Kinetic characterization of sporulation in *Streptomyces albido flavus* SMF301 during submerged culture

Yong Taik Rho and Kye Joon Lee

Author for correspondence: Kye Joon Lee. Tel: +82 2 880 6705. Fax: +82 2 882 9285.

We report the first quantitative analysis of the relationship between environmental changes and sporulation of a streptomycete, *Streptomyces albido flavus* SMF301, in submerged culture. A chemically defined medium was constructed for sporulation, over 10^6 spores ml^-1 being formed in the submerged batch culture. Kinetic parameters calculated from batch and chemostat cultures showed that specific submerged spore formation rate (q_sp) was inversely related to the specific mycelial growth rate (μ). The optimum growth rate for submerged spore formation was 0.05 h^-1, when the maximum value of q_sp was 1.0 x 10^4 spores g^-1 h^-1. The turnover rate of biomass at maximum growth yield was 0.029 h^-1, when 5 x 10^4 spores were formed from 1 g mycelium. The present quantitative analysis of submerged spore formation using a controlled system opens the way for biochemical and molecular biological studies related to the morphological differentiation of *Streptomyces* spp.

Keywords: *Streptomyces albido flavus*, submerged culture, kinetics of sporulation, differentiation

INTRODUCTION

Secondary metabolite production (physiological differentiation) in *Streptomyces* spp. accompanies the formation of spores (morphological differentiation), with both being induced by shift-down of some essential nutrients (Champness, 1988; Champness et al., 1989; Chater, 1984; Demain, 1982). Although most streptomycete-derived antibiotics are produced in submerged liquid cultures, studies on the mechanisms involved in the morphological differentiation of *Streptomyces* spp. have mostly been carried out on solid medium (Chater, 1989, 1991; Chater et al., 1988, 1989; Tan & Chater, 1993).

Some species of *Streptomyces* can sporulate in submerged culture, and this has been physiologically analysed in certain cases (Babcock & Kendrick, 1988; Kendrick & Ensign, 1983; Koepsel & Ensign, 1984; Huber et al., 1987; Ochi, 1987; Daza et al., 1989; Glazebrook et al., 1990). However, the kinetics of sporulation of *Streptomyces* spp. in response to changes in culture conditions have not yet been thoroughly studied. Hence, adequate conclusions have not been made regarding the quantitative relationship between environmental changes and sporulation in these micro-organisms. In this context, we selected a strain of *Streptomyces* sp. which produced abundant spores both in submerged culture and on solid media (Shin & Lee, 1986; Jeong et al., 1988; Rho et al., 1988, 1989). This strain was classified as *Streptomyces albido flavus* by numerical analysis of the operating taxonomy unit characters (Williams et al., 1983a, b) using *TAXON*, an identification probability matrix (Rho et al., 1992). The aerial spores formed on solid medium and submerged spores formed in liquid culture were characterized in terms of their cellular composition and resistance to extreme conditions (Lee & Rho, 1993). In the work reported here, we evaluated the optimum culture conditions for submerged spore formation and analysed kinetic parameters which help elucidate the quantitative relationship between environmental changes and spore formation.

METHODS

Micro-organisms and media. The micro-organism used was *Streptomyces albido flavus* SMF301 (Rho et al., 1992; Lee & Rho, 1993). Rich medium consisted of (w/v): 1% glucose, 0.2% peptone, 0.1% yeast extract and 0.1% beef extract, with 1.8% agar for solid cultures. Chemically defined medium contained (w/v): 1–2% glucose, 0.15% NH_4 Cl, 0.013% KH_2 PO_4, 0.009% Na_2 HPO_4, 0.06% MgSO_4.7H_2 O and 0.0001% trace elements (FeSO_4.7H_2 O, MnCl_2.4H_2 O, CaCl_2.2H_2 O and ZnSO_4.7H_2 O). The initial pH of media was adjusted to 7.0
and amino acids were purchased from Sigma. All other chemicals were of reagent grade. Each experiment was repeated three times and the mean values are given.

