

They found the C<sub>15</sub>-saturated branched chain acid as a most abundant acid (24–49%). Smaller amount of acids were C<sub>16</sub>, C<sub>15</sub> and C<sub>17</sub>-saturated branched-chain acid. Also the normal saturated fatty acids (C<sub>12</sub>, C<sub>14</sub>, C<sub>18</sub> and C<sub>20</sub>) as well as unsaturated acids (C<sub>16:1</sub>, C<sub>18:1</sub>) were detected, but only at low levels. Mycolic acids i.e. long-chain 3-hydroxycarboxylic acids having a long alkyl branch on C-2, formed in some corynebacteria, were absent in *P. acnes*<sup>21</sup>. Our GC-MS analysis confirms the previous pharmacological finding that the bioactive compounds in *P. acnes* are prostaglandin-like. Furthermore, we have been able to present evidence about the structure of the common part of these molecules, namely the aliphatic chain. In spite of the fact that this chain is a general feature of the prostaglandin family, it was not possible to find any other member of this group with the same spectral pattern for the remaining part of the molecule. These data agree with recent proposal of Kuehl et al.<sup>22</sup> that, in contrast to early concepts, other prostanoids (i.e. products derived from PGG<sub>2</sub>) than classical prostaglandins play a key role in the etiology of inflammation.

- 1 The competent technical assistance of Mr N. Engström and Mr G. A. de Vogel is gratefully acknowledged.
- 2 S. Abrahamsson, L. Hellgren and J. Vincent, *Experientia* 34, 1446 (1978).
- 3 S. Abrahamsson, N. Engström, L. Hellgren and J. Vincent, in manuscript.

- 4 E. Berlin, L. Hellgren, O. Thulesius and J. Vincent, *Experientia* 36, 197 (1980).
- 5 L. Hellgren, G. Selstam and J. Vincent, *Experientia* 35, 1096 (1979).
- 6 J. Belsheim, A. Dalen, H. Gnarpe, L. Hellgren, O.-J. Iversen and J. Vincent, *Experientia* 35, 1587 (1979).
- 7 L. Hellgren, B. Lindblom, J. Vincent and L. Wilhelmsson, *Experientia* 35, 1298 (1979).
- 8 S. Abrahamsson, R. J. Gryglewski, L. Hellgren, J. Splawinski, J. Vincent and B. Wojtaszek, *Experientia* 37, 164 (1981).
- 9 S. Abrahamsson, K. Gréen, L. Hellgren and J. Vincent, *Experientia* 36, 58 (1980).
- 10 K. C. Srivastava and V. K. S. Shukla, *Z. analyt. Chem.* 293, 45 (1978).
- 11 J. M. Bailey, E. W. Bryant, S. J. Feinmark and A. N. Makheja, *Prostaglandins* 13, 479 (1977).
- 12 R. C. Murphy, S. Hammarström and B. Samuelsson, *Proc. natl. Acad. Sci. USA* 76, 4275 (1979).
- 13 J. G. A. M. Raaijmakers, *J. Chromat.* 138, 355 (1977).
- 14 W. E. C. Moore and E. P. Cato, *J. Bact.* 85, 870 (1963).
- 15 Z. Strápatzaki-Cocovini, *Annls. Inst. Pasteur, Paris* 93, 647 (1957).
- 16 K. Takizawa, *J. Derm.* 4, 193 (1977).
- 17 L. Larsson, P.-A. Mårdh and G. Odham, *J. clin. Microbiol.* 7, 23 (1978).
- 18 C. W. Moss and W. B. Cherry, *J. Bact.* 95, 241 (1968).
- 19 C. W. Moss, V. R. Dowell, Jr, D. Farschtchi, L. J. Raines and W. B. Cherry, *J. Bact.* 97, 561 (1969).
- 20 C. W. Moss, V. R. Dowell, Jr, V. J. Lewis and M. A. Schekter, *J. Bact.* 94, 1300 (1967).
- 21 M. Goodfellow, M. D. Collins and D. E. Minnikin, *J. gen. Microbiol.* 96, 351 (1976).
- 22 F. A. Kuehl, Jr, J. L. Humes, G. C. Beveridge, C. G. Van Arman and R. W. Egan, *Inflammation* 2, 285 (1977).

## Low pH in fungal bud initials

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**Summary.** Quenching of fluorescence of the pH probe, 4-methylesculetin, in bud initials of *Allomyces* hyphae and yeast (*Saccharomyces*) vegetative cells confirms the cytoplasmic acidity (pH not more than 5) in such amitochondrial structures.

