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EXPERIMENTAL ARTICLES

Endospore Formation by *Streptomyces avermitilis* in Submerged Culture

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Abstract—The ability of streptomycetes to form endospores during their life cycle was studied in submerged cultures of *Streptomyces avermitilis*. Submerged *S. avermitilis* spores were most intensely formed (1) during the culture development cycle on synthetic medium CP1 with glucose under phosphate limitation and (2) in autolysing cell suspensions of high density obtained by tenfold concentration in phosphate buffer (pH 7.2) with 0.2% $CaCl_2$ of stationary-phase cells grown in synthetic medium. Endospores of *S. avermitilis* formed in submerged cultures shared the major characteristics of specialized microbial resting forms: heat resistance, resistance to lysozyme, ability to retain the main species-specific features, and ultrastructural organization characteristic of endospores. They can be considered a resting form of streptomycetes alternative to the spores formed exogenously on aerial mycelium in surface cultures.

Key words: streptomycetes, *Streptomyces avermitilis*, submerged endospores, spore ultrastructure, autolysis, nutrient limitation.

Investigation of cell differentiation in actinomycetes grown in submerged culture is important for understanding the ontogeny, ecology, and evolution of these prokaryotes, as well as for solving practical tasks related to their use as producers of biologically important metabolites.

Formation of spores—specialized cells used for reproduction and survival of unfavorable ambient conditions—is an expression of the ability of actinomycetes to undergo cell differentiation [1]. Spores can be formed either exogenously or endogenously. Spore formation by actinomycetes grown on solid agar media has been studied best of all. Unlike bacterial endospores [1, 2], these spores are classified with exospores [2]. In mycelial prokaryotes, endogenous spore formation as a specialized multistage process of cell differentiation that follows the pattern characteristic of the sporogenesis in bacilli and consists in absorption of a forespore by the maternal cell has been first demonstrated for thermoactinomycetes [3]. For the genus *Streptomyces*, the single instance of endospore formation in a submerged culture of the *S. globisporus* strain 0234 and one of its natural variants, *S. globisporus* 0234A, has been described [4]. The submerged endospores of these streptomycetes had two spore membranes separated by the cortex—typical structural components of bacterial and thermoactinomycete spores. They also contained dipicolinic acid, which is believed to be responsible for the high heat resistance of endospores in bacilli [5]. However, submerged spores found in *S. globisporus* were lysed by lysozyme and were sensitive to heating, which was accounted for by their low calcium content [4]. Although *Streptomyces* is the most voluminous and best studied actinomycete genera, other publications referring to endogenous spore formation by streptomycetes are absent. It should be noted that spore formation by actinomycetes was studied predominantly under the conditions of surface growth on agar media. The ability of these organisms to produce reproductive resting forms in a submerged culture has raised doubt for a long time; when observed, the structures with the characteristics of resting cells were regarded as spore-like mycelial fragments or the result of mycelial degeneration [2]. The lack of information on streptomycete submerged spores is partly due to the fact that studies of submerged cultures were usually performed for biotechnological purposes and did not deal with the developmental biology of these organisms. Only several cases of production of submerged resting forms by *Streptomyces* are presently known [4, 6–10]. However, since streptomycetes are widespread in nature, including ecological niches where submerged growth occurs [11], this survival strategy is possibly characteristic for these organisms.

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The goal of this study was to investigate the ability of *S. avermitilis* to form spores when grown in liquid media and to study the sporogenesis type and characterize the main features of spores and the conditions promoting spore formation.

MATERIALS AND METHODS

This study was performed with the type culture of *Streptomyces avermitilis* JCM 5070 (Merck Sharp & Dohme Res. Labs. MA-4680) maintained on oat agar [12]. The submerged culture of the streptomycete was grown in 250-ml flasks containing 50 ml of maize medium no. 2 or synthetic CP1 medium supplemented with glucose [12] on a shaker (180 rpm) at 28°C for 7–15 days. Vegetative mycelium grown for 48 h in liquid maize medium no. 2 (K-2) was used as inoculum. The mycelium was washed with saline, resuspended in sterile water, and added to fermentation flasks (5%, vol/vol). The time course of culture growth was assessed by changes in dry cell mass (DCM) after drying at 105°C. The number of viable cell was determined by the number of the colony-forming units (CFUs) after plating cell suspension onto oat agar.

Microscopic analysis was performed using a Zetopan phase-contrast microscope (Reichert, Austria) and by fluorescence microscopy. In the latter case, cells were fixed with 96% ethanol and stained with acridine orange and DAPI (Serva), and specimens were analyzed using a Lumam-I2 fluorescent microscope with blue and UV excitation.

For electron microscopy, the biomass of *S. avermitilis* submerged culture was concentrated, washed with distilled water, fixed in 1% OsO_4 in acetate–veronal buffer (pH 6.0), and treated with 0.5% uranyl acetate in the same buffer. Cells were dehydrated with increasing ethanol concentrations and embedded into Epon resins. Cell sections were stained with lead citrate and analyzed using a JEM 100C electron microscope (instrumental magnification, 30000).

