Short Communication

The Response of Wild Type Male Gametes of Allomyces to Sirenin

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The hermaphroditic, haploid, sexual generation of the watermolds Allomyces macrogynus and A. arbuscula bear orange male gametangia and colorless female gametangia which discharge motile male and female gametes into an ambient liquid environment (1, 8). The female gametes, beginning prior to their release from the gametangia, secrete sirenin, a sperm-attractant (3, 4), whose structure was recently established (6) and which is depicted in figure 1. The production of sirenin (7) was done with the almost entirely female isolate #F-1 (3) derived from a cross between A. macrogynus (n=28) and A. arbuscula (n=16) and the bioassay, during the time it was needed, with an almost entirely male isolate derived from the same cross. These particular hybrids have not been studied cytologically or genetically but the chromosome behavior of various crosses between the parents has been described (2).

The purpose of this communication is to show that male gametes from the parent species do respond chemotactically to sirenin which was synthesized by the female hybrid strain. At the same time, the experiments raise the possibility that there may be species-specific sirenins.

The gametophytic generation of A. macrogynus, strain Burma 3 and A. arbuscula, strain Ceylon 1 were grown on Difco Yps nutrient agar plates. Gametangia were scraped from these plates and placed in DS solution (3) in a small glass petri dish. Male gametangia were then picked up in a micro pipette and 500 placed in 0.6 ml of DS in containers of a proper size to take the assay apparatus (fig 2). Discharge of the gametes was then allowed to take place for 3 hours. The male gametangia must be separated in approximately 40 minutes, the time between immersion in DS solution and the first emergence of gametes. In this period it was possible to select 1500 gametangia thus per-

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1 The original cross used isolate Burma 1Da (1). Professor Ralph Emerson has informed me that isolate Burma 3 was collected in the same locality as Burma 1Da and that both isolates are almost certainly completely equivalent.

Fig. 1. The structure of sirenin. C_{12}H_{24}O_{2}. MW 236. Bond representation: \(\cdots\), in the plane of the paper; \(\uparrow\), coming out of the plane toward the reader; \(\downarrow\), going back of the plane away from the reader. Two sites of potential isomerism occur. In the attachments to the apex of the cyclopropyl ring, it is the methyl that projects out over the cyclohexyl ring rather than the isohexyl side chain. The attachment of the hydroxymethyl group to the double bond in the isohexenyl side chain is trans to the ethylene group.

Fig. 2. The apparatus for the bioassay. The glass ring is 10 mm deep and 22 mm in diameter. In practice, 12 of these are cemented to a glass plate for ease of manipulation under the microscope. The apparatus will be described in detail elsewhere (5). The basic feature is that through the center of it is a hole one-fourth inch in diameter and three-eighths inch long which is closed at its lower end by a piece of dialyzing tubing held in place by a small dental rubber band. The sirenin solutions are placed in the well above the membrane. A sperm suspension is placed in the ring up to a level just above the membrane. The membrane itself is one-eighth inch from the floor of the ring. When the well is full and covered with a cover slip, the sperm can be seen with a low power microscope and the number per unit area of membrane counted.
The characteristic of sireniin produced by arbuscula can be to arbuscula. The number of gametes attached to 0.47 mm² of membrane after 60 minutes is reported. Each figure is the average of 3 separate determinations.

<table>
<thead>
<tr>
<th>Species</th>
<th>Male gametes on membrane</th>
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<tr>
<td><em>A. macrogyinus</em></td>
<td>Expt. 1: 113, Expt. 2: 235</td>
</tr>
<tr>
<td><em>A. arbuscula</em></td>
<td>Expt. 1: 4, Expt. 2: 25</td>
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The response of the male gametes of *A. macrogyinus* is vigorous. In actual numbers it is of the same order of magnitude previously observed for M-4 male strain gametes to the same concentration of sireniin. In contrast, the male gametes of *A. arbuscula* are poorly reactive. The results show that sireniin produced by a hybrid female strain is highly effective with at least one of the parent species. The response of the *A. arbuscula* gametes is far less than can be accounted for by the fact that their concentration is half that of the *A. macrogyinus* gametes. Thus, if strain M-4 male gametes at 40,000 and 20,000 per ml are assayed against the same concentration of sireniin the lower concentration of gametes gives a reading equal to 60 % of the higher concentration whereas the response of *A. arbuscula* to sireniin (table I) is only 4 to 11 % of that of *A. macrogyinus*.

The poor response of *A. arbuscula* male gametes can be explained at present in 2 possible ways. First is the assumption that each species has its own characteristic sireniin. This would mean that the F-1 female strain produces a sireniin that is the same or similar to that of the *A. macrogyinus* parent. Alternatively, it can be assumed that both species produce the same sireniin but that the male gametes of *A. arbuscula* are much less sensitive to sireniin.

Experiments of the type described are difficult to do. The small number of replicates that can be assembled in any one experiment and the relatively low concentration of gametes that can be attained are serious impediments to critical work. Efforts are now in progress to produce sireniin with the wild-type species and to obtain male gametes on a mass scale from the hermaphroditic parent species for assay purposes. If several technical problems can be solved, it will be possible to find out if there are species-specific sireniins.

**Acknowledgments**

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**Literature Cited**