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Detachment of *Polytomella agilis* flagella induced by cell crowding

Aggregations of *Polytomella agilis* occur in cultures with population densities of about 200,000 to 1.5 million cells/ml (Gittleson and Jahn 1968). When these cultures are placed in containers with a low surface area to volume ratio such as burettes the cells accumulate at the bottom in densities as high as 278 million cells/ml (Gittleson, Woodruff and Hoover 1970). This mass accumulation produces cell crowding which affects the geometric design of aggregation (Gittleson and Ressler 1972). Cell crowding also brings about detachment of flagella, changes in cell body shape and increases in cell volume which are described in this report.

**Methods**

Experimental organism and growth medium. *P. agilis* (Dölein, 1916) was originally obtained from the Indiana Algal Collection, Indiana University, Bloomington. The growth medium consisted of 0.1 g sodium acetate, 0.2 g yeast extract and 0.1 g tryptone per 100 ml of glass-distilled water. This medium was autoclaved at 121°C for 20 min at a pressure of 16 p.s.i. Stock cultures were maintained in 125 by 20 mm screw-cap test tubes and grown in 1000 ml batches in 2000 ml Erlenmeyer flasks for experimentation. Temperature ranged from 20 to 22°C. Glasware was washed in Alconox, rinsed in tapwater 10 times, rinsed twice in dilute nitric acid to remove soap residue and finally rinsed with glass distilled water three times.

Experimental procedure. The preparation of isolated flagella was accomplished by the method of mass accumulation previously described (Gittleson, Woodruff and Hoover 1970). In this study, ultra-high population densities of *P. agilis* (about 210 million cells/ml compared to about 1.5 million cells/ml in the growing culture) were obtained by transferring 48–53 h cultures to 500 ml burettes. The cells which accumulated at the bottom of the burette in 15 min were withdrawn through the stopcock and placed in embryological watch glasses. After standing two hours the isolated flagella and abnormal cells were studied. Cell counts and cell volumes were measured using a Coulter counter model B and volume distribution plotter model H. Volume calibration was obtained with pollen of paper mulberry (diameter 12–13 µm). Isoton (Coulter Inc.) was used as a diluent. No significant changes in volume of *P. agilis* were associated with Isoton. Photomicrographs were taken through a Zeiss phase contrast microscope using Kodak 35 mm Plus-X film.
Results

The effect of cell crowding on flagella. A major effect of cell crowding after 2 h is the detachment of large numbers of flagella from the cell bodies (Pl. I 1). An average number of 42,000 flagella/ml was counted with the Coulter counter. These isolated flagella range from 7.0 to 8.0 µm in length. An oval-shaped basal body was a prominent feature (Pl. I 2). The basal body appeared translucent except for an opaque band extending between two nodes. When this opaque band could be viewed on the upper surface of the basal body, the basal body appeared coin-shaped with the opaque band running along the edge. Some of the isolated flagella continued to beat and those that became attached to the slide or coverslip by the basal body continued to undulate.

The effect of cell crowding on the cell body. Normally \textit{P. agilis} is slightly elliptical in shape with an average anterior to posterior length of 10 µm. Cell crowding caused swelling and distortion of the cell bodies. After 12 h of crowding the average anterior to posterior length increased to 17.5 µm. Cell volume increased an average of 10% and many cells ultimately ruptured.

Discussion

A number of investigators have reported how flagella and cilia may be detached from cells by chemical and physical treatment. These studies have been reviewed by Blum 1971 who noted that invariably cilia and flagella detach between the end of the basal body and the beginning of the flagellar shaft. In contrast, \textit{P. agilis} under conditions of extreme crowding lose their flagella with basal body intact. The mechanism of detachment appears to involve high degrees of physical stress exerted on the flagella by adjacent cell bodies. There is also the possibility of a chemical effect of metabolites which might occur in extraordinary concentrations in such a dense population of cells. The changes in cell shape and cell volume after many hours suggest that there are metabolic changes. The question still remains whether \textit{P. agilis} which have lost their entire flagellar apparatus including basal body are able to regenerate new flagella.

Summary

Crowding of \textit{Polytomella agilis} into cell densities of 210 million cells/ml induced detachment of flagella. The isolated flagellum consists of a flagellar shaft and a basal body. The coin-shaped basal body is translucent except for an opaque band running along the edge. Cell size and volume increase under the conditions of this experiment.
RESUMÉ

Le concentration de la *Polytomella agilis* jusqu'à la densité de 210 millions de cellules/ml provoque le détachement des flagelles. Un flagellum détaché en plus de la partie libre contient son corps basal. Le corps basal est discoïde et transparent à l'exception d'une bande opaque qui borde sa circonférence. Sous les conditions de cet expériment les dimensions et le volume des cellules augmentent.

REFERENCES


EXPLANATION OF PLATE I

1: Photomicrograph of isolated flagella of *Polytomella agilis*
2: Diagram of an isolated flagellum viewed through oil immersion objective
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