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OLFACTORY RESPONSES OF PHYCOMYCES BLAKESLEEANUS

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SUMMARY. Upon exposure to low levels of various volatile compounds such as n-heptanol, methanol, $CHCl_3$, mercaptoheptane, etc., the sporangiophore of Phycomyces blakesleeanus responds with a transient and reproducible decrease in its elongation rate. All 22 volatile substances tested (except H_2O) elicited negative responses. The amplitude of the responses depends on the compound and its concentration. A characteristic concentration, required for 50% inhibition, correlates remarkable well with the human olfactory threshold (coefficient of correlation $r = 0.89$ ($P < 0.001$)). Perhaps some process in olfaction is common to this fungus and higher systems.

Receptor cells of the olfactory epithelium are responsible for the transduction of olfactory signals in higher vertebrates. Olfactants are thought to change the membrane potentials of these cells but the molecular mechanism is unknown. Model systems for chemical transduction include bacterial chemotaxis (1), algal and fungal sexual attraction (2), protozoan chemotaxis, especially paramecium excitability (3), slime mold chemotaxis (4), and the newly described electrical response of Nitella (5). None of these, however, involve volatile chemical signaling.

Clearly volatile substances affect all exposed living systems. CO_2 and SO_2 in air influences the transpiration rate of higher plants. Recently it has been shown that aldehydes and terpenoids at concentrations as low as 1.4 ppm decrease transpiration in wheat (6). A major difficulty in elucidating the mechanism of these responses seems to be the lack of a good model system. Such a system should provide a large and measurable behavioral response which can be correlated with other concurrent physiological responses, belongs to an organism convenient to obtain and to grow, relates easily to other systems, and is amenable to biochemical genetic analysis.

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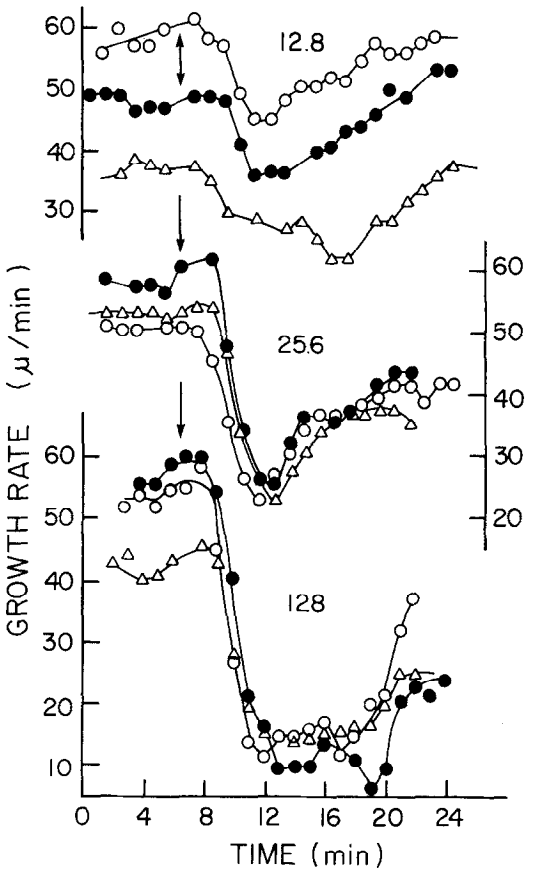
A recent publication of ours argues that the avoidance response of Phycomyces blakesleeanus is mediated by a volatile self-emitted effector molecule which enhances its growth rate (7). In the course of attempting to identify this gas, we discovered that the sporangiophore of Phycomyces is exquisitely sensitive to several volatile chemicals. Moreover, a relationship exists with human olfaction. Although some of the gases tested are emitted by Phycomyces, none can be the avoidance gas since all elicit a negative growth response.

MATERIALS AND METHODS

Phycomyces was grown on 4% potato dextrose agar (Difco) supplemented with 0.25 ppm thiamine HCl in 3 cm^3 vials. All sporangiophores but the specimen to be tested were plucked and discarded. The specimen, 2.5 - 3.5 cm tall, was enclosed in a glass container, 7.5 x 7.5 x 2.5 cm, made from microscope slides, with a piece of filter paper affixed to one side and 0.5 mm slit in the glass top on that side. The system was completely closed except for the slit. The temperature was 24° ± 1C. The sporangiophore was allowed to adapt for 30 min and then the rate of growth was measured utilizing a telescope equipped with a Filar micrometer. The average growth rate was about 50 $\mu \cdot min^{-1}$. Before introduction, chemicals were mixed with a inert adjuvent perfluorotributylamine. 100 µl of the mixture containing the appropriately diluted odorant was delivered by a syringe to the filter paper where it rapidly volatilized. We calculate that a constant concentration is reached within the house in 7 sec. The rate of effusion through the slit was measured by injecting [^{14}C]-toluene into the house, withdrawing 1 cm^3 samples at times 0, 2, 5, 10, 20, 40, and 60 min, and determining radioactivity using a scintillation counter. The half-time observed was 49 min. Since the response to odorants was generally over in 15-25 min, we infer that effusion has no significant effect on our conclusions.

