Determination of cAMP and protein content in dormant chlamydospore and non-dormant chlamydospore of Duddingtonia flagrans

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Abstract

Duddingtonia flagrans, a nematode-eating fungus, is an effective component of animal parasitic nematode biocontrol agents. In the dried formulation, the majority of spores are in an endogenous dormant state. This study focuses on dormant chlamydospore and non-dormant chlamydospore of D. flagrans to investigate the differences in cAMP and protein content between the two types of spores. In this study, cAMP and soluble proteins were extracted from the non-dormant chlamydospore and dormant chlamydospore of D. flagrans isolates SDH035 and DH055, respectively. The cAMP Direct Immunoassay Kit and Bradford protein concentration assay kit (Coomassie brilliant blue method) were used to detect the cAMP and protein content in two types of spores. Results showed that the content of cAMP in dormant spores of both isolates was significantly higher than that in non-dormant spores ($p<0.05$). The protein content of dormant spores in DH055 bacteria was significantly higher than that of non-dormant spores ($p<0.05$). In addition, the protein content of dormant spores of the SDH035 strain was slightly higher than that of non-dormant spores, but the difference was not significant ($p>0.05$). The results obtained in this study provide evidence for the biochemical mechanism of chlamydospore dormancy or the germination of the nematophagous fungus D. flagrans.

Determination of cAMP and protein content in dormant chlamydospore and non-dormant chlamydospore of *Duddingtonia flagrans*

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Abstract:

Duddingtonia flagrans , a nematode-eating fungus, is an effective component of animal parasitic nematode biocontrol agents. In the dried formulation, the majority of spores are in an endogenous dormant state. This study focuses on dormant chlamydospore and non-dormant chlamydospore of *D. flagrans* to investigate the differences in cAMP and protein content between the two types of spores. In this study, cAMP and soluble proteins were extracted from the non-dormant chlamydospore and dormant chlamydospore of *D. flagrans* isolates SDH035 and DH055, respectively. The cAMP Direct Immunoassay Kit and Bradford protein concentration assay kit (Coomassie brilliant blue method) were used to detect the cAMP and protein content in two types of spores. Results showed that the content of cAMP in dormant spores of both isolates was significantly higher than that in non-dormant spores (p <0.05). The protein content of dormant spores in DH055 bacteria was significantly higher than that of non-dormant spores (p <0.05). In addition, the protein content of dormant spores of the SDH035 strain was slightly higher than that of non-dormant spores, but the difference was not significant (p >0.05). The results obtained in this study provide evidence for the biochemical mechanism of chlamydospore dormancy or the germination of the nematophagous fungus *D. flagrans* .

Keywords : *Duddingtonia flagrans* ; cAMP; protein; Dormant chlamydospore; Non-dormant chlamydospore

Introduction

Duddingtonia flagrans belongs to a species of nematophagous fungi, which produces three-dimensional viscous nets to capture nematodes. At present, *D. flagrans* is a member of*Duddingtonia* , which has no sexual reproduction stage. The fungus was first described in 1948 [1], and during culture, the mycelium expands to form an intermediate chlamydospore. The chlamydospore is usually spherical, with an inner diameter of 24–32 μm and a wall thickness of 2 μm. Wang et al. (2015, 2019) reported that immature chlamydospore was elliptical with a smooth surface and few tubercles, whereas mature spore was spherical with many tubercles on the surface [2,3]. Chlamydospores were produced from the third day, and they increased with the extension of culture time. Compared with chlamydospores, the production of conidia, which can be produced within a week of culture, was small. The chlamydospore of this bacterium has an asexual reproductive structure. Given their high stress resistance, conidia are an important form of survival in nature and a source of laboratory inoculants. Many studies have shown that *D. flagrans*chlamydospores that are taken by animals do not reproduce in the body, but they can still survive as feces are excreted from the body. When the external environment is suitable, the spores can germinate and produce predatory structures, thereby killing larvae in feces [4-8]. Chlamydospore feeding as a feed additive to animals has been shown to be effective in reducing infectious larvae (L3) on pasture under laboratory and field conditions [9-11]. Therefore, these fungi are an important biocontrol strain for the biological control of animal parasitic nematodes. Cyclic adenosine phosphate (cAMP) is an important second messenger in cells, which plays a role in regulating physiological activities and material metabolism within cells. The content of cAMP in cells is small, which plays a variety of signal regulation and transduction roles. For eukaryotic fungi, cAMP has been shown to