RESULTS

Optimum culture conditions for mycelial growth and submerged spore formation

*S. albidoflavus* SMF301 grew well in the chemically defined medium containing glucose, fructose, maltose, starch or mannitol, but not in that containing sucrose, lactose or myo-inositol (Rho et al., 1992). Since glucose was the best energy and carbon source, the effects of glucose concentration on mycelial growth and spore formation in submerged batch culture were evaluated. Mycelial growth and sporulation in submerged culture was not repressed by glucose at concentrations of up to 40 g l⁻¹ when the culture pH was not controlled (Fig. 1a). However, repression was observed with higher concentrations of glucose (Fig. 1a). Repression at high concentrations of glucose was not evident when the culture pH was...

*Fig. 1. Effects of glucose concentration on the formation of biomass (▪) and spores (□) in batch cultures of* *S. albidoflavus* SMF301. (a) Initial culture pH was adjusted to 7.0 at the beginning of incubation, but was not controlled thereafter; (b) culture pH was maintained at 7.0 throughout the incubation period. The results shown were from 5 d cultures.

*Fig. 2. Effects of culture pH on the formation of biomass (▪) and spores (□) in batch cultures of* *S. albidoflavus* SMF301. The results shown were from 5 d cultures.
Submerged spore formation by *S. albidojavlus*

1. **Fig. 3.** Effects of the concentration of (a) ammonium ions and (b) inorganic phosphate on the formation of biomass (■) and spores (□) in batch cultures of *S. albidojavlus* SMF301. The results shown were from 5 d cultures.

2. **Fig. 4.** Changes in the concentration of ammonium ions (○), glucose (●), biomass (■) and spores (□) in batch cultures of *S. albidojavlus* SMF301.

3. **Fig. 5.** Steady-state values of ammonium ions (○), glucose (●), biomass (■) and submerged spores (□) in glucose- and NH₄Cl-limited chemostats of *S. albidojavlus* SMF301.

4. **Fig. 6.** Effect of specific growth rate on *qₐₐₘₙ* (○), *qₐₕₕ* (●), *Yₐₕ* (■) and *Yₐₕₕ* (□) in glucose- and NH₄Cl-limited chemostats of *S. albidojavlus* SMF301.

5. **Kinetic parameters for mycelial growth and submerged spore formation**

   The changes in concentration of glucose, ammonium ions, biomass and submerged spores in a batch culture using the chemically defined medium are shown in Fig. 4.
The number of submerged spores decreased with the initiation of mycelial growth, indicating germination of spores. Mycelial growth revealed a typical batch culture pattern, entering stationary phase when ammonium ions were completely utilized. However, the formation of spores was evident when the residual concentration of glucose was still relatively high.

Since the formation of submerged spores appeared to be closely related to the concentration of ammonium ions, experiments with ammonium-ion-limited chemostats were carried out in order to evaluate more precisely the relationship between the kinetic parameters. The steady-state values of glucose, ammonium ions, biomass and submerged spores are shown in Fig. 5. The steady-state concentrations of biomass decreased at lower dilution rates (below 0.1 h\(^{-1}\)), but submerged spore formation was inversely related to dilution rate (specific growth rate). The optimum dilution rates were 0.1 h\(^{-1}\) for mycelial growth and 0.05 h\(^{-1}\) for submerged spore formation.

Kinetic parameters calculated from the steady-state data of the chemostat culture are shown in Fig. 6. Specific ammonium ion uptake rate \(q_{\text{amn}}\) and specific glucose uptake rate \(q_{\text{glu}}\) increased as the dilution rate increased. The maximum value of \(q_{\text{glu}}\) was 0.2 g g\(^{-1}\) h\(^{-1}\) at 0.1 h\(^{-1}\), whereas the maximum value of \(q_{\text{amn}}\) was 0.018 g g\(^{-1}\) h\(^{-1}\) at 0.15 h\(^{-1}\). The maintenance energy coefficient \((\mu_m)\) was calculated to be 0.046 g g\(^{-1}\) h\(^{-1}\) by extrapolating the values of \(q_{\text{glu}}\) to zero growth rate. Maximum growth yield \((Y_{\text{glu}})\) was calculated to be 0.036 g g\(^{-1}\), and the rate of biomass turnover \((a)\) was estimated to be 0.029 h\(^{-1}\). By plotting \(q_{\text{spo}}\) against \(\mu\) (Fig. 7a), \(q_{\text{glu}}\) (Fig. 7b) and \(q_{\text{amn}}\) (Fig. 7c), it was clear that \(q_{\text{spo}}\) was inversely related to all these parameters. The maximum value of \(q_{\text{spo}}\) (1.0 x 10\(^6\) g l\(^{-1}\) h\(^{-1}\)) was obtained at 0.05 h\(^{-1}\), when 5 x 10\(^6\) spores were formed from 1 g of mycelium.