The formation of submerged spores by *S. avermitilis* in autolysing cell suspensions of increased (tenfold) density was studied using submerged *S. avermitilis* cultures at the stationary phase grown in shaker flasks on K-2 or glucose-containing CP1 media. The biomass was separated by aseptic centrifugation at 3000 *g* for 20 min and resuspended in (1) native growth medium; (2) phosphate buffer (pH 7.25) supplemented with 0.2% $CaCl_2$; or (3) phosphate buffer (pH 7.25) supplemented with 0.2% $CaCl_2$ and oleic acid, a chemical analogue of the autolysis autoinducer [13]. Oleic acid in ethanol was added to a final concentration of 600 nmol/ml (ethanol concentration in this case was 0.3% (vol/vol)). The cell suspension was stored at 4°C. Cell viability in the control (without concentration) and experimental samples was determined prior to storage and after one and three months of storage. The heat resistance of resting forms produced in stored samples was determined by calculating the time of 50 (LD_{50}) and 90% (LD_{90}) decrease in CFUs after heating the suspension at 70°C.

Spore formation in submerged *S. avermitilis* cultures under nutrient limitation was studied using CP1 synthetic medium containing glucose, potassium nitrate (1 g/l) as a source of nitrogen, K_2HPO_4 (0.5 g/l) as a source of phosphorus, and glucose (20 g/l) as a source of carbon. To achieve limitation by one of the nutrients, its concentration was decreased fivefold.

To compare the heat resistance and lysozyme sensitivity of various types of *S. avermitilis* resting forms, we obtained aerial spores (exospores) of a ten-day-old *S. avermitilis* culture grown on oat agar in the presence of yeast extract, as described in [12]; submerged spores and vegetative cells grown for 1–7 days in liquid CP1 medium supplemented with glucose were also used. Heat resistance of spores of different morphotypes and that of vegetative mycelium were assessed by the changes in the number of CFUs in suspensions after heating in a UV-4 ultrathermostat at 70°C for 5, 10, 15, and 20 min. In lysozyme sensitivity tests, cells were treated with lysozyme (1 mg/ml; Sigma) and then incubated at 37°C for 1 h. Lysozyme sensitivity was assessed by the changes in the number of viable cells (CFUs) after enzymatic treatment.

Results were considered statistically significant when the standard deviation σ did not exceed 11% within a data group and 5% between data groups ($p \le 0.05$).

RESULTS

The production of resting forms in life cycles of a submerged *S. avermitilis* culture was studied using streptomycetes grown either on maize medium no. 2 rich in organic nitrogen and phosphorus or on CP1 synthetic medium containing only mineral salts and glucose. On maize medium, intense growth of mycelial mass consisting of uniform hyphae was observed. Aging of the culture resulted in fragmentation of the mycelium with subsequent autolysis. When grown in liquid synthetic medium, biomass was accumulated less actively, and changes in the structure of *S. avermitilis* mycelium were observed. Phase microscopy revealed protrusions or swellings on tips of individual hyphae as early as on the third day of growth; by the fifth day, ovoid or spherical highly refractive bodies were formed in hyphae (Fig. 1). On the seventh day of growth, refractive cells were found in lysed mycelial hyphae. The use of fluorescent dyes made it possible not only to visualize these spore-like cells but also to monitor the stages of their formation, from the terminal hyphal protrusion to completely developed structures (Fig. 2). Upon maturation of spores, their refractivity increased and their envelopes underwent structural changes, which were correlated with a decreased permeability for dyes (Fig. 2f).

Electron microscopy of refractive *S. avermitilis* cells showed that their ultrastructure and ways of formation

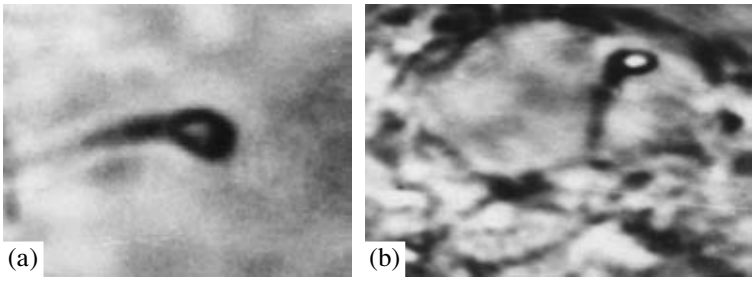

Fig. 1. Submerged spores of *S. avermitilis*. Phase microscopy. Scale, 1 μm = 4 mm.

