Interference of *Phytophthora* sexual reproduction

The *Phytophthora* mating hormone α2 is an antagonist of the counterhormone α1

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The crop destroyer *Phytophthora* uses mating hormones α1 and α2 to commence its sexual reproduction. The α1-induced sexual reproduction of the A2 mating type was unexpectedly found to be interfered with by the counterhormone α2 that the A2 type itself produces to induce the sexual reproduction of the A1 type. A plausible mechanism is proposed based on structure-activity relationships.

Key words: plant pathogen; *Phytophthora*; mating hormone; sexual reproduction

Phytophthora, a genus of filamentous fungus-like microorganisms, is well known as a plant pathogen. It has been reported to cause devastating diseases worldwide, such as potato late blight and sudden oak death.[1,2] The heterothallic species of *Phytophthora*, consist of two mating types, A1 and A2 that, when paired, produce sexual spores called oospores, which are a persistent form of progeny that can survive in extreme environments for a prolonged period.[3] This feature of its sexual reproduction makes it difficult to control this plant pathogen in agricultural fields. The sexual reproduction of *Phytophthora* is regulated by mating hormones. The mating hormone produced by the A1 mating type, designated α1, can induce the sexual reproduction of the counter A2 mating type, whereas the sexual reproduction of the A1 mating type can only be induced by the hormone α2 produced by the A2 mating type.[4] The structures of α1 (**1**)[5,6] and α2 (**2**)[7], as well as their biosynthetic pathway,[7] have been resolved by our group (Fig. 1). The sexual reproduction of *Phytophthora* rarely occurs in the field but is easily observed in the laboratory. When the A1 and A2 mating types are co-cultured across a membrane filter (to avoid direct contact), both types produce oospores within a few days. In the course of such mating experiments, we observed that the A1-induced oospore formation of the A2 type was suppressed in the presence of α2 (**2**), which is originally a product of the A2 type. This phenomenon was also observed when the A1 type was replaced with α1 (**1**). These results suggest that the A1-induced sexual reproduction of the A2 type is caused by **1** and interfered with by **2**, so it appears that **2** is an antagonist of **1**. We herein report a detailed analysis of this unexpected phenomenon using a paper disk hormone assay.

The hormonal activity of α1 (**1**) was evaluated by a previously described method.[5] Briefly, **1** was applied to a paper disk at a dose of 30 ng/disk, placed on a colony of *P. nicotianae* ATCC 38606 (A2 mating type) and incubated at 25 °C for 3 days. An area of the colony around the paper disk was cut out and the number of oospores that formed in the entire area was counted. The effect of α2 (**2**) was evaluated by the simultaneous administration of **2** at doses of 10, 30, and 100 ng/disk. Hormone α2 (**2**) dose-dependently suppressed the α1-induced oospore formation of *P. nicotianae* (Fig. 2A). To obtain further information on this phenomenon, its structure-activity relationship was examined using α1 and α2 derivatives **3**–**12** (Fig. 1), which were prepared previously.[8] All the results including those for **2** are presented in Fig. 2B. Three acetates, 1-*O*-acetyl α2 (**3**), 16-*O*-acetyl α2 (**4**), and 1,16-di-*O*-acetyl α2 (**5**), showed inhibitory activity against the α1 activity. These activities were compared by plotting the relative activity against the molar ratio of **2** (or a derivative) to **1** (Fig. 2C), which indicated that the three acetate derivatives showed comparable activity to natural **2** and an approximately equivalent amount of these molecules can inhibit 50% of the α1 activity. Interestingly, no inhibitory activity was observed for 11-*O*-acetyl α2 (**6**), suggesting that of the three hydroxy groups of α2 (**2**), the 11-OH group is essential for this antagonistic activity. Since no significant inhibitory activity was observed for monocarbamates of α2 (**7** and **8**) unlike the monoacetates **3** and **4**, we hypothesized that this group might be too large to fit the ligand-binding pocket of the α1 receptor to interfere with α1 function. Another possibility, the biotransformation of the acetates to active α2 (**2**), could be excluded because no significant difference in activity was observed between **2** and the acetates despite the possibility that the concentration of **2** generated from an acetate is lower than that in the direct use of **2**. The C2-C3 double bond of **2** was found to be another essential substructure because the dihydro derivative **9** showed no inhibitory effect despite the presence of the 11-OH group and the absence of the huge carbamoyl group. Besides the α2 derivatives, three α1 derivatives (**10**-**12**) were tested and no-inhibitory activity was observed, supporting the importance of the double bond of α2 (**2**).