**DISCUSSION**

Differing results have been obtained for the effect of glucose concentration on the formation of spores in submerged culture of a range of *Streptomyces* spp. Daza *et al.* (1989) found that sporulation of several species of *Streptomyces* in submerged culture was not inhibited by high concentrations of glucose. In contrast, Glazebrook *et al.* (1990) found that sporulation of *Streptomyces venezuelae* in submerged culture was repressed by high concentrations of glucose, and that this repression was not merely a secondary result of acid accumulation. The same result was also obtained with *Streptomyces griseus* (Babcock & Kendrick, 1988). In addition, cellular differentiation of *Streptomyces coelicolor* A3(2) in surface culture was inhibited by high concentrations of glucose (Chater *et al.*, 1988).

In the present experiments, we found that sporulation of *S. albidoflavus* SMF301 in submerged culture was pH-dependent, with an optimum at 7.0, and that sporulation was repressed by glucose up to 80 g l\(^{-1}\), when the culture pH was maintained at 7.0. Therefore, it was concluded that sporulation of *S. albidoflavus* SMF301 in submerged culture is more affected by culture pH than by high concentrations of glucose; however, the molecular mechanism remains to be elucidated.

It has been reported that sporulation of *S. griseus* in submerged culture is initiated by ammonium ion starvation (Kendrick & Ensign, 1983), and that an excess of nitrogen is inhibitory to submerged spore formation in *S. venezuelae* (Glazebrook *et al.*, 1990). We found that mycelial growth in submerged culture of *S. albidoflavus* SMF301 was enhanced by higher concentrations of ammonium ions or inorganic phosphate, whereas sporulation was repressed significantly by these conditions. Our data indicated that nitrogen or phosphate starvation influenced the shift from mycelial growth to submerged spore formation.

Prior to the present investigation, kinetic studies on the sporulation of *Streptomyces* spp. in submerged culture had not been reported, although the physiological analysis of sporulation of several species of *Streptomyces* in submerged culture has been carried out (Babcock & Kendrick, 1988; Kendrick & Ensign, 1983; Koepsel & Ensign, 1984; Huber *et al.*, 1987; Ochi, 1987; Daza *et al.*, 1989; Glazebrook *et al.*, 1990). From our batch and continuous culture kinetic analysis, it was very clear that the specific rate of formation of spores was inversely related to the specific growth rate (\(\mu\)), and that \(q_{\text{spo}}\) was optimum (1.0 x 10\(^6\) spores g\(^{-1}\) h\(^{-1}\)) when \(\mu\) was 0.05 h\(^{-1}\). The rate of biomass turnover was 0.029 h\(^{-1}\), when 5 x 10\(^6\) spores was formed from 1 g of mycelium.

This study is the first attempt to analyse quantitatively the relationship between mycelial growth and spore formation of *Streptomyces* spp. in submerged culture. The strain used produced abundant spores in submerged culture compared to other species of *Streptomyces*. Such quantitative analysis in controlled, submerged liquid
culture using a simple, chemically defined medium offers a system in which to elucidate the biochemical and molecular basis of morphological differentiation in \textit{Streptomyces} spp.

**ACKNOWLEDGEMENTS**

We should like to thank Professor K. F. Chater, Professor M. Goodfellow and Dr M. E. Bushell for discussions throughout the study; Mr J. A. Foley for proofreading the manuscript, and Mr S. G. Kang for computer analysis. This work was supported by a grant from the SRC (Research Centre for Molecular Microbiology) supported by the Korea Science and Engineering Foundation (KOSEF).

**REFERENCES**


Received 30 July 1993; revised 23 March 1994; accepted 25 March 1994.