increase the frequency of appressorium differentiation of the entomopathogenic fungus *Metarhizium anisopliae* [12]. cAMP plays an important role in the growth, development, and pathogenicity of plant pathogenic fungi. cAMP also participates in the growth and development of plant pathogenic fungi, as well as regulates their pathogenicity and the germination of asexual spores [13]. Decreased intracellular cAMP levels affected the growth of *Colletotrichum lagenarium* and *Magnaporthe grisea* appressorium, which led to a decrease in fungal pathogenicity [14,15]. In *C. gloeosporioides* , kinase A, which is also known as cAMP-dependent protein kinase A, regulates its morphogenesis and plays an important in its pathogenicity [16]. cAMP is also required for the saprophytic germination and appressorium formation of *C. gloeosporioides*[17]. In *Fusarium graminearum* , the Ras GTPase guanine nucleotide exchange factor FgCdc25 regulates fungal development, deoxynivalenol (DON) production, and plant infection by modulating the cAMP and MAPK signaling pathways [18]. The transcription factors Tri6, Tri10, and AreA mediate DON synthesis by ammonium and cAMP signaling in *F. graminearum* [19,20]. Mutants deleted by the PKR regulatory subunit of cAMP-PKA in *F. graminearum* had severe defects, but they often produced spontaneous suppressors [18]. The cAMP/protein kinase A (cAMP/PKA) signaling pathway has been well studied because of its conserved and crucial roles in the pathogenesis and development of human pathogenic fungi [21-27]. In *Candida albicans* , their ability to switch between yeast and hyphal forms is regulated by multiple signaling cascades, including cAMP/PKA and other signaling pathways [28]. A protein kinase has two "inactivate" catalytic subunits in *C. albicans* , each bound to a regulatory subunit. In addition, environmental cues such as amino acids can trigger the cAMP/PKA signaling pathway by activating adenylyl cyclase to convert ATP to cAMP [21, 24]. Teasaponin can inhibit filamentation and biofilm formation by reducing the intracellular cAMP level in *C. alicense* [29]. To date, there have been no reports on the content of cAMP in the nematophagous fungi *D. flagrans* .

Fungal dormancy is the most sophisticated specific state that ensures the preservation of life under the action of unfavorable factors. Spore dormancy can be divided into endogenous dormancy and exogenous dormancy. Endogenous dormancy refers to the fact that the germination of spores is mainly influenced by internal factors such as their own permeability, self-inhibitory factors, and metabolic damage. The presence of water is not sufficient for spore germination in the case of endogenous dormancy, as this process is under the control of the cAMP system and other special autoinhibitory compounds, thereby delaying the exit of the spore from the dormant state [30]. Exogenous dormancy indicates that spore germination is mainly affected by external conditions such as temperature, humidity, oxygen, nutrients, environmental inhibitors, and symbiotic microorganisms. This dormancy is disrupted when the effect of an unfavorable factor ceases. During biological control preparations with the pachyspore of *D. flagrans* as the effective component, most of the spores are in endogenous dormant state. The dormant spores have a long shelf life when stored in a dry environment, which is conducive to the transportation, preservation, and promotion of the final product. The fungal cells in the state of biosis and the dormant cells are different in chemical composition. This difference is primarily reflected in the different contents of sugars such as trehalose, lipid, and triglycerides. Nitrogen-containing compounds such as amino acids and their derivatives have been less studied in spore dormancy and germination than non-reducing disaccharides. This study focuses on *D. flagrans*chlamydospores and dormant chlamydospores as the research objects, aiming to explore the differences in cAMP and protein content between the two types of spores. The result of this study provides a basis for revealing the biochemical mechanism of the dormancy of the chlamydospore structure of this bacterium.

1 Materials and methods

1.1 Test strains

The *D. flagrans* isolates used in this study were SDH035 (CGMCC KY288614.1) and DH055 (CGMCC: KY419119.1). The gene database entry number is KU881774.1, which was isolated and purified from sheep feces by using an improved spreading separation technique [2] (Wang et al., 2015). The isolate was stored in a refrigerator at 4 on a test tube containing 2% corn meal agar (Beijing Wanjia First chemical Biotechnology Co., LTD.). The mycelium and spores were taken from the preserved inclined plane, inoculated on a potato dextrose agar (PDA, Beijing Landbridge Technology Co., LTD.) plate containing chloramphenicol, and

incubated at 28 for 7 days.