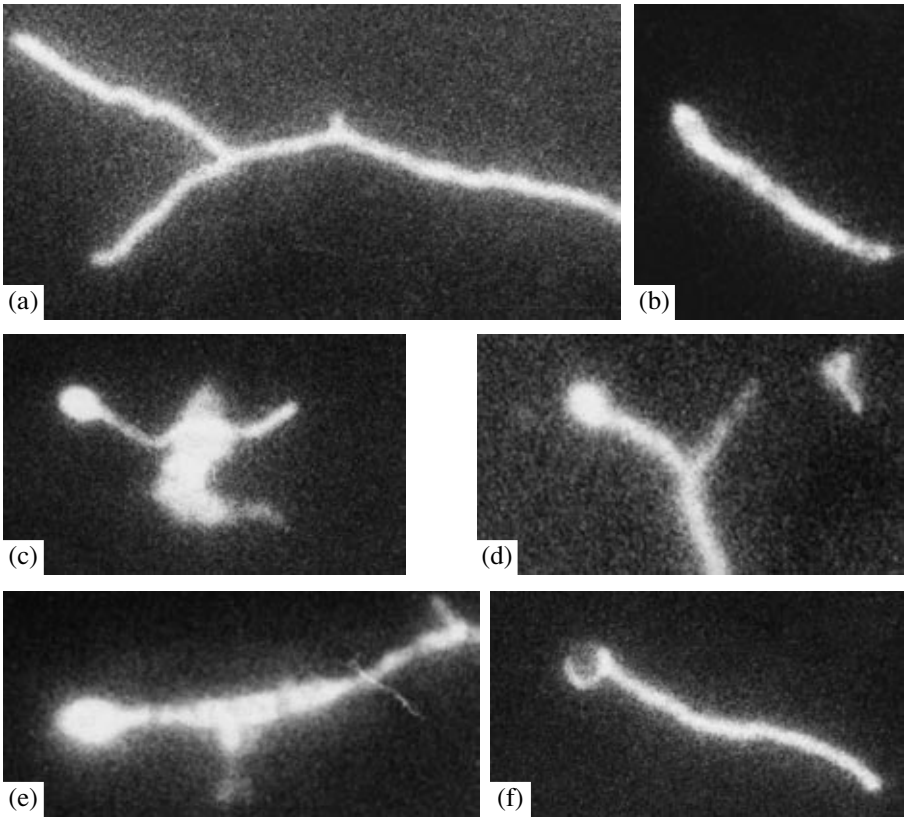

Fig. 2. Stages of the formation of submerged spores by *S. avermitilis.* Fluorescent microscopy, DAPI staining. Scale, 1 μm = 4 mm. (a) A fragment of mycelium of the 48-h culture; (b) swelling of a hyphal tip, beginning of spore formation; (c–e) stages of spore maturation; (f) mature spore, no fluorescence in the core.

were analogous to those of endospores of thermophilic actinomycetes (Fig. 3) [3]. On transverse sections of the hyphae shown in Figs. 3a and 3b, the initial stage of forespore formation proceeding via invagination of the cytoplasmic membrane and forespore absorption can be seen. Subsequent spore maturation was accompanied by an increase in the electron density of the cytoplasm and formation of characteristic endospore structures, including the cortex located between the outer and inner spore membranes (Figs. 3c, 3d).

To enhance sporogenesis, we applied techniques based on the use of extracellular autoregulators involved in the production of resting forms: autoinducers of autolysis (factor d_2) and anabiosis (factor d_1) [13–15]. The first technique was based on the possibility of enhanced sporogenesis in autolysing dense suspensions (concentrated ten times), where the level of autoregulators was higher than in a growing culture due to their release from the autolysed portion of the mycelium [14, 17, 18]. To achieve spontaneous or induced autolysis, stationary-phase mycelium was resuspended in the native culture liquid or in buffer solutions, respectively [18]. In the last case, to achieve different autolysis rates, oleic acid (600 nmol/ml) was used as a chemical analogue of the autolysis autoinducer. The number of viable and heat-resistant cells was deter-

