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pH and acid phosphatase determinations after growth of Aspergillus nidulans on solid medium

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Abstract

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The pH of the medium after growth of more than 1000 A. nidulans colonies (wild-type and several mutants grown on non-buffered solid medium, adjusted to pH 6.5 and supplemented with different carbon and nitrogen sources) was estimated by introducing a pH indicator into the agar through the fungal colony. The pH values obtained, whose precision depends on the indicator used, revealed nearly the same pH profile as measured with a pH meter after fungal growth in liquid medium i.e., when the pabaA1 strain grows on solid minimal medium supplemented with acetate as carbon source, the pH of the medium reaches 8, but when the strain is grown on solid medium supplemented with glucose as carbon source and ammonium chloride as nitrogen source the pH drops to 5. When nitrate or nitrite was the nitrogen source the pH was about 7, but with 10 mM ammonium (+)-tartrate the pH was about 6. These results were expected because agar has no buffering capacity and should not affect the changes occurring in extracellular pH during the growth of A. nidulans on solid medium. Indeed, superimposing curves were obtained when 20 ml of minimal medium (Cove, 1966. Biochim. Biophys. Acta 113:51-56) with or without 1.3% agar (after its disaggregation and twice diluted in both cases with decationized water) was titrated with 10 mM HCl. However, although A. nidulans grows well on non-buffered solid minimal medium supplemented with 10 mM ammonium chloride as the nitrogen source, this is not the case when the fungus is grown in liquid minimal medium supplemented with this same nitrogen source, where the pH drops to about 3 and does not permit mycelial growth. Because tartaric acid, added as the ammonium salt, has no buffering capacity at pH values above 5.2 (the two pKa equal to 2.2 and 4.2), we may assume that its presence in both solid and liquid medium somehow inhibits proton production and/or extrusion and permits mycelial growth, a role probably played by agar as well. It is accepted that A. nidulans utilizes both agar and tartrate as carbon sources through the pathways of acetate metabolism i.e., as sources of acetyl-CoA (Payton et al. 1976. J. Gen. Microbiol. 94:228-233). It also appears that, as already proposed for N. crassa (Han et al. 1987. Curr. Genet. 11:521-527), acetyl-CoA triggers in A. nidulans a series of adaptive events including alkalinization of the culture medium (A. Rossi and H.N. Arst Jr., unpublished results). This signaling appears to occur even in the presence of preferential carbon sources (Han et al. 1987. Curr. Genet. 11:521-527) and may permit fungal growth on ammonium chloride as the nitrogen source.

To measure levels of acid phosphatase excreted by the pabaA1 strain of A. nidulans, 26 colonies grown on complete medium were transferred with a wire replicator to phosphate-free solid minimal medium (10 ml/plate) supplemented with 10 mM ammonium (+)-tartrate as nitrogen source and the pH adjusted to 5.0 or 6.5. After growth for 16, 24 and 48 hours at 37°C, the solid medium was homogenized with 5 ml of decationized water for 10 minutes with stirring and centrifuged at 24,000 g for 15 min. Enzyme assays of the supernatant were carried out in 6 mM p-nitrophenyl phosphate, 100 mM maleate and 2 mM EDTA at 37°C, pH 6.0 (Caddick and Arst 1986. Genet. Res. Camb. 47:83-91). Tables I and II show nearly the same profile of acid phosphatase excretion regardless of whether the measurement was made after incubation on solid or liquid medium, i.e., a burst of excretion was observed at pH 5.0 and a lag at 6.5. Thus, if we consider that the same enzyme form is being excreted at both pH values, it appears that the prompt response to extracellular pH is a function of the velocity of excretion and not of the number of copies of the enzyme molecule synthesized. For this reason, stimulation of acid phosphatase secretion by a decrease in the pH of the medium, as first demonstrated in N. crassa (Nahas et al. 1982. J. Gen. Microbiol. 128:2017-2021), is better visualized when the colonies of A. nidulans are stained for this enzyme after growth on solid medium for 16 hours (Table I) or when the mycelium is incubated for two to three hours in liquid medium (Table II), both media lacking phosphate (Caddick et al. 1986. Mol. Gen. Genet. 203:346-353). However, if for example, the colonies of A. nidulans were stained after growth for 48 hours on solid medium or incubated in liquid medium for 6 to 8 hours, both media lacking phosphate (Tables I and II), we would have to assume that an increase in the pH of the medium would increase acid phosphatase excretion, therefore not insuring a regulatory mechanism favorable to the fungus.

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Table I. Phosphatase activity and colony diameter of the pabaA1 strain of A. nidulans as a function of growth pH and incubation time at 37°C.

Growth pH	5.0			6.5		
Incubation time[a] (hours)	16	24	48	16	24	48
phosphatase activity[b]	145 ± 10	165 ± 10	180 ± 15	35 ± 8	98± 11	280 ± 12
colony diameter (mm)	3 ± 1	8 ± 1	18 ± 2	3 ± 1	7 ± 1	19 ± 1

a - 10 ml of non-buffered solid minimal medium, lacking phosphate, was supplemented with 10 mM ammonium (+)-tartrate as nitrogen source and the pH adjusted to 5.0 or 6.5

b - acid phosphatase activities are expressed in nMol p-nitrophenol liberated at 37°C/plate/minute. The standard deviations were calculated from 4 replicates.

Table II. Effect of extracellular pH and of incubation time on the secretion of acid phophatase in A. nidulans

incubation time (hours)	incubation pH			
	5.0		6.5	
	phosphatase activity[a]	mycelial dry weight (mg)	phosphatase activity[a]	mycelial dry weight (mg)
2	6 ± 1	332 ± 25	2 ± 0.5	280 ± 22
4	22 ± 3	412 ± 20	18 ± 2	380 ± 30
6	26 ± 2	456 ± 30	40 ± 3	472 ± 28
8	21 ± 1	568 ± 32	42 ± 4	592 ± 42
10	20 ± 1	636 ± 40	48 ± 2	596 ± 30

a - Mycelia of the pabaA1 strain were grown for 12 hours at 37°C, pH 6.5 in minimal medium (1×10^9 conidia/100 ml medium were shaken at 160 rpm in 500 ml erlenmeyer flasks) and then transferred to the same medium lacking phosphate, and incubated further for the period and at the pH indicated. Enzyme assays of the filtered medium were carried out as described in the text. Activities are expressed in nMol p-nitrophenol liberated at 37°C/mg mycelial dry weight/min. The standard deviations were calculated from 3 replicates.

From the results described here, it is clear that measurement of extracellular pH changes and of the levels of acid phosphatase secreted on solid medium give reproducible results. This permits a comparison of the excretory patterns of any enzyme during growth on solid medium. Also, these results allow us to propose that the characteristics of the pH regulatory system of A. nidulans (Caddick et al. 1986. Mol. Gen. Genet. 203:346-353; MacRae et al. 1988. Gene 71:339-348) appear to differ from those observed in N. crassa (Nahas et al. 1982. J. Gen. Microbiol. 128:2017-2021).

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