In the *Phytophthora* sexual reproduction system, both hormones originated from

phytol, namely α2 (**2**) is biosynthesized from phytol by A2 mating type and then converted to α1 (**1**) by A1 mating type (Fig. 3A).[7] The hormones α1 (**1**) and α2 (**2**) exclusively induce the sexual reproduction of A2 and A1 mating types, respectively. Since this suggests the presence of the hormone receptors that strictly recognize the corresponding hormone, the result described above is surprising to us. The hormonal activity of α1 (**1**) is likely to be triggered by its recognition by the α1 receptor ($R^{\alpha1}$) expressed in the A2 mating type, and several structural features essential for the α1 function have been reported.[8] We propose a model to explain the above-mentioned interference of the α1-mediated sexual reproduction by α2 (**2**) (Fig. 3B). The middle part (including two essential stereo centers of C7 and C11) of **1** is recognized by the recognition site of the receptor $R^{\alpha1}$, and the left portion (including another essential α-methyl-branching ketone at C3-C4) of **1** binds to the active site of $R^{\alpha1}$, leading to the sexual reproduction of the A2 mating type (Fig. 3B, upper chart). When α2 (**2**) is present in the same time, the middle part of **2** is also recognized by the recognition site of $R^{\alpha1}$, because this part is structurally shared with **1** (Fig. 3B, lower chart). On the other hand, the left part of **2** is not capable of activating the active site of $R^{\alpha1}$, probably due to the absence of the ketone functionality, although this part retains the ability to bind to the active site. The C2-C3 double bond of **2** could play a role comparable to the ketone of **1** at least for the receptor binding ability. This antagonistic binding of **2** results in blocking **1** to induce the sexual reproduction of the A2 mating type. This may be a unique system inherent in the A2 mating type to avoid unprofitable sexual transformation by masking its receptor with its own mating hormone **2**.

In summary, we found the *Phytophthora* mating hormone α2 (**2**) to be an antagonist of the counter mating hormone α1 (**1**). Some acetate derivatives of **2** also showed an inhibitory effect comparable to **2**. Based on the structure-activity relationship, we propose a plausible mechanism for this phenomenon, namely that **2** might competitively bind to the α1 receptor without activation of the sexual reproduction system of the A2 mating type. These results imply that α2-related compounds antagonistic to the sexual reproduction inducer α1 (**1**) give a hint for

developing candidates to control the *Phytophthora* sexual reproduction that may yield more virulent offspring.

Author contribution

L.Z. and M.O. designed the research and wrote the manuscript. L.Z. performed the experiments and analyzed the data. A.Y. synthesize the derivatives.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Figure legends

Fig. 1. Structures of α hormones and their derivatives

Fig. 2. Interference of α1-induced sexual reproduction.

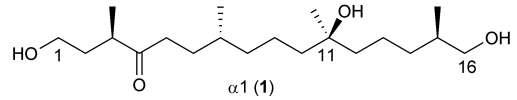
Notes: (A) Photomicrographs of *P. nicotianae* ATCC 38606 (A2 mating type). No oospore formation was observed in the absence of α1 (**1**) (left). The α1-induced oospore formation (30 ng/disk of **1**, center) was suppressed by the simultaneous administration of 100 ng/disk of **2** (right). (B) Graphical representation indicating the effect of **2** and α hormone derivatives (**3-12**) on α1-induced oospore formation. α1 (30 ng/disk) was administered on a colony of *P. nicotianae* ATCC 38606 (A2 mating type) with the indicated amount of an α hormone derivative. At 3 d post-inoculation, the number of oospores that formed around the paper disk was counted and three of five replicates were used after removal of the maximum and minimum values. The control data are essential in each experiment because they are usually sensitive to mycelial stocks used. (C) The relative oospore formation was plotted against the molar ratio of the compounds **2**–**5** to **1**. The curves were drawn by sigmoid curve fitting.