1.2 Production and purification of chlamydospores

1.2.1 Non-dormant chlamydospore

First, Sabourg's liquid culture medium was prepared by weighing 10.0 g of peptone, 40 g of glucose, and 1000 mL of distilled water. The prepared medium was autoclaved at 121 for 15 min, and the pH was finally adjusted to 7.0. The sterilized Sabourg's culture medium was divided into 500 mL triangular flasks before sterilization, with each bottle containing 100 mL.

Using a sterilized punch (aperture 5 mm × 5 mm), the PDA agar containing the aforementioned mycelium was perforated into uniform small circular blocks, and a sterile technology was used to inoculate the agar blocks onto a triangular flask containing 100 mL of Sabourg's liquid medium (10 g of peptone, 40 g of glucose, and 1000 mL of distilled water at pH 7.0) on an ultra-clean workbench. Eight blocks were inoculated per bottle. Then, the flask was placed on a shaking table (HZP-250 type, Shanghai Jinghong Experimental Equipment Co., Ltd.), shaken, and cultivated at 28 and 200 rpm for 72 h, and the cultivation was stopped. The flask was stored for no more than 3 days at 4 .

Corn and wheat were mixed in a 2:1 ratio, with a material-to-water ratio of 1.5:1. The mixture was soaked overnight in tap water and divided into 1000 mL triangular flasks. Each triangular flask contains 200 g of grain (calculated as dry matter) and sterilized under high pressure at 121 for 20 min. When the culture medium cools to room temperature, the preserved seed solution is inoculated in a 1:9 ratio (v/w) into a flask containing grains and then cultured at 28 for 20 days. When a large amount of mycelium grows on the grains and when a yellow powdery culture is obtained, the cultivation can be terminated. 0.05% sterilized Tweene-80 solution was added to a triangular flask and swirled for 1–2 min to wash the mycelium and spores from the grain. The crude bacterial suspension was collected by repeated washing three times and filtered by using a double gauze to remove the mycelium. Then, the suspension was divided into centrifugal tubes, centrifuged at 1000 rmp for 5 min, and then removed by enzymolysis. 2% snail enzyme and 2% cellulase were mixed with the spore suspension to achieve a final concentration of 1%. The two enzymes were oscillated in a 35 water bath oscillator for 2 h and then centrifuged with sterilized distilled water (8000 rpm, 2 min) three times to remove the remaining enzymes and discard the supernatant. The concentration of chlamydospore was adjusted by adding distilled water, and the number of chlamydospore was counted by using a blood cell counting plate. The average of five times was used to calculate the concentration of spores per milliliter. The total amount of chlamydospore and the content of spores in the sample before the test (spores/mL) were calculated by measuring the volume of spore suspension and stored at 4 for use.

1.2.2 Dormant chlamydospore

The culture grown in a grain medium for 30 days was placed in a shallow dish and treated at 28 and RH30% for 10 days. Then, the culture was dried for 4 days under circulating air at 35 , and the water content was determined to be less than 3%. The spore germination rate was calculated in accordance with the method described by Wang et al. (2019) [3]. The germination rate should be below 5%, sealed, and stored in a dry place for later use. The spores were purified and counted in the same way as non-dormant spores.

1.3 Extraction and determination of cAMP from chlamydospore

The purified chlamydospore-concentrated suspension was collected, added with liquid nitrogen, and ground repeatedly in a mortar until flocculation. Appropriate amount of PBS, 10 μL of 10 mmol/L EDTA, and 200 μL of 100 mmol/L phenylmethyl sulfonyl fluoride were added, and ultrasonic crushing (power 400 w) was carried out in an ice water bath for 10 min, with an interval of 2 s. After the spores broke the wall, they were bathed in water at 80 for 2 h, cooled, and centrifuged at 8000 r/min for 10 min. The supernatant was absorbed, and 1 mL of deionized water was added to the residue for centrifugation and extraction two times. The obtained supernatant is the extraction liquid of cAMP.

