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Promotion of Asexual Development and Inhibition of Sexual Development of *Aspergillus nidulans* by Short-Chain Primary Amines

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Effects of short-chain primary amines on *Aspergillus nidulans* development were analyzed. Propylamine induced asexual development and inhibited sexual development. Even on medium containing lactose as the sole carbon source, on which little conidial heads are formed and sexual structures are formed preferentially, or when sexual development was induced, propylamine induced asexual development and inhibited sexual development. These effects of propylamine seemed to be due to accumulation of mRNA of the *brlA* gene, which has been identified as a positive regulator of asexual development, and due to the reduction of the *veA* mRNA level. The *veA* gene has been identified as an activator of sexual development and also as an inhibitor of asexual development. Other primary amines, methylamine and ethylamine, showed identical effects on development where short-chain primary amine also promoted asexual development and inhibited sexual development.

Key words: asexual development, *Aspergillus nidulans*, *brlA*, ethylamine, methylamine, propylamine, sexual development, *veA*

Aspergillus nidulans having two distinctive reproduction cycles, sexual reproduction and asexual reproduction, has been used as a model organism to study development (Adams *et al.*, 1998). In asexual development, a central regulatory pathway consisting of three genes, *brlA*, *abaA* and *wetA*, has been identified and sequential expression of them is required for formation of conidiophores and conidia (Adams *et al.*, 1988; Mirabito *et al.*, 1989). In sexual development, the *veA* gene has been proposed to act negatively in asexual development (Champe *et al.*, 1981; Yager 1992). However, the *veA* gene has been identified recently as a positive regulator of sexual development and also as a negative regulator of asexual development. A *veA*-overexpressor can form cleistothecia in a liquid culture or in the presence of a salt at high concentration under which conditions no or little cleistothecia, respectively, are formed in wild types. In addition, a veA-null mutant forms no sexual structures but forms more conidiophores than a wild type (Kim *et al.*, 2002).

Several environmental factors influencing the developmental processes have been identified. One of the environmental factors is the induction condition of sexual development with the simultaneous inhibition of asexual development (Jeong *et al.*, 2000; Han *et al.*, 2001). Other factors affecting sexual development are types and concentrations of carbon sources (Han *et al.*, 1990). When a higher concentration of glucose, such as 3%, or lactose is added as the sole carbon source, more sexual structures are formed, simultaneously with the inhibition of conidial head formation, than when 1% glucose is added (Han *et al.*, 1990). Nitrogen sources also affect *A. nidulans* development (Han *et al.*, 1990). Addition of casein hydrolysate leads to the formation of more sexual structures. During the examination of various nitrogen sources to identify which nitrogen source affects *A. nidulans* development, it has been found that propylamine influenced development. Therefore, in this study, effects of short-chain primary amines on development were analyzed, and it was found

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that such a short-chain primary amine had significant effects on development. Furthermore, it was also verified that effects of such short-chain primary amines on development were probably due to the increase and decrease in the transcript levels of the *brlA* gene and the *veA* gene, respectively.

A wild type strain of *A. nidulans*, FGSC (Fungal Genetics Stock Center, Kansas, KS) A4, was cultured at 37°C in minimal medium (MM), MM containing 0.1% casamino acid (MMCA), or complex medium (CM) (Pontecorve *et al.*, 1953). As a carbon source, 1% glucose, 3% glucose, or 1% lactose (MM-Lac) was added to MM. When necessary, 2% (w/v) propylamine, ethylamine or methylamine was added.