Budding is a common morphogenetic process, continuous in filamentous fungi and cyclic in yeasts<sup>1</sup>.

We have recently shown by semivital staining with pH indicators that the ultimate, budding tips of filamentous fungi are more acidic than the subapical, mitochondria-rich zone<sup>2</sup>. As this suggested a decreasing pH gradient toward the apices of elongating hyphae, we have attempted to confirm such results using the more refined technique of a fluorescence pH probe. Gerson and Burton<sup>3</sup> have already used with success the fluorescence of 4-methylesculetin (6,7-dihydroxy-4-methylcoumarin) for ascertaining pH in moving plasmodia of the slime mold *Physarum*. We thought that the wide outgrowing apices, budding laterally below differentiated zoosporangia of *Allomyces*, as well as the emergent buds from vegetative cells of *Saccharomyces*, would be especially fitted for an extension of our previous work.

The *Allomyces arbuscula* cultures were obtained from germinating zoospores which produce young mycelia after 24 h of culture at 25°C in liquid glucose-casein hydrolysate-yeast extract (GCY) medium<sup>4</sup>. Bunches of apically differentiated hyphae, starting to bud new hyphal branches laterally, were transferred on quartz slides into drops of fresh medium saturated with 4-methylesculetin (Senn Chemicals). The fluorescence of this reagent fades below

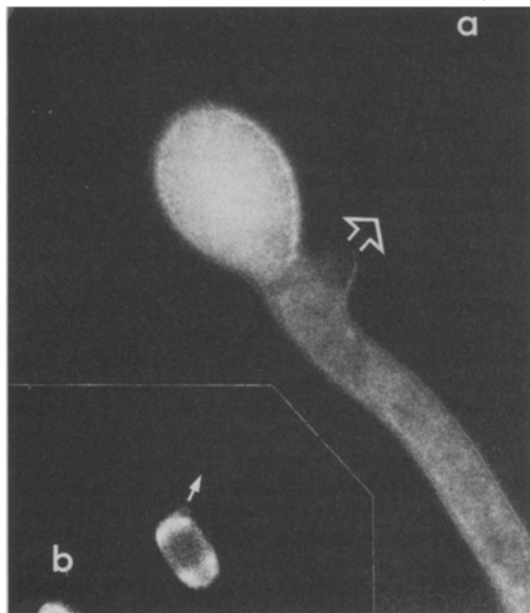
5.5<sup>3</sup> and is therefore practically extinct in the GCY medium (pH 5.2).

Using exciting UV-light at 350 nm and filters for emitted fluorescent light in the band of 420–450 nm on an O-lux Leitz microscope<sup>5</sup>, we observed a vivid greenish-grey fluorescence in the differentiating zoosporangia (relatively alkaline pH) and their supporting hyphae, in sharp contrast to the total lack of fluorescence in the young apices which budded laterally (full extinction in their tip, fig. a). Such quenching of fluorescence of 4-methylesculetin in outgrowing apices, and especially at their ultimate tips indicates that their cytoplasmic (cytogel?) pH is not above 5.0. Alizarin yellow S (sulphonated) stained similar apices orange yellow, a color indicative of a pH-value also around 5; this is in full agreement with previous results obtained with bromocresol purple<sup>2</sup>. In comparison, the subapical cytoplasm and especially its mitochondrial rodlets, stained pinkish violet with alizarin, were most fluorescent with 4-methylesculetin (pH more than 6.0).

From these data with outgrowing hyphae of *Allomyces*, we can tentatively surmise the existence of a decreasing pH gradient from the subapical, mitochondrial zone of the hyphae (average pH at least 6) to the apical hyaloplasm, a presumed cytogel<sup>2</sup>, at a pH close to 5.

In a 2nd set of experiments, buds emerging cyclically from

yeast cells were studied. Such cells were obtained by subcultivating a wine strain of *Saccharomyces cerevisiae* for 3 h at 20°C on slants of malt (2%)-peptone (2%) agar medium (pH 6.2). A small loopful of the actively budding population of yeast cells was dispersed into an aqueous drop of the reagent used. The greenish-blue staining with bromocresol green ( $10^{-4}$ , w/v), dark red with chlorophenol red ( $10^{-4}$ , w/v) and pinkish violet with alizarin yellow S ( $10^{-4}$ , w/v) observed in the peripheral cytoplasm of non-budding cells and budding mother cells are all in agreement with the overall intracellular pH value of 5.8, previously determined by physico-chemical methods in resting baker's yeast<sup>6</sup>. However, the yellow staining of young buds with all indicators indicates an internal pH not higher than 5.0 in such emerging structures.