The protein was removed from the cAMP extract by using the Sevag method [31], in which chloroform and

n-butanol were first mixed at a volume ratio of 4:1 to produce a mixture of chloroform and n-butanol. The extract solution of cAMP was mixed with the mixed solution of chloroform n-butanol at a volume ratio of 4:1, shaken for 30 min, and then centrifuged at 12000 r/min for 5 min to separate the chloroform phase from the water phase. The water phase was taken into a new centrifuge tube to obtain a cAMP extract, which removes the protein.

The cAMP Direct Immunoassay Kit (Colorimetric, BioVion) was used to detect cAMP in chlamydospore. The standard solution was prepared in accordance with the kit instructions. The absorbance of the standard solution was measured using the enzyme marker (RT-5000, Beijing Yuanpinghao Biotechnology Co., LTD.), and the standard curve was drawn. The OD value of the samples to be tested was measured using the enzyme marker, with three parallel samples for each sample, and the average value of each parallel sample was determined three times.

Determination of soluble proteins in chlamydospore

The spore wall was broken, centrifuged, and washed in accordance with the abovementioned methods to remove the spore wall, and the resulting supernatant is the spore protein extraction solution. Protein concentration was determined using the Coomassie Brilliant Blue method in accordance with the instruction of the Bradford Protein Concentration Assay Kit (BioVion products). First, the protein standard stored at -20 is melted and prepared into a series of standard solution. 200 μL of G250 staining solution was added into each hole of the 96-well plate, and various parameters of the enzyme marker were set. The 96-well plate was placed into the enzyme-labeled instrument for testing, and the data were recorded and saved. The OD value of the measured standard solution was used to draw the standard curve. The volume of the added sample was recorded during sample determination, and three parallels were repeated per well. Then, the OD value of the sample to be tested was determined using the enzyme marker by adding a dye solution in the same way as the standard product, and the test was repeated three times for each parallel sample. Furthermore, the protein concentration was determined using the Coomassie Brilliant Blue method in accordance with the instructions of the Bradford Protein Concentration Determination Kit (BioVion product).

1.5 Data analysis

In this experiment, the content of spores in the original sample was measured by the number of spores/mL, whereas the horizontal coordinate of the standard curve was the concentration of W/V, that is, pmol/50 μL (cAMP assay) or mg/mL (protein assay). First, the absorbance values measured by different samples were substituted into the standard curve to find the concentration of cAMP or protein in different samples (pmol/50 μL or mg/mL), and then the protein or cAMP content in different samples was determined (pmol or mg) in accordance with the volume of the sample when determining the absorbance. Considering that the number of spores in the sample is measured per milliliter, it is finally converted to the protein or cAMP content (pmol or mg) per 10^7spores. The mean value was measured, and the difference in protein or cAMP content between dormant and non-dormant spores was compared by ANOVA in SPSS.

2 Results

2.1 Determination of cAMP content in spores

The standard cAMP concentration (0.3125–0 pmol/50 μL) and its corresponding absorbance were linearly correlated. Based on the measured data, the standard curve is shown in Figure 1, and the following regression equation is obtained: Y = -0.2214X + 0.1949. In the formula, Y represents the measured absorbance value, X represents the standard cAMP concentration (pmol/50 μL), and $R^2 = 0.9368$.

The cAMP content of the measured sample was obtained from the standard curve. Finally, cAMP concentrations in dormant and non-dormant chlamydospores of SDH035 and DH055 isolates were obtained, and the results are shown in Figures 2A and 2B. As shown in the figure, the content of cAMP in resting spores of both strains was significantly higher than that in non-resting spores ($p < 0.05$).

2.2 Determination of protein content in spores

After measurement, the concentration curve of standard protein is shown in Figure 3. In the regression equation y=1.362x + 0.9675, R2=0.9555, Y represents the measured average absorbance value, and X represents the standard protein concentration.

Based on the absorbance values, the standard curve was substituted to obtain the protein concentration of the measured samples. The average value of total protein in each 10^7 spores was finally obtained (Figures 4A and B). As shown in the figure, the protein content of dormant spore in DH055 was significantly higher than that of non-dormant spore (p <0.05), and the protein content of dormant spore in SDH035 was slightly higher than that of non-dormant spore, but the difference was not significant (p >0.05).