Propylamine promotes asexual development

Since addition of casein hydrolysate can influence development (Han *et al.*, 1990), it was of interest to know which nitrogen source affects development. Among several nitrogen sources analyzed, propylamine at 0.3~2% was found to influence development. As shown in Table 1, the effect of 2% propylamine on asexual development was not observed on MM supplemented with 1% glucose as the carbon source, since there were so many conidial heads on MM containing no propylamine. However, more conidial heads were formed in the presence of propylamine than in the absence of propylamine on MM containing 3% glucose where fewer conidial heads are formed than on MM containing 1% glucose, clearly indicating that propylamine promotes asexual development. To confirm further the promoting effect of propylamine on conidial head formation, the effect of propylamine was examined under two conditions where conidial head formation is inhibited. First, conidia were cultured on MM-Lac containing propylamine. Second, sexual development was induced. As shown in Fig. 1, more conidial heads were formed on MM-Lac in the presence of propylamine than in the absence of propylamine. When sexual development was induced, more conidial heads were formed in the presence of propylamine than in the absence of propylamine (Fig. 1). These results indicate that propylamine suppresses the inhibitory effect of lactose or induction of sexual development on conidial head formation, and raised a possibility that propylamine influences *brlA* and/or *veA* expression. Therefore, total RNA isolated from mycelia cultured on the surface of liquid CM for 14 h with or without 2% propylamine was hybridized with a *brlA*-specific probe and a *veA*-specific probe. The *veA*-specific probe was the 500 bp *Eco*RI/*Eco*RV-digested fragment of the *veA* gene (GenBank Accession number AF335465), and the *brlA*-specific probe was a 1.3 kb PCR-amplified fragment of the *brlA* gene (GenBank accession number M20631). The nucleotide sequences of the primer pair used for amplification of the *brlA*-specific probe DNA were 5-GGA TCC ATG CGA AAT CAG TCC AGC CT -3 (brlAF) and 5- AAG CTT TCA TTC ATC CCA GCC GTC CA -3 (brlAR). As shown in Fig. 2, propylamine caused accumulation of the *brlA* transcript while reducing the *veA* transcript level. This result suggests that promotion of conidial head formation by propylamine is due to accumulation of the *brlA* transcript, which was identified as *brlAβ*, and due to the reduction of the *veA* transcript level. An overexpressor of the *brlA* gene forms conidiophore-like structures in a liquid culture when *brlA* expression is induced by threonine (Adams *et al.*, 1988). Therefore, it is also very interesting to examine the effect of propylamine in a liquid culture, since propylamine seemed to have an identical effect as threonine on *brlA* expression. However, when propylamine was added to a liquid culture, we failed to observe conidiophore-like structures (result not shown).

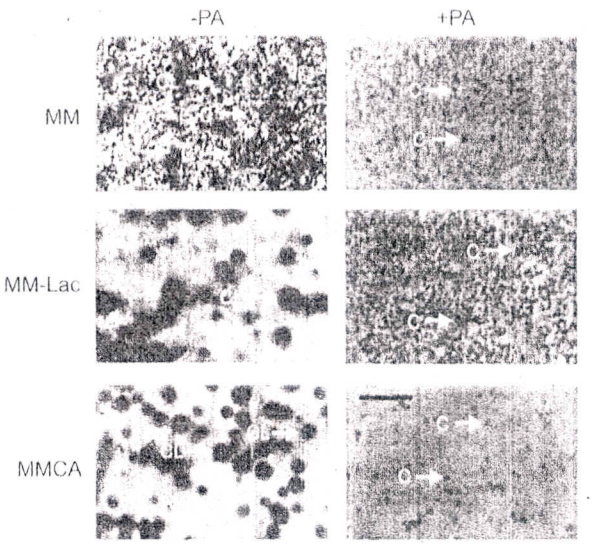

Fig. 1. Effects of propylamine on development of *A. nidulans* under conditions where sexual structures are formed preferentially. First, about 10^6 conidia were plated homogeneously on MM containing lactose (MM-Lac) as the sole carbon source, and incubated for 3 days. Second, about 10^6 conidia were plated homogeneously on MM containing no or 2% propylamine and incubated for 3 days (MM). Finally, mycelial balls obtained by culture of 10^6 conidia in liquid CM for 14 h were transferred on MMCA containing no or 2% propylamine, and plates were sealed with parafilm and aluminum foil for 20 h to induce sexual development (Jeong *et al.*, 2000). After removal of sealing, plates were incubated further for 2 days (MMCA). C, conidiophore; CL, cleistothecium; PA, propylamine. The length of the scale bar is 1 mm.