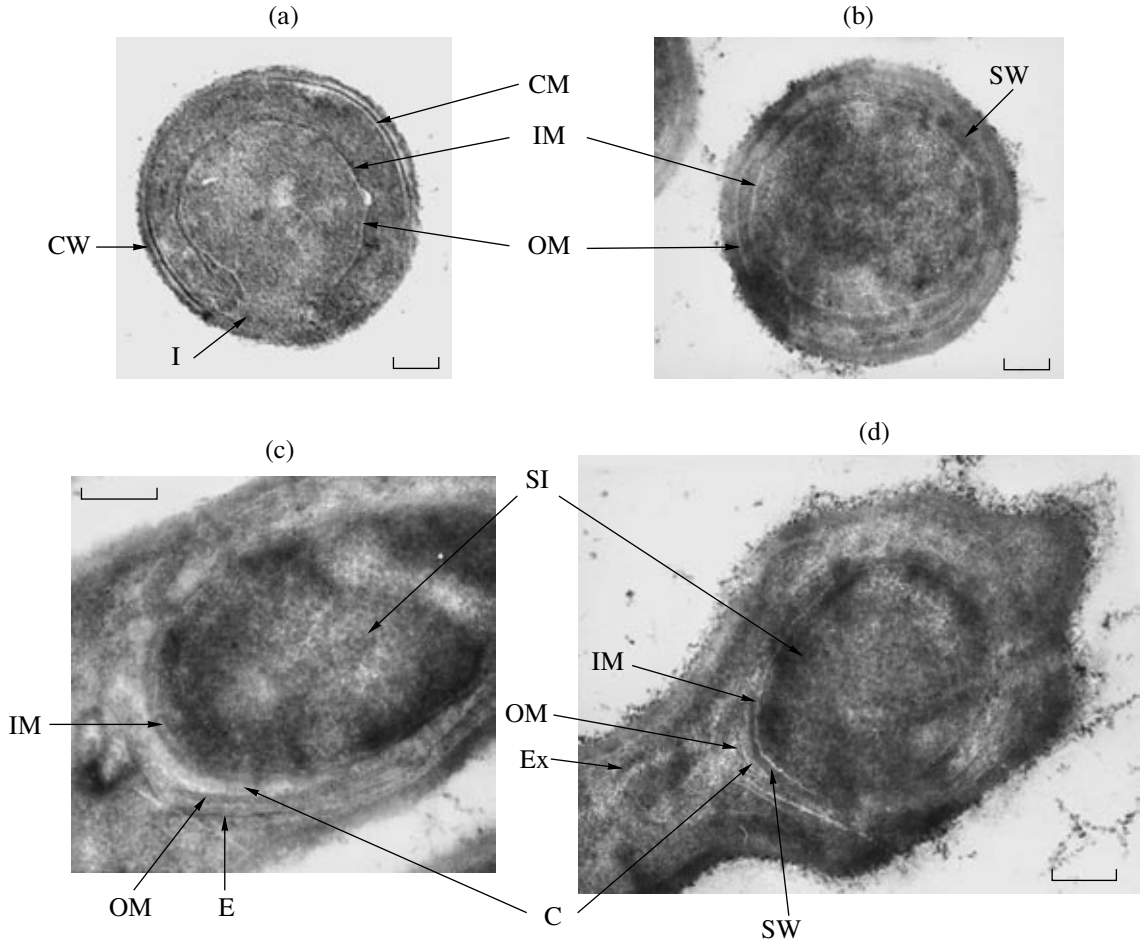

Fig. 3. Stages of submerged spore formation in *S. avermitilis.* Ultrathin sections. (a) Invagination of the cytoplasmic membrane; (b) absorbed forespore; (c, d) formation of the cortex (C) between the inner (IM) and outer (OM) spore membranes. Designations: CM, hyphal cytoplasmic membrane; I, invagination of CM; CW, hyphal cell wall; SW, spore cell wall; IM, inner spore membrane; OM, outer spore membrane; C, cortex; SI, spore interior; E, spore envelope; Ex, exosporium. Scale bar, 0.2 μm.

mined in suspensions of actinomycetes (grown on CP1 synthetic medium supplemented with glucose or on rich organic maize medium no. 2) in the course of autolysis during storage for three months at 4°C (Tables 1, 2). Comparison of the number of viable cells found after three-month storage showed that their titer in suspensions of *S. avermitilis* cells grown on CP1 medium supplemented with glucose was higher by two orders of magnitude than in suspensions of *S. avermitilis* cells grown on maize medium no. 2; in the samples suspended in culture liquid, the titer was lower than in the samples suspended in buffer solutions used to induce autolysis. In the experiments with oleic acid, no significant change in the number of CFUs was found; i.e., the rate of autolysis did not affect the production of submerged spores.

Another parameter that can be used to assess the processes of submerged spore formation is the heat resistance of spores, an index characterizing the resistance of the resting forms to detrimental effects. Analysis of this parameter for resting *S. avermitilis* cells formed in autolysing suspensions, as determined using the LD_{50} and LD_{90} values characterizing cell death after heating at 70°C (Table 2), confirmed the patterns found when determining the number of CUFs in stored suspensions. The number of heat-resistant cells in *S. avermitilis* samples grown on CP1 synthetic medium not only did not decrease during storage but, in some cases, even increased; these findings were indicative of qualitative and quantitative changes in the composition of the cell population in the direction of heat-resistant spore forms. Conversely, heat resistance was low in the suspensions of streptomycete cells grown on maize medium, and the number of CFUs decreased during storage, possibly because the number of submerged spores was small in these samples (Table 2).

The second way to stimulate sporogenesis in submerged *S. avermitilis* cultures was based on growth limitation by one of the nutrients; such a limitation had been previously demonstrated to enhance spore formation in actinomycetes [7]. In our experiments, a decrease in glucose concentration to 0.4% resulted in a

Table 1. Number of viable *S. avermitilis* cells (log CFU) in concentrated autolysing suspensions stored for three months at 4°C

Sample	Viable cells (log CFU) in suspension of cells obtained by cultivation on					
	CP1 with glucose			Maize medium no. 2		
	Stationary-phase culture, 5 days	Storage for		Stationary-phase culture, 5 days	Storage for	
		1 month	3 months		1 month	3 months
Original cell suspension	6.6	4.65	4.45	6.5	4.0	2.0
Cells concentrated in native culture liquid	7.8	5.9	4.4	7.9	4.05	2.2
Cells concentrated in phosphate buffer	8.0	7.15	5.5	8.0	5.5	3.0
Cells concentrated in phosphate buffer containing oleic acid	7.75	5.4	5.3	8.0	5.6	2.35

