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Inhibition of Asexual Sporulation and Growth of *Aspergillus niger* and *Aspergillus oryzae* by Propylamine

SONG, MYUNG HOON, KUPPUSAMY SELVAM, HYO-YOUNG JEONG, AND KEON-SANG CHAE*

Division of Biological Sciences and Basic Science Research Institute, Chonbuk National University, Chonju, Chonbuk 561-756, Kore a

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Abstract Effects of propylamine on conidial head formation and growth of *Aspergillus niger* and *Aspergillus oryzae* were analyzed. Propylamine inhibited conidial head formation in these two fungi, and the inhibitory effect of propylamine was not suppressed by the addition of potassium chloride at high concentration, which promotes conidial head formation in *A. nidulans* and *A. oryzae*. Propylamine also inhibited the growth of *A. niger* and *A. oryzae* by 41% and 32%, respectively, when the concentration of propylamine was 2%.

Key words: Asexual development, *Aspergillus niger*, *Aspergillus oryzae*, propylamine

Since some plant diseases are spread out principally by asexual spores, asexual sporulation in plant-pathogenic fungi has been one of target processes to develop new agricultural chemicals. To form asexual spores, proper cell wall structures are required, and in turn, pigments should be synthesized well and deposited appropriately in the cell wall, since pigment formation and the cell wall structures as well as the cell wall structures and development are closely related, although the pigment deposition is not absolutely required [1-4, 9].

On the other hand, low or high concentration of a salt such as potassium chloride has been identified as one promoting asexual development in *Aspergillus nidulans* or *Aspergillus oryzae* [6, 7, 10]. In the presence of a salt at high concentration, *A. nidulans* develops asexually with simultaneous inhibition of sexual development, which is also applicable to *A. oryzae*, in that a salt promotes conidial head formation in this organism [10]. Nitrogen sources also affect the development of *A. nidulans* [5]. When casein hydrolysate is supplemented as a nitrogen source, *A. nidulans* forms more sexual structures than when sodium nitrate is used. During the examination of various nitrogen sources to identify which nitrogen source affects development of *A. nidulans*, it has been found that propylamine can influence the development of *A. nidulans* [11]. In this study, the effect of propylamine on asexual sporulation was analyzed in *A. niger* and *A. oryzae*. In addition, the effect of propylamine on growth was also examined.

Aspergillus oryzae FGSC (Fungal Genetics Stock Center, Kansas, KS, U.S.A.) A815 and *A. niger* ATCC 6275 were cultured at 30°C on minimal medium (MM) to reduce the complexity [8]. When necessary, 1 or 3% glucose and 2% propylamine were added. To obtain large numbers of conidia from *A. oryzae*, 0.6 M potassium chloride was added into MM [10]. Conidia were harvested from solid cultures by using Tween 80 solution (0.08%, v/v).

To observe developmental phenotypes of the two fungi, about 10^7 conidia were inoculated evenly on appropriate solid MM, and the center of the culture plate was photographed after incubation of the plates for 3 days [10]. To analyze the effect of propylamine on growth, the radial growth was measured and expressed as a mean diameter of colonies on five plates. The equal numbers of conidia, 6×10^4 conidia in ten microliter, were inoculated at the center of five plates, the plates were incubated for 3 days, and the diameters of colonies were measured.

During the study on development of *A. nidulans*, some environmental factors, such as various types of carbon sources and the concentration of a carbon source, have been analyzed and proved to affect development [5]. The higher concentration of glucose in the medium increases sexual structure formation, e.g., cleistothecia [5]. Beside carbon sources, it was also of interest to know which nitrogen source affected development. Among several nitrogen sources examined, propylamine was found to influence development of *A. nidulans*. Therefore, in this study, it was examined whether propylamine influences development in *A. niger* and *A. oryzae*. As shown in Fig. 1, propylamine inhibited conidial head formation of *A. niger*.

*Corresponding author
Phone: 82-63-270-3340; Fax: 82-63-270-3345;
E-mail: chaeks@moak.chonbuk.ac.kr

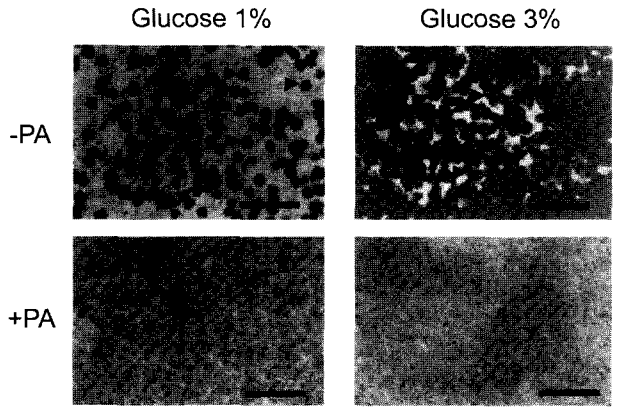

Fig. 1. Effect of propylamine on development of *Aspergillus niger*.