Propylamine inhibits sexual development

During the examination of the propylamine effect on asexual development, it was also found that propylamine inhibits sexual development, as shown in Table 1 and Fig. 1. Even when plates were incubated for up to 7 days, no sexual structures were formed in the presence of propylamine. The minimal concentration of propylamine required for the inhibition of sexual development was 0.5% (data not shown). The developmental phenotypes of mycelia

Table 1. Phenotypes of *A. nidulans* strains on various media in the absence and in the presence of propylamine[a]

Developmental structure	PA[b]	MM		MMCA[c]	MM-Lac[d]
		Glucose 1%	Glucose 3%		
Conidial heads	-	+++	+	++	+
	+	+++	+++	+++	+++
Cleistothecial initials	-	++	+++	++	++
	+	-	-	-	-

[a]Approximately 10^6 conidia were inoculated onto 40 ml of solid medium and incubated at 37°C for 4 days without induction of sexual development. The number of conidial heads was determined per 1 mm^2 area: +, <50; ++, 100 to 200; +++, >200. The number of cleistothecial initials was determined per 1 mm^2 area: –, 0; +, <5; ++, 5 to 10; +++, >10.
[b]2% propylamine added or not.
[c]Mycelial balls obtained by a culture of 10^6 conidia in liquid CM for 14 h were transferred on MMCA containing no or 2% propylamine, and the plates were sealed with parafilm and aluminum foil for 20 h to induce sexual development. After removal of sealing, the plates were incubated further for 2 days.
[d]Minimal medium containing 1% lactose as the sole carbon source.

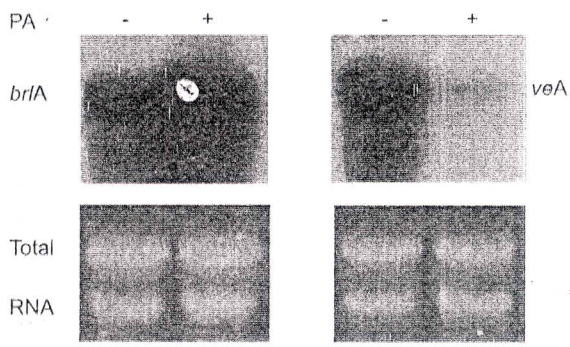

Fig. 2. Effect of propylamine on *veA* and *brlA* expressions. About 10^6 conidia were cultured on the surface of liquid CM for 14 h with or without 2% propylamine. Total RNA was isolated from the mycelia and hybridized (Chirgwin *et al.*, 1979; Sambrook *et al.*, 1989) with a *brlA*-specific or a *veA*-specific probe. PA, propylamine.

cultured under three different conditions where sexual structures are formed preferentially were examined in the presence of propylamine. These three conditions were on 3% glucose as a sole carbon source, on MM-Lac, or with the induction of sexual development by preventing an inoculated plate from illumination and gas exchange (Han *et al.*, 1990; Han *et al.*, 1994; Han *et al.*, 2001). First, even when 3% glucose was added as the carbon source, no sexual structures were formed in the presence of propylamine (Table 1). Second, if propylamine was present, no sexual structures was formed on MM-Lac (Table 1 and Fig. 1). Finally, even when sexual development was induced, no sexual structures were formed in the presence of propylamine as shown in Table 1 and Fig. 1. These results clearly indicate that propylamine inhibits sexual development even under conditions where sexual development is favored. In addition, the result that propylamine reduced the amount of the *veA* transcript, as shown in Fig. 2, suggests that the inhibition of sexual development by propylamine is due to the repression of *veA* expression.

Effects of other primary amines

To learn whether other primary amines affect development, effects of ethylamine and methylamine were analyzed. When ethylamine or methylamine was added in MM in addition to both a carbon source and a nitrogen source, such an amine compound showed identical effects to propylamine on development in that lots of conidial heads and no sexual structures were formed even when sexual development was induced (data not shown). These results indicate that a short-chain primary amine promotes asexual development and inhibits sexual development as is the case with propylamine. At present, it is still uncertain by what mechanism such a short-chain primary amine affects the transcript levels of the *brlA* gene and the *veA* gene and, in turn, development. However, what seems to be clear is that effects of primary amines are not probably due to their potential to be used as carbon sources and/or nitrogen sources, since *A. nidulans* forms more sexual structures and fewer conidial heads when a higher concentration of a carbon source is provided (Han *et al.*, 1990).

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