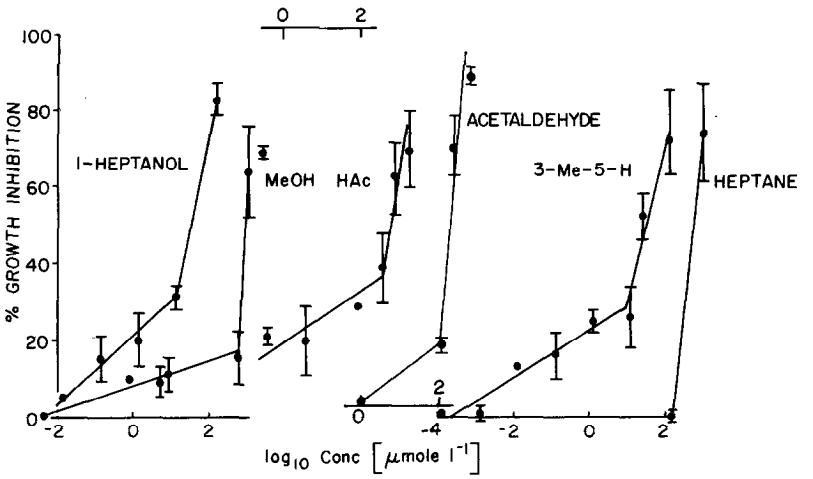
RESULTS

Experiments were done blindly: observers were given random experiments to perform; they were unaware of expected results and were not advised on possible interpretations. Figure 1 is part of a series of experiments using 3-methyl-5-heptanone. One and a half to 3 min after the injection the growth rate of the sporangiophore starts to decrease; at about 5 min, a minimum is reached. For moderate stimulation the growth rate quickly returns to normal. However, if the diminution of growth rate is greater than about 60-70%, the minimum is attained at the same time (Fig. 1, bottom), but growth may continue at slow rates for as long as one hour before returning to normal. No



1. The negative growth responses of Phycomyces blakesleeanus to several concentrations of 3-methyl-5-heptanone in perfluorotributylamine. Numbers denote final concentration in $\mu mole \cdot l^{-1}$. The arrows indicate the time of injection. The nine experiments shown were done on separate sporangiophores. Injection of perfluorotributylamine resulted in no response ($\pm$ 5%) in numerous controls.

doses in the concentration range considered here appeared toxic, for all specimens eventually returned to a near normal growth rate. More than 400 olfactory experiments were performed, each on a separate sporangiophore. All concentrations were at least a factor of 5 below vapor saturation levels under the experimental conditions. For several volatile organic compounds, the plot of the percent growth rate diminution vs. log concentration [$\mu mole\ l^{-1}$] appears biphasic (Fig. 2). The biphasic nature of the response is unlike the electrical responses of Nitella (5), however, it should be recalled that the



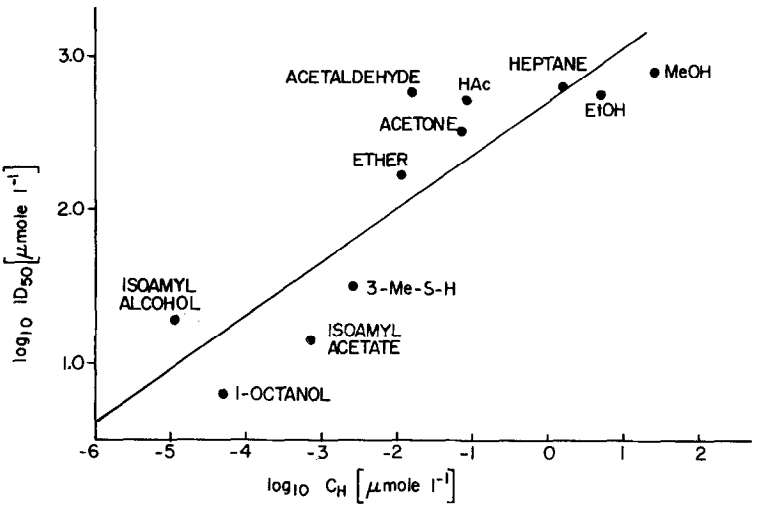
2. Growth inhibition as a function of the logarithm of the olfactant concentration in μmole·l^{-1}. Six examples are shown. Flags at data points represent two standard deviations, three to six measurements are averaged. Points with no error bars indicate 1-2 determinations. Each measurement is with a different sporangiophore. 3-Me-5-H is 3-methyl-5-heptanone. Concentrations required for 50% inhibition (ID_{50}) were interpolated using a least square analysis of curves such as these. Note that in the worst case (n-heptane) such analysis leads to only a 5% maximum error in log ID_{50}.

observable parameter in Phycomyces is a physiological behavior which may have been transduced nonlinearly from a linear primary process. Thresholds are difficult to evaluate precisely because of the shallowness of the curves. Most are of the order of 100 pmol·l^{-1} (10^{11} molecules cm^{-3}).