Fig. 3. A model for interference of α1-mediated hormonal activity by α2 in *Phytophthora* sexual reproduction system.

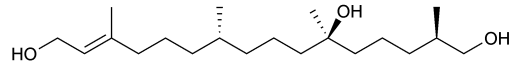
Notes: (A) Outline of *Phytophthora* sexual reproduction system. Two mating types, A1 and A2, secrete the mating hormones α1 (**1**) and α2 (**2**), respectively (solid lines), to promote the sexual reproduction of the counter mating type (dotted lines). α2 (**2**) was found to interfere with the α1-promoted sexual reproduction of the A2 type (solid line marked with "?"). (B) A schematic model for interference of α1-mediated hormonal activity by α2. α1 (**1**) binds to the receptor ($R^{\alpha 1}$) expressed in the A2 mating type to promote its sexual reproduction; the substituents and asymmetric centers indicated with asterisks are essential for the hormonal activity [8] (upper chart). The ligand **1** in the complex is replaced by α2 (**2**), in which the double bond may tightly

bind to and block the active unit of the receptor $R^{\alpha 1}$, interfering with the α1-induced sexual reproduction (lower chart).

Phytophthora mating hormones:

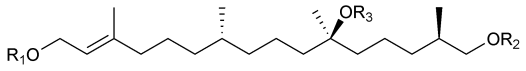


α1 (**1**)

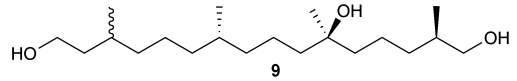


α2 (**2**)

Derivatives of α2 (**2**):



3: R_1 = Ac, R_2 = R_3 = H
4: R_1 = R_3 = H, R_2 = Ac
5: R_1 = R_2 = Ac, R_3 = H
6: R_1 = R_2 = H, R_3 = Ac
7: R_1 = *p*-BrC_6H_4NHCO, R_2 = R_3 = H
8: R_1 = R_3 = H, R_2 = *p*-BrC_6H_4NHCO



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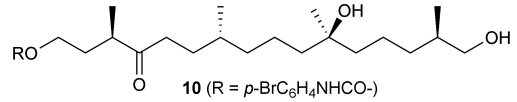
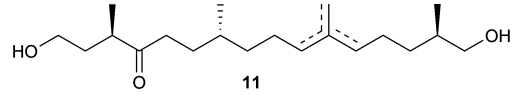
Derivatives of α1 (**1**) (hormonally inactive):



10 (R = *p*-BrC_6H_4NHCO-)



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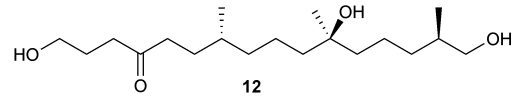