Table 2. Resistance to heating at 70°C (LD_{50} and LD_{90}, min) of *S. avermitilis* resting cells

Sample	Time before death (min) for cells grown on					
	CP1 with glucose			Maize medium no. 2		
	Prior to storage	Storage for 1 month	Storage for 3 months	Prior to storage	Storage for 1 month	Storage for 3 months
Original cell suspension (7 days)			LD_{50}			
	11.25	8.25	12.75	6.0	7.0	5.0
			LD_{90}			
	18.5	13.25	17.5	11.5	9.25	9.25
Cells concentrated in native culture liquid			LD_{50}			
	11.75	9.0	11.5	5.75	6.5	3.5
			LD_{90}			
	18.75	14.0	16.5	10.7	9.25	9.0
Cells concentrated in buffer			LD_{50}			
	11.5	11.75	12.5	6.75	8.0	3.0
			LD_{90}			
	18.75	20.75	19.0	13.5	14.0	9.5
Cells concentrated in buffer containing oleic acid			LD_{50}			
	12.75	9.5	14.5	6.0	8.0	5.0
			LD_{90}			
	19.5	20.25	19.5	14.5	14.0	11.25

certain decrease in the growth rate (Fig. 4) but had no positive effect on the development and formation of submerged spores by submerged *S. avermitilis* culture.

A fivefold decrease in nitrate concentration (to 0.02%) had the greatest effect on culture growth (Fig. 4); however, in this case, intense formation of submerged spores was not observed as well. Electron microscopy revealed the formation of electron-transparent inclusions in the nonlysed portions of the mycelium, characteristic for old hyphae of streptomycete mycelium (Fig. 5).

When *S. avermitilis* was grown in CP1 liquid medium with a decreased phosphorus content, changes in mycelial micromorphology were the most pronounced. A decrease in the concentration of K_2HPO_4 to 0.01% enhanced the production of submerged spores by the culture (Fig. 6). It is worth mentioning that spore formation was observed not only on the tips of mycelial hyphae but also on numerous short side branches, where spores were formed, swelling in a budlike manner during maturation and thereby resembling sporogenesis in thermoactinomycetes [2, 3]. In this variant, a

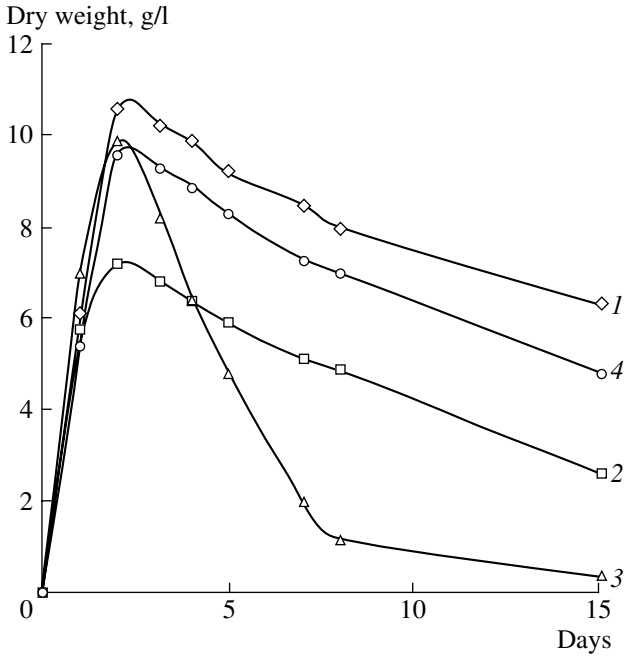

Fig. 4. The time course of biomass accumulation by *S. avermitilis* grown on CP1 medium in the presence of fivefold decreased concentrations of carbon, nitrogen, and phosphorus sources. (*1*) Complete medium; (*2*) a decreased concentration of the source of nitrogen (KNO_3, 0.02% (wt/vol)); (*3*) a decreased concentration of the source of phosphorus (K_2HPO_4, 0.01% (wt/vol)); and (*4*) a decreased concentration of the source of carbon (glucose, 0.4% (wt/vol)).

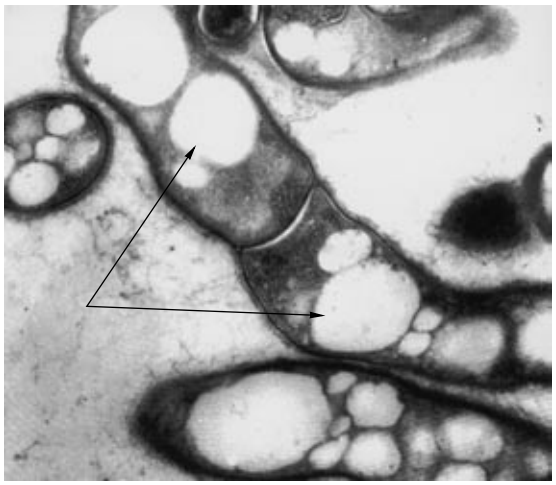

Fig. 5. Septating mycelium of a 7-day submerged *S. avermitilis* culture grown under nitrogen limitation (KNO_3, 0.02% (wt/vol)). Ultrathin section, 30000×. Arrows show electron-transparent inclusions in the cytoplasm.