About 10^7 conidia were inoculated homogeneously on the whole plate of MM containing 1% or 3% glucose as well as no or 2% propylamine. The plates were incubated for 3 days and photographed. Arrowheads indicated conidial heads. The length of the scale bar is 1 mm. PA, propylamine.

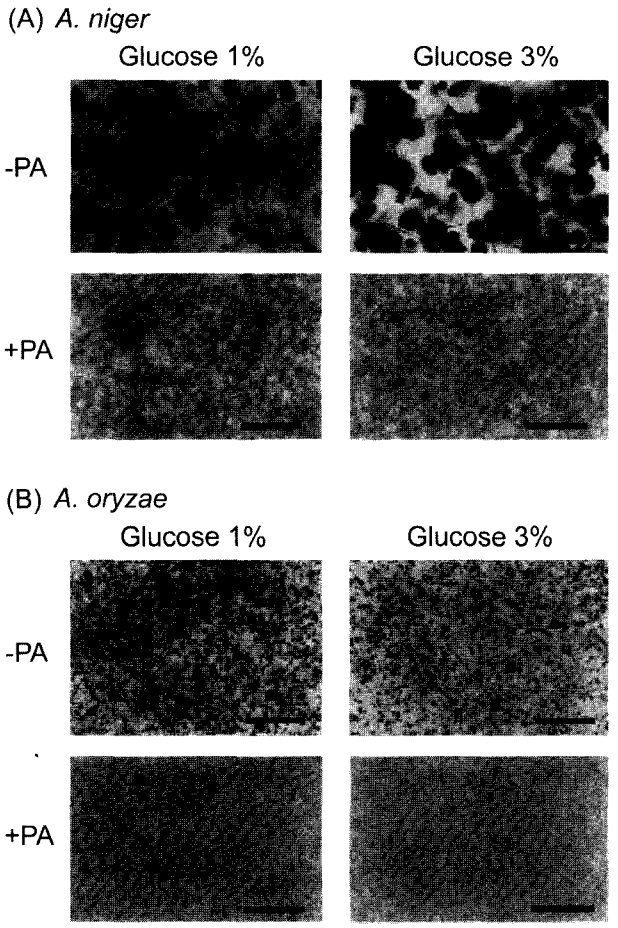

Fig. 2. Effect of propylamine in the presence of potassium chloride on development of *Aspergillus niger* and *Aspergillus oryzae*.

Fungi were grown on the same media as in Fig. 1, except with supplementation of 0.6 M potassium chloride. Arrowheads indicated conidial heads. The length of the scale bar is 1 mm. PA, propylamine.

Table 1. Inhibition of the radial growth of *A. niger* and *A. oryzae* by propylamine.

Strain	Glucose 1%		Glucose 3%	
	-PA[a]	+PA[b]	-PA	+PA
Aspergillus niger	1.9±0.1 (100%)	1.4±0.1 (73.7%)	2.2±0.1 (100%)	1.3±0.1 (59.1%)
Aspergillus oryzae	1.7±0.1 (100%)	1.2±0.1 (70.6%)	1.9±0.1 (100%)	1.3±0.1 (68.4%)

The equal numbers of conidia, 6×10^4 conidia in one microliter, were inoculated at the center of five plates, the plates were incubated for 3 days, and the diameters of colonies were measured. The value was the mean diameter in centimeter±standard deviation of colonies on five plates.

[a]In the absence of propylamine.

[b]In the presence of 2% propylamine.

A. niger did not form conidia in the presence of 1% glucose and 2% propylamine, while a few conidial heads of reduced size were formed in the presence of 3% glucose and 2% propylamine. However, the inhibition of conidial head formation of *A. oryzae* could not be observed, since *A. oryzae* formed very little conidial heads on MM supplemented with 1 or 3% glucose.

As reported previously [6, 10], the high concentration of a salt such as potassium chloride promotes conidial head formation of *A. nidulans* and *A. oryzae*. It was of great interest to find out whether potassium chloride at high concentration can suppress the propylamine effect, since propylamine seems to be antagonistic to potassium chloride. As shown in Fig. 2, the inhibitory effect of propylamine on conidial head formation of *A. niger* and *A. oryzae* was also observed even in the presence of potassium chloride, indicating that propylamine inhibited conidial head formation in these two fungi, and that potassium chloride could not suppress the propylamine effect.

Mycelia grow at the apical tips of mycelia and by branching in the middle of mycelia. Therefore, whether propylamine inhibited the apical growth was first investigated by measuring diameters of colonies formed in the presence and in the absence of propylamine. As shown in Table 1, diameters of colonies of *A. niger* and *A. oryzae* in the presence of propylamine were reduced by 41% and 32%, respectively, of those in the absence of propylamine, regardless of the concentration of glucose, clearly indicating that propylamine inhibited the apical growth of mycelia, although it was still not clear whether propylamine inhibits the mycelial branching.

The inhibitory effect of propylamine on development was observed even at the low concentration of 0.3% propylamine, and the growth inhibition became more severe when the concentration of propylamine was increased (data not shown). All of the results described in this study clearly demonstrated that propylamine inhibited conidial head formation and growth of *A. niger* and *A. oryzae*.

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