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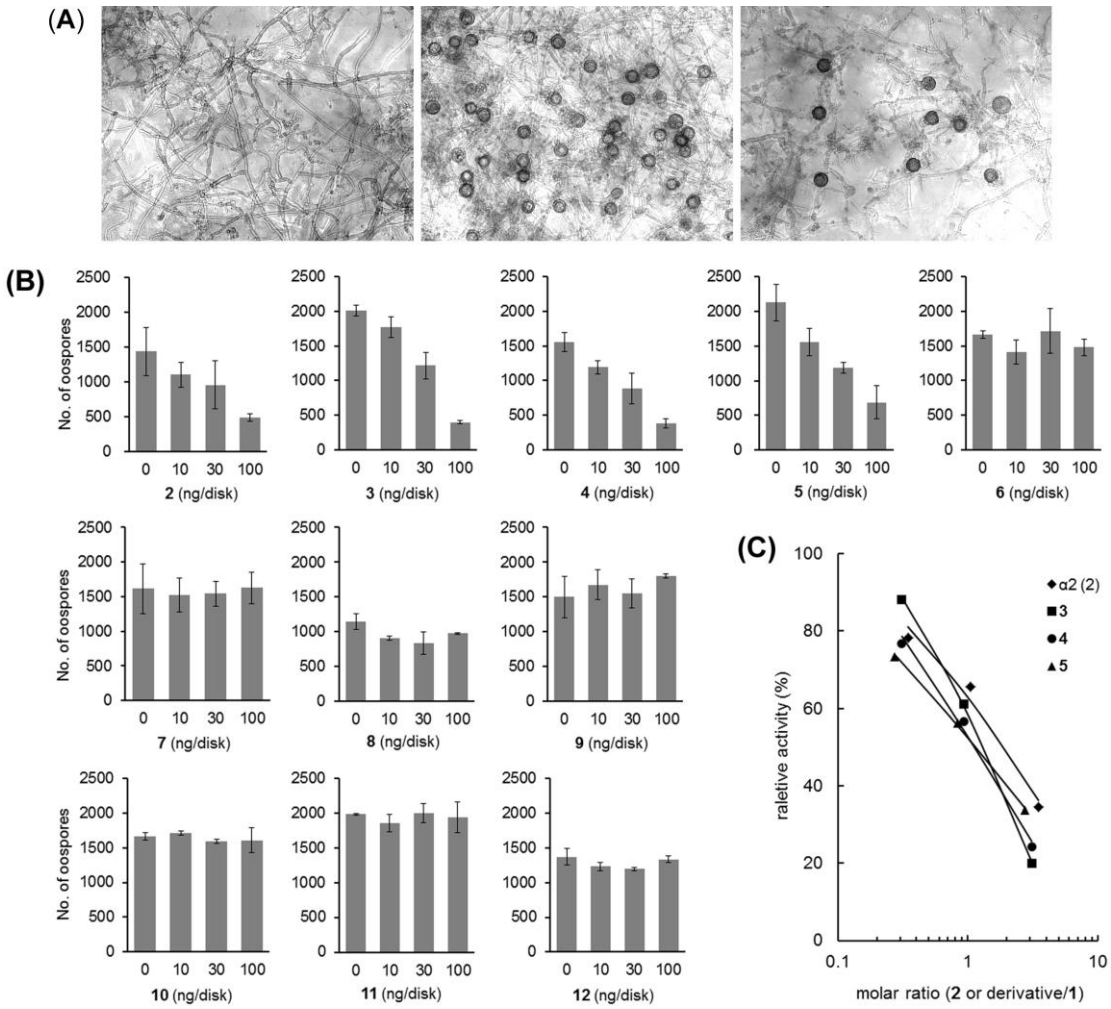
Figure 1



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2 Figure 2

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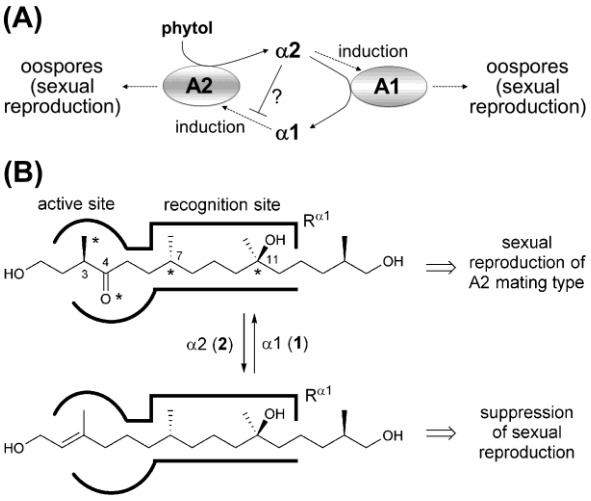

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6 Figure 3