined at 36 h of the cultures [c] determined after 5 d of the cultures [d] Control medium was formulated with 2% glucose, 0.1% NH_4Cl, 0.13% KH_2PO_4, 0.09% Na_2HPO_4, 0.06% $MgSO_4$ $7H_2O$, 0.0001% FeSO4 7H2O, 0.0001% $MnCl_2$ $4H_2O$, 0.0001% $CaCl_2$ $2H_2O$, and 0.0001% $ZnSO_4$ $7H_2O$.

Table 2. Effects of cysteine addition on mycelium growth, the spore formation, the biosynthesis of pyruvate dehydrogenase complex (PDH) and α-ketoglutarate dehydrogenase complex (KGDH) in the submerged culture of *Streptomyces albidoflavus* SMF301.

Cysteine conc. mM	DCW (g l^{-1})	Spore (Number ml^{-1})	PDH activity (units ml^{-1})	KGDH activity (units ml^{-1})
0.	3.62	1.5×10^8	0.44	0.20
0.1	3.76	1.3×10^8	0.48	0.42
1.	4.56	1.6×10^6	0.53	0.36
10	5.11	1.75×10^4	1.76	1.75

The strain was cultured in chemically defined medium containing nitrogen source for 5 d at 28°C.

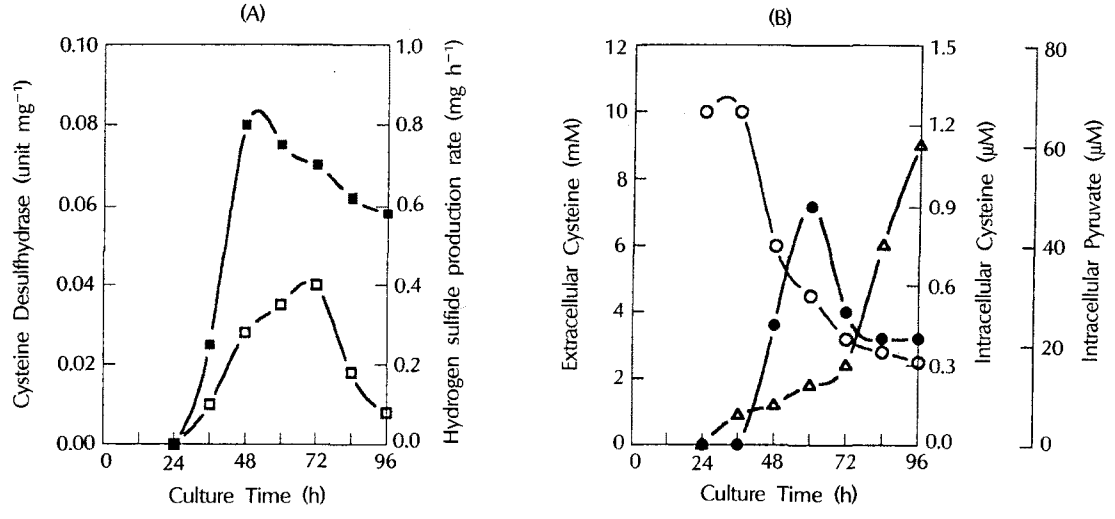

Fig 4. Changes (A), in the activity of cysteine desulfhydrase (▲) and hydrogen sulfide production rate (□); (B), in the concentration of extracellular cysteine (●), intracellular cysteine (●), and intracellular pyruvate (△) in the submerged culture of *Streptomyces albidoflavus* SMF301.

Cysteine solution was added to the culture at 24 h to give final concentration 10 mM.

KGDH in the culture without the cysteine addition was relatively low compared to those in the culture with cysteine. The higher activities of PDH and KGDH in the culture containing cysteine indicated that pyruvate produced from cysteine was metabolized via the TCA cycle with activated rates. Hence mycelium growth was enhanced and spore formation was repressed, although a lag period was necessary to recover the lowered redox potential. However, the TCA cycle operated at a suboptimal level due to the lower activities of PDH and KGDH in the culture of cysteine limitation. Consequently spore formation might be triggered by the limitation of building blocks for the mycelium growth.

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