decrease in phosphate concentration primarily activated autolysis rather than limited growth, which resulted in an almost complete lysis of the culture by the eighth day of cultivation (Fig. 4). Transmition electron-microscopy showed that phosphorus deficiency in the nutrient medium promoted formation of endogenous spores in the hyphae by separation of a portion of the parental hypha by the cytoplasmic membrane followed by its adsorption by the parental hypha and synthesis of all the structures characteristic of endospores (Figs. 7a, 7b). A characteristic morphological feature of submerged endospores of *S. avermitilis* was a peculiar shape of the exosporium (Fig. 7).

High heat and lysozyme resistances are characteristic features of bacterial endospores. However, in the only work describing the formation of submerged endospores by *S. globisporus*, it was stated that they were sensitive to heating and lysed by lysozyme [4]. Our results demonstrated a high heat resistance of submerged spores of *S. avermitilis* (Fig. 8, Table 3). It should be mentioned that the heat resistance of submerged *S. avermitilis* spores substantially exceeded the heat resistance of not only vegetative cells but also of aerial spores. The survival rate of vegetative mycelial cells dramatically decreased as soon as after 5 min of heating at 70°C (Fig. 8), and 90% of mycelial cells died after 10 min of heating (Table 3). The survival of an insignificant portion of vegetative mycelium after longer heating (Fig. 8) can be possibly explained by the presence of heat-resistant mycelial fragments in the samples. The presence of such fragments had been reported for *Nocardia*-like mutants of *Streptomyces* spp. [2]. Although aerial spores (exospores) of *S. avermitilis* were more heat-resistant than vegetative cells, they were still sensitive to heating: a significant drop in their viability was observed after 15 min of heating (Fig. 8), and 90% of aerial spores lost their viability after 20 min of heating (Table 3). The effect of an increased temperature on the endospores formed by submerged *S. avermitilis* culture was different. A noticeable decrease in the endospore viability was observed as early as in the first 5 min of heating; then, the death rate stabilized at a low level, and over 50% of the endospores retained viability after 20 min of heating (Fig. 8, Table 3).

Table 3. Heat resistance of vegetative and resting *S. avermitilis* cells under heating at 70°C

Heat resistance indices	Vegetative mycelium	Aerial spores (exospores)	Submerged spores
LD_{50}, time of death, min	3.7	7.8	>20
LD_{90}, time of death, min	10	20	–

Analysis of the lysozyme effect on aerial and submerged spores of *S. avermitilis* showed that both types of resting forms were resistant to the enzyme, whereas the vegetative mycelial cells were completely lysed by lysozyme (Table 4).

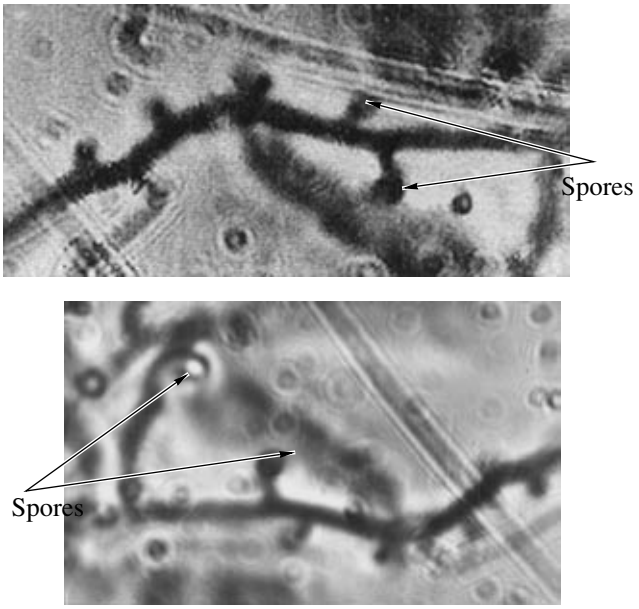

Fig. 6. Submerged spores of *S. avermitilis* grown on CP1 medium with glucose in the presence of fivefold decreased concentration of the source of phosphorus (K_2HPO_4, 0.01% (wt/vol)). Phase-contrast microscopy. Scale, 1 μm = 4 mm.

DISCUSSION

The strain of *S. avermitilis* producing a complex of macrolide antibiotics, avermectins, was first isolated and described by a group headed by S. Omura during screening antiparasitic drugs performed by Merck Sharp & Dohm Research Laboratories in 1979 [16]. Its taxonomic description was based on the *S. avermitilis* strain MA-4680, designated by the authors as the type strain. According to this description, aerial cylindrical spores with a smooth spore envelope, formed exogenously on the spiral sporophores of aerial mycelium, are characteristic of *S. avermitilis.* Our study of the physiological and morphological properties of the *S. avermitilis* type strain JCM 5070 performed in this work confirmed its initial description.