3 Discussion

cAMP is an important second messenger in cells, which plays a role in regulating physiological activities and material metabolism within cells. Studies on some fungi have shown that the intracellular cAMP expression level is rapidly increased through the Ga/Ras pathway, which in turn activates the cPKA catalytic subunit [32,33] (Hatanaka and Shimoda, 2001; Xue et al., 1998). The activation of cAMP leads to the degradation of trehalose in the cell and the synthesis of glucose [34]. cAMP can regulate various functions of fungi, including endogenous and exogenous carbon source utilization, spore formation, spore germination, and phototaxis. In *Saccharomyces cerevisiae* , several signaling pathways allow cells to sense glucose levels in the environment and initiate a transcriptional response. These pathways include the activation of cAMP/PKA and glucose expression (Snf1 kinase gene/MigI), which controls transcription [35]. Similar to yeast, the germination of the conidia of *Aspergillus nidulans* and*Neurospora crassa* is also involved in the activation of the cAMP signaling pathways and trehalose degradation [36]. Related studies have shown that the spore germination and appressorium formation of plant pathogens, particularly *M. grisea* , are mediated by the cAMP pathway and the cascade reaction of Pmk1 and Mps1 kinases [37,38].

In this study, the content of cAMP in the dormant spores was significantly higher than that in the non-dormant spores of the two*D. flagrans* isolates. Li et al. (2016) proved that the cAMP content in chlamydospore was negatively correlated with the germination rate, and the cAMP content in resting spores (black chlamydospore) was higher than that in non-resting spores (yellow chlamydospore) [39]. Although the methods used to determine the strains in the two studies were different, the former using enzyme-labeled colorimetry and the latter using gas chromatography showed similar results. Virdy et al. (1999) reported that the cAMP content of dormant spores of the fungus*Dictyostelium discoideum* is more than 11 times higher than that of newly formed spores [40]. Spores can achieve spontaneous germination in 14–18 days, and cAMP has a typical surge effect. However, the duration is not long, and the high level of cAMP significantly decreases during successful spore germination. These results support the hypothesis that externally activated (e.g., heating) and automatically activated spores germinate through different mechanisms. During heat activation, the transcription of ACG (a gene encoding adenylate-activating enzymes) was strongly correlated with the cAMP content in spore. For young wild-type spores that cannot spontaneously germinate, high cAMP levels are often maintained. Therefore, once spore germination was inhibited, cAMP levels increased. For example, when the activated spores were placed in a hypertonic environment to inhibit their activity, the concentration level of cAMP increases in dormant spores. Barhoom and Sharn (2004) reported that cAMP early activated the germination and expansion of *A. nidulans*spore [41]. Their results also show that plant surface signals induce spore germination specific to the pathogenic fungus*Colletotrichum gloeosporioides* f. sp. aeschynomene in a cAMP-dependent manner, and that cAMP is necessary for saprophytic germination and appressorium formation.

C. gloeosporioides have two different germination strategies, namely, pathogenic development and saprophytic germination. The growth of this bacterium in liquid soybean extract can induce pathogenic germination, but no appressorium is observed [41]. Appressorium is only produced on the surface of a solid medium containing a soybean extract. The formation of appressorium in *M. rosea* requires the induction of functional Pmk1 MAP kinase and cAMP. Mutants with the deletion of Pmk1 and various cAMP pathways do not form appressorium, whereas the addition of cAMP can save some mutants and enhance the formation of appressorium [37, 38, 42]. Treatment of the conidia of*C. gloeosporioides* with cAMP

changed the pattern of germination and appressorium formation. In particular, cAMP induced a large number of conidial enlargement on the bean infusion medium, and enlargement was one of the signs before spore germination. Studies of *C. gloeosporioides* and other fungal germination and signaling pathways have shown that germination in the early stages of saprophytism involves the activation of the cAMP pathway [41]. In the early stage, pathogenic germination is regulated by the cAMP-independent pathway and not by cAMP, and cAMP can interrupt pathogenic germination. Fillinger et al. (2002) studied trehalose degradation and germination tube development in the conidium of three mutant strains of *A. nidulans* (chaA^{-} *PKA*$^{-}$ *and*Δ*schA*$^{-}$) *germinatedonthemediumwithminimumconcentrationofglucosetoevaluatetheroleofthecAMPsignal*