Quenched fluorescence of the pH probe 4-methylesculetin in the bud initials of: *a* hypha of *Allomyces arbuscula* outgrowing laterally (directional arrow) below an apically differentiating zoosporangium; *b* vegetative cell of *Saccharomyces cerevisiae* at the emergence stage (directional arrow on polarized outgrowth).  $\times 1000$ .

When budding yeast cells were plunged into a drop of saturated 4-methylesculetin in distilled water, mother cells exhibited a vivid greenish-grey fluorescence in their cytoplasmic 'shoulders' while the bud initials remained fully extinct (fig. b). Only larger buds, just penetrated by mitochondria<sup>7</sup>, showed significant fluorescence.

That the lack of observable fluorescence in the bud initials is essentially due to their low pH and not to insufficient concentration of the probe in such small structures can reasonably be assumed based on the observation, facilitated by rolling cells, of some fluorescence in the narrow collar transition between the vividly fluorescing 'shoulders' of mother cells and their extinct buds. As for the possible selective segregation into young buds of quenching compounds absorbing light at the excitation or emission wavelength, its effects should be minimized by the overwhelming concentration of fluorescent probe available to the cells in the saturated drops. Assuming therefore that the lack of observable fluorescence in the bud initials is essentially a pH effect, the internal pH of the buds can be estimated at no more than 5.

An understanding of the origin of the increased acidity found in outgrowing buds might involve our recently proposed concept of a randomly occurring event of positioning of mitochondria initiating vectorial dissipation of their extruded protons toward facing plasmalemma<sup>8</sup>. At this consequently polarized site (functionally, while electrically depolarized?), a proton sink would thus be created and self-entrained by further drainage of the protons through the plasmalemma which finally bulges with its acid-plasticized wall, into an outgrowing amitochondrial bud.

- 1 G. Turian, in: The Fungal Spore, chapt. 16, p. 715. Ed. D.J. Weber and W.M. Hess. Wiley-Interscience, New York 1976.
- 2 G. Turian, *Experientia* 35, 1164 (1979).
- 3 D.F. Gerson and A.C. Burton, *J. Cell Physiol.* 91, 297 (1977).
- 4 M. Ojha and G. Turian, *J. gen. Microbiol.* 122, 263 (1981).
- 5 Thanks are due to Dr F. Barja (Laboratoire d'Anatomie et Physiologie comparées) for technical advice.
- 6 E.J. Conway and M. Downey, *Biochem. J.* 47, 355 (1950).
- 7 Ph. Matile, H. Moor and C.F. Robinow, in: The Yeasts, chapt. 6, p. 222. Ed. A.H. Rose and J.S. Harrison. Academic Press, London and New York 1969.
- 8 G. Turian, *Ber. schweiz. bot. Ges.* 203 (1980).

## Adenosine 3',5'-cyclic monophosphate levels in maize roots<sup>1</sup>

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**Summary.** Endogenous levels of adenosine 3',5'-cyclic monophosphate (cAMP) in maize (cv. LG 11) root cells, grown in light and dark conditions, were found to be 309 and 387 pmoles/g of fresh tissue respectively.

The occurrence and physiological role of adenosine 3',5'-cyclic monophosphate (cAMP) in bacteria<sup>3</sup> and mammals<sup>4</sup> have been well established. Despite numerous studies, the presence of cAMP in plants remains uncertain and controversial<sup>5,6</sup>. However, it has been found recently that cAMP does exist in wide varieties of plant species, such as *Phaseolus vulgaris*<sup>7,8</sup>, *Lolium multiflorum*<sup>9</sup>, *Funaria hygrometrica*<sup>10</sup>, *Ochromonas malhamensis*<sup>11</sup> and *Zea mays*<sup>12</sup>.

The aim of the present work is to examine the presence of cAMP in the growing roots of *Zea mays*, the elongation of which being clearly regulated by a hormone balance<sup>13</sup>.

Caryopses of *Zea mays* L. (cv. LG 11) were grown in darkness at 22°C<sup>14</sup>. After germination, seedlings were kept in the dark or in white light (Sylvania 220 V/40 W;  $1.84 \pm 0.12 \text{ Wm}^{-2}$  at the root level<sup>15</sup>). When the primary roots reached  $12 \pm 1 \text{ mm}$  length, they were excised on an