The results of the study of submerged growth of *S. avermitilis* demonstrated that, in submerged culture, this streptomycete is able to produce submerged spores—resting forms alternative to exospores—whose formation is promoted under the conditions of concentrated suspensions subject to autolysis or by phosphorus starvation. In the second case, in a fraction of the cells in starved cultures, the processes of autolytic degradation were also activated; in the remaining intact mycelial fragments, differentiation followed the pattern of endospore formation. Coupling between autolysis and cell differentiation was first reported for myxobacterial spore formation [15] and then for coryneform bacteria and yeast [14, 18]. Our results showed that this phenomenon is also characteristic of mycelial prokaryotes (streptomycetes).

For the analysis of submerged spore formation in limited cultures, it should be taken into account that the effect of the major nutrient sources greatly depends on their nature and the physiological characteristics of strains. It was shown that a decrease (to 0.2%) in the concentration of glucose used as the sole source of carbon had no effect on sporogenesis in submerged cultures of some streptomycetes [6, 7, 10] and *S. avermitilis* (as demonstrated in this study). However, in the case of *S. viridochromogenes*, a decrease in glucose concentration suppressed the formation of submerged spores [8].

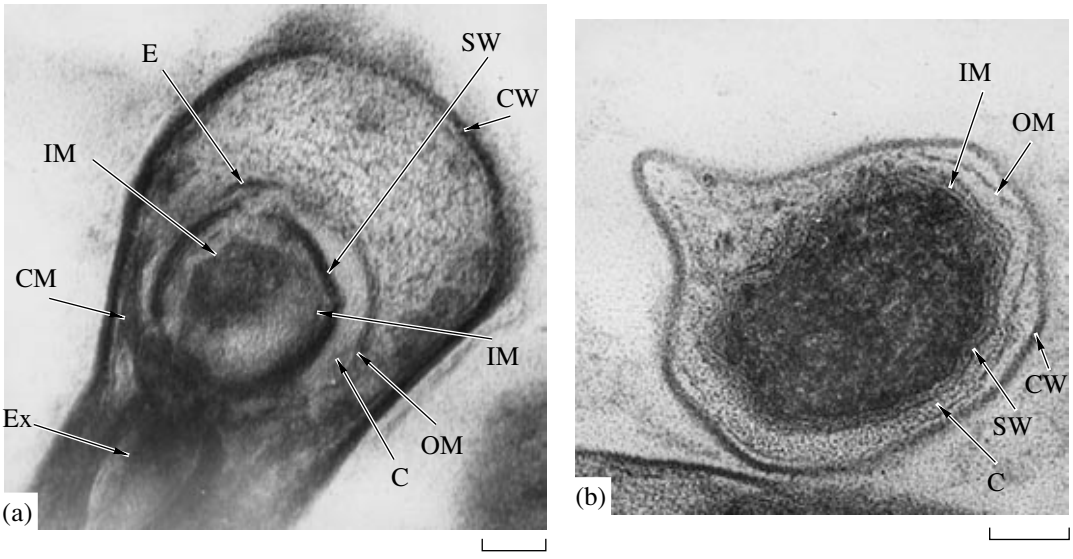

Fig. 7. Formation of submerged endospores of *S. avermitilis* under phosphorus limitation. Ultrathin sections: (a) forespore, the stage of cortex formation; (b) mature spore. For abbreviations, see Fig. 3. Scale bar, 0.2 μm.

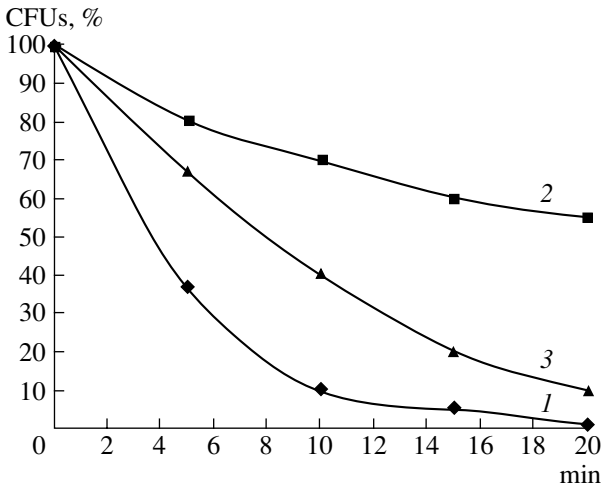

Fig. 8. Viability (CFUs, %) of (*1*) vegetative mycelium and (*2*) submerged and (*3*) aerial spores of *S. avermitilis* during heating at 70°C. 100% CFU corresponds to 5.2×10^5 for vegetative mycelial cells; 5.7×10^3 for submerged spores; and 5.8×10^5 for surface (aerial) spores. Vegetative mycelium and submerged spores were obtained on glucose-supplemented liquid CP1 medium under shaking; surface (aerial) spores were obtained on glucose-supplemented CP1 agar.