Based on the research of *S. cerevisiae* , the regulation of adenylate activity depends on the GTP enzymes Ras1 and Ras2. However, several studies have shown that the G-protein 2 subunit Gpa2 regulates yeast growth and pseudofilament development through the cascade amplification of the cAMP/PKA response to glucose [43]. For *A. nidus* , the alpha subunit of the three heterotrimers of G protein, namely, FadA, GanA, and GanB, has been identified, but its role in regulating AC remains unclear [13]. Some studies have shown that RasA is involved in the regulatory development of *A. nidulans*[44]. However, Fillinger et al. (2002) showed that RasA did not regulate the activity of AC in *A. nidulans* , and cAMP levels did not increase when RasA was overexpressed [13]. RasA may regulate the development of *A. nidoris* by regulating the activated mitogen kinase pathway, which has also been observed in several other fungi. Fillinger (2002) pointed out that the cAMP/PKA signaling pathway is a major but not necessary component for the asexual spore germination of the filamentous fungus *A. nidus* [13]. For *A. nidus*spores, more than one cAMP target pathway and another PKA-catalyzed subnodal pathway are required for germination. The polytropic effect of cAMP was also manifested in the withdrawal of spores from continuous dormancy involving cAMP and AC [45]. The cAMP content of*Streptomyces* spores is low, but the cAMP level initially increases during spore germination and then decreases during subsequent colony growth [46]. The spores of the AC mutant strain of*Streptomyces Str. coelicolor* showed no germination, but when cAMP with a concentration of more than 1 mM was added to the culture medium, spore germination was observed [45]. When this mutant grows on the agar surface, the colony morphology changes [45]. In later research, a receptor for cAMP of *Str. coelicolor* was discovered, which is a protein homologous to the*Escherichia coli* cyclic adenylate receptor protein (CRP). The Crp gene knockout mutant exhibited similar defects in spore germination, whereas other physiological effects were also observed on the mutant. The abovementioned research concludes that the cAMP-AC-CRP system plays an important role in controlling the spore germination of *Str. coelicolor* [47]. The high cAMP content in dormant spores is necessary to initiate germination, and once the spores germinate, the cAMP content decreases. Although the content of cAMP in germinating spores was not determined in this study, *A. flagrans* dormant chlamydospores contain high levels of cAMP compared with non-dormant spores. The results of this study indicate that cAMP is necessary to initiate the germination of this fungus, and its content may rapidly decrease after germination, with a mechanism similar to that of other fungi.

Protein is one of the three major nutrients in living organisms, and little research has been conducted on its content in fungal spores. In this study, the protein content in the chlamydospores of two *D. flagrans* strains was determined using the Coomassie brilliant blue method. The results showed a higher soluble protein content in the dormant spores, indicating a difference in protein content between dormant and non-dormant spores. In a study on the spore germination of*Botryodiplodia theobromae* [48], dormant spores had two distinct protein bands (A and B). Protein band A decreased with the prolongation of spore germination time, while protein A was not present or detected in the mycelium. The content of protein B in dormant spores is second only to protein A, and the amount of protein B also decreases with the prolongation of germination time. However, some proteins are similar to protein B in the mycelium. The abovementioned two proteins do not exist in fresh spores and hyphae, so such proteins are similar to storage proteins in many higher plant seeds. During the germination of plant seeds, amino acids produced by the degradation of stored proteins in plant seeds are used as synthetic sources of new proteins required for germination. In some non-fungal organism studies, diatom dormant cells are rich in organelle proteins such as membrane proteins, ribosomal proteins, energy-related proteins, pigment proteins, and phosphoproteins. These proteins may be

involved in the accumulation of chlorophyll during the formation of dormant cells. Some bacterial spores, such as *Bacillus megaterium* and *B. cereus* , contain specific proteins that degrade during germination, and their amino acids are used to synthesize new proteins [49,50]. The results of the study of *D. flagrans* are also similar to those of the abovementioned studies. However, the types of proteins in the two types of spores have not been studied. Moreover, the protein content, types, and functions in dormant spores remain unknown. Thus, further research is necessary.

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Compliance with ethical standards

Conflict of interest statement The authors declare that they have no financial or commercial conflicts of interest.

Ethics statement This article does not contain any studies with human participants or animals performed by any of the authors.

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Figure 1 Standard curve measured by cAMP

Figure 2 cAMP content in dormant and non-dormant chlamydospores. (A) SDH035; (B) DH055

Figure 3 Standard curve of protein concentration

Figure 4 Protein content in dormant and non-dormant chlamydospores.

(A) DH055 spore protein content; (B) SDH035 spore protein content