To characterize the stimulus-response behavior, the concentrations of various effectors required to produce a 50% transient attenuation of growth (ID_{50}) were determined. A plot of log ID_{50} vs. the log of the human olfactory threshold, C_H [μmole·l^{-1}], is linear through several orders of magnitude (Fig. 3). Linear regression analysis yields the equation log ID_{50} = 0.351 log C_H + 2.74. The linear correlation coefficient, r, is 0.89 (N = 11, P < 0.001).

DISCUSSION

Olfaction in Phycomyces and in humans thus appear to be related. Such relationships between measured responses to olfactants and human olfactory



3. Log ID_{50} versus the log of the human olfactory threshold, log C_H, both in $\mu mole \cdot l^{-1}$. The equation log ID_{50} = .351 log C_H + 2.74 was derived by linear regression. The linear correlation coefficient r = +0.89 (N=11) corresponds to P (r, N) < 0.001, where P (r, N) is the probability that if two variables are uncorrelated, a random sample of N observations will yield a correlation coefficient greater than $|r|$. Values of human threshold are from reference (8), except 1-octanol (9) and heptane (10). Note that values in (8) are low by 10^2. For compounds tried and not shown here, either insufficient data was gathered or human threshold data could not be found. Every relevant experimental determination was used.

threshold are not unique to Phycomyces, even among the lower eukaryotes. Ueda and Kobatake (11) report similar correlations in Tetrahymena, Physarum, and Nitella. These correlations provide strong evidence that from protist to Homo sapiens olfactory transduction includes at least one very similar process.

ID_{50}'s of Phycomyces do not correlate well with dielectric constant, dipole moment or several other physical parameters. They do bear a relationship to the positive surface tension increments induced by odorants in phospholipid monolayers as measured by Koyama and Kurihana (12). These authors argue that olfaction is mediated by olfactants adsorbed to or intercalating into the cell membrane phospholipid bilayers of receptor cells. However, the possibility remains that the correlated parameters may be more appropriately related to a more fundamental physical parameter not yet deciphered. Never-

theless, any attempt to elucidate human olfaction must also explain olfaction in Phycomyces.

An important caveat has been pointed out by Beets (13). Human threshold measurements at the nares do not account for the differential "trapping" of odorific compounds in the nasal cavity. A truer picture of the response to a given olfactant at the receptor, he feels, would be obtained by measuring concentration and receptor potentials at the receptor cells. Unfortunately very little such data exists and current studies all refer to threshold at the nares.

While the ID_{50} values are all well above the human threshold concentrations, Phycomyces threshold concentrations are as much as 10^3 - 10^4 lower. The response of Phycomyces to several odorants exhibits one form of adaptation. For example, stimulation by heptanethiol elicited an inhibition response of 31%; after the growth returned to normal, a second injection of the same concentration yielded only a 10% response. A strong light growth response occurs even at the chemically induced minimum growth rate. Mutants of Phycomyces which have aberrant light and avoidance responses are available and some have been assigned to complementation groups. The mutant strain C149, which is termed "stiff" because it fails to respond to these physiological stimuli, responds identically as wild type to several olfactory stimuli.

In agreement with our previous report (7), water vapor gave no response up to 100% relative humidity. We tested 11 additional volatile substances (14) and all elicited a negative growth response; no volatile compound produced a positive growth response or no response in our laboratory. (Ethylene has been reported to give a slight positive response (15)). The fluorocarbon perfluorotributylamine elicited no response presumable because it is nonvolatile at 24°.

The major advantage of Phycomyces as a model system is that a behavioral response to olfactants is easily and reproducible measured in an organism

quite amenable to biophysical, biochemical and genetic manipulation. The stalk of the sporangiophore is nonseptate, essentially a giant single cell several cm tall. All transduction processes must occur in the single cell. Electrical measurements of Phycomyces have already been reported in another context (16); obviously a requirement now is to determine longitudinal and transverse membrane potentials and impedance changes after chemical stimulation. Squeezed Phycomyces cytoplasm forms a protoplasmic drop which becomes bound by a membranous material, much like the protoplasmic drops of Nitella, whose electrical properties have been studied (5, 17). (However, Phycomyces will eventually regenerate a complete plant from the drop (18, 19).

The latency, duration and amplitude of the olfactory responses are much the same as those of other responses in Phycomyces; hence, the observations are consistent with a common transduction pathway. The availability of characterized mutants in the general transduction system should be useful in understanding the olfactory response of Phycomyces. The addition of another sensory system to the known repertoire of Phycomyces should be an important aid in determining the ordering and the biochemical components of the transduction pathway. In many respects the light stimulus - response system of Phycomyces is the best understood of all eukaryote sensory systems (19, 20). The olfactory sense appears to be a promising complement.

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