AN IMPROVED METHOD FOR CHARACTERIZATION OF CITRATE PRODUCTION BY CONIDIA OF ASPERGILLUS NIGER

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SUMMARY : A modification of a method is presented, which enables the characterization of a given conidial material of Aspergillus niger with respect to its capacity to produce citric acid in submerged culture. The procedure can be applied to selection experiments as well as to counteract degeneration during strain maintenance.

INTRODUCTION :

Citric acid is produced almost exclusively by fermentation using selected strains of Aspergillus niger [4]. These industrial mutants are selected by extensive mutation-selection programs, and the maintenance of such organisms has proved a problem. Upon longer storage the conidial material shows 'degeneration' (i.e. inferior citric acid synthesis). The reasons for this are still unclear. Since selected mutants often have reduced activity, this is mostly explained by selective advantage of more vigorous back mutants accompanying successive transfers [2].

To counteract this decay in productivity the strain must be frequently reisolated. In the case of citric acid fermentation this requires a simple and specific method for assessing the citric acid production capacity of conidia

during reisolation. Since procedures involving liquid media are more time consuming, methods based on direct analysis of the conidial material would be preferred.

Such methods are scarcely found in the literature. A method based on colonial growth of single conidia on nutrient soaked paper sheets has been described by James, Rubbo and Gardner [1. Citric acid was detected by means of an acid-base indicator. However, this method exhibited some disadvantages: the acid zones were only partially due to citric acid accumulation and sometimes consisted largely of mineral acids. Moreover characterization of conidia was only possible with strains of low productivity.

These difficulties could be overcome when the method was subjected to some modifications. The present paper shows that the modified procedure proves a useful tool in the characterization of conidial material during reisolation or screening processes.

DESCRIPTION OF THE METHOD : For the preparation of paper disks circular sheets of Chromatographic paper (Macherey and Nagel, MN 866, 2 mm) with a diameter of 120 mm were placed in glass petri dishes and sterilized. After cooling they were soaked with 23 ml of sterile Shu-Johnson medium

Throughout these experiments Aspergillus niger ATCC 11 414 [6] or Aspergillus niger W, a wild strain from the collection of the authors' institute, were used. Immediately before use, conidial suspensions were prepared according to [3] and diluted to 2 X 10 conidia per milliliter. Inoculations were made by means of a slowly operating peristaltic pump. Amounts of 0.5 *µl* (containing one conidium) could be applied when the paper was lightly touched with the tip of a capillary tube (0.7 mm diameter) Usually 12 - 13 spots were applied on to one sheet. The papers were then incubated for 1.15 hours at 30°C.

Before the assay of citric acid,replica cultures were made.

This was best done by pressing the paper upon another sheet prepared as described above. This procedure transferred conidia but also mycelial fragments.

Paper disks were dried for two hours at 100 C. Charring was avoided. Citric acid was assayed by spraying the plates with 4 % (w/v) p-dimethyl-aminobenzaldehyde in acetic acid anhydride [5] . After heating the paper between a glass and an aluminum plate (with holes for vapours) for one minute at 140 C, a purple colour was obtained indicating citric acid. The reaction is very sensitive (1 µg citric acid on paper) and not interferred with by mineral acids or other organic acids commonly produced by Aspergillus niger.

The acid producing capacity of different colonies was determined by dividing the diameter of the acid zone by the diameter of the colonies. The figure so obtained is called the 'acid unitage[1] (AU-value) according to [1] .

RESULTS AND DISCUSSION

By means of this modified method it was possible to select higher producing conidia from a low producing population, as well as counteract degeneration, by selectively transferring only conidia of high AU-values. A standard experiment for each type is given in Fig. 1 and *2*_. Details are given in the legends of the figures. Provided that the environmental conditions are kept constant, a reasonable correlation between the AU-values and the citric acid producing capacity could be demonstrated (Fig. 3). Although the method appears to be limited to a certain range of AU-values, it was possible to evaluate much higher citric acid yielding capacities as compared to the original technique.

As the method of paper culture technique is widely applicable, it may be of general interest to workers with filamentous organisms, because it can save time and space. The precision of the method depends mainly on the procedure used for the assay of the desired product.