Different sources of nitrogen, such as ammonium salts and organic nitrogen, provide for a high growth rate of streptomycetes in liquid culture and, therefore, ensure rapid biomass accumulation but do not facilitate sporogenesis [19]. Conversely, the use of nitrate salts has a stimulatory effect on submerged exospore formation [7, 10, 19]. In this work, culturing *S. avermitilis* in a liquid medium containing maize extract resulted in abundant production of mycelial mass; however, no submerged spores were found in this case. When a depleted synthetic medium containing potassium nitrate was used, submerged spores were found on the third day of cultivation.

The results on the growth and development of *S. avermitilis* under conditions of phosphorus limitation, which enhanced sporogenesis, can be probably correlated with the data on the effect of phosphate starvation on the formation of submerged spores by *S. griseus* [7, 20]. In those works, phosphorus deficiency effectively triggered sporogenesis, which was accompanied by the activation of the genes that control intracellular accumulation of proteins belonging to the so-called "starvation stress response group"; these proteins are required for the rearrangements of the cell structures during the transition of cells to differentiation.

Table 4. Lysozyme sensitivity of vegetative and resting *S. avermitilis* cells

Cells	Viability, CFU (%)	
	Before lysozyme treatment	After lysozyme treatment
Vegetative mycelium	5.2×10^5	0
Submerged spores	5.7×10^3	4.6×10^3 (80)
Aerial spores (exospores)	5.8×10^5	6.0×10^5 (100)

Our results and published data indicate that streptomycetes can form sporelike cells both during surface growth and in a submerged culture. The process of production of resting forms during surface growth of actinomycetes is well studied, whereas only a few cases of submerged sporogenesis are known [4, 6–10]. Except for the spores produced by *S. globisporus* and its variant, the ultrastructural organization of which is characteristic of endospores [4], all other submerged spores were formed in a way similar to that of aerial spores, i.e., were representative of exospores [6–10]. The published data on the resistance of submerged exospores of streptomycetes to increased temperatures and lysozyme are contradictory (Table 5). For instance, Kendrick *et al.* [7] found that *S. griseus* submerged spores were resistant to ultrasound (US) but were lysed by lysozyme. They explained this phenomenon by a thinner spore envelope compared to aerial spores of the same actinomycete. Submerged spores of *S. antibioticus* were resistant to US; their heat resistance, however, was less than that of surface spores but higher than that of vegetative mycelium [10]. Heat-resistant exospores were found in submerged cultures of some strains of *S. griseus, S. acrimycini*, and *S. albus* [6]; *S. viridochromogenes* exospores were also resistant to US and lysozyme [8]. Conversely, sporelike cells of *S. coelicolor* and *S. lividans* formed in a liquid culture in the presence of calcium were heat-sensitive [6]. In spite of the similarity of submerged endospores of *S. globisporus* and its natural variant to bacterial endospores in ultrastructural organization and chemical composition, they were found to be sensitive to lysozyme and to heating at 60°C for 30 min [4]. The authors explain these results by a possibly low content in spores of calcium ions able to form chelates with dipicolinic acid [4]; these chelates are believed to determine the resistance of bacterial endospores to high temperatures [5].

We have reliably demonstrated the ability of *S avermitilis* to produce submerged spores; the rate of this process depended on culture conditions. The optimal conditions promoting the formation of submerged spores were determined: culturing on phosphorus-limited medium and cell suspensions undergoing autolysis. The morphological and ultrastructural characteristics of spores in the time course of their formation were determined. Submerged *S. avermitilis* spores are similar to the endospores of bacilli and thermoactinomycetes with respect to ultrastructural organization and the type of formation. *S. avermitilis* endospores are resistant to heating and lysozyme.

Analysis of these data allows us to conclude that the presence of two alternative types of sporogenesis in *S.*

Table 5. Examples and main characteristics of submerged spores formed by streptomycetes

Strain	Ultrastructural organization	Characteristic			Source
		Heat resistance	Resistance to US	Resistance to lysozyme	
S. griseus ATCC 1037	Similar to aerial spores (exospores)	+	ND	ND	[6]
S. griseus IMRU 3570		+	ND	ND	[6]
S. griseus JI 2212		+	ND	ND	[6]
S. acrimycini JI 2236		+	ND	ND	[6]
S. albus G		+	ND	ND	[6]
S.lividans JI 1326		–	ND	ND	[6]
S.coelicolor JI 2280		–	ND	ND	[6]
S. griseus NRRLB-2682		ND	+	–	[7]
S.viridochromogenes NRRL B-1511		+	+	+	[8]
S.venezuelae ISP 5230		ND	+	+	[9]
S. antibioticus ETHZ 7451		±	+	–	[10]
S. globisporus 0234A	Endospores	–	+	–	[4]

Note: +, resistant; ±, more resistant than vegetative cells but less resistant than exospores; –, not resistant; ND, no data.

avermitilis, similarly to that previously described for *S. globisporus* [4], is not an exception and possibly occurs in other species of streptomycetes. However, this problem requires further investigation. As in the case of microorganisms of other taxa, the ability of streptomycetes to undergo cell differentiation both in submerged and surface cultures reflects their high adaptive potential. The ability to produce alternative spores increases the ability to survive unfavorable conditions, which ensures the persistence of the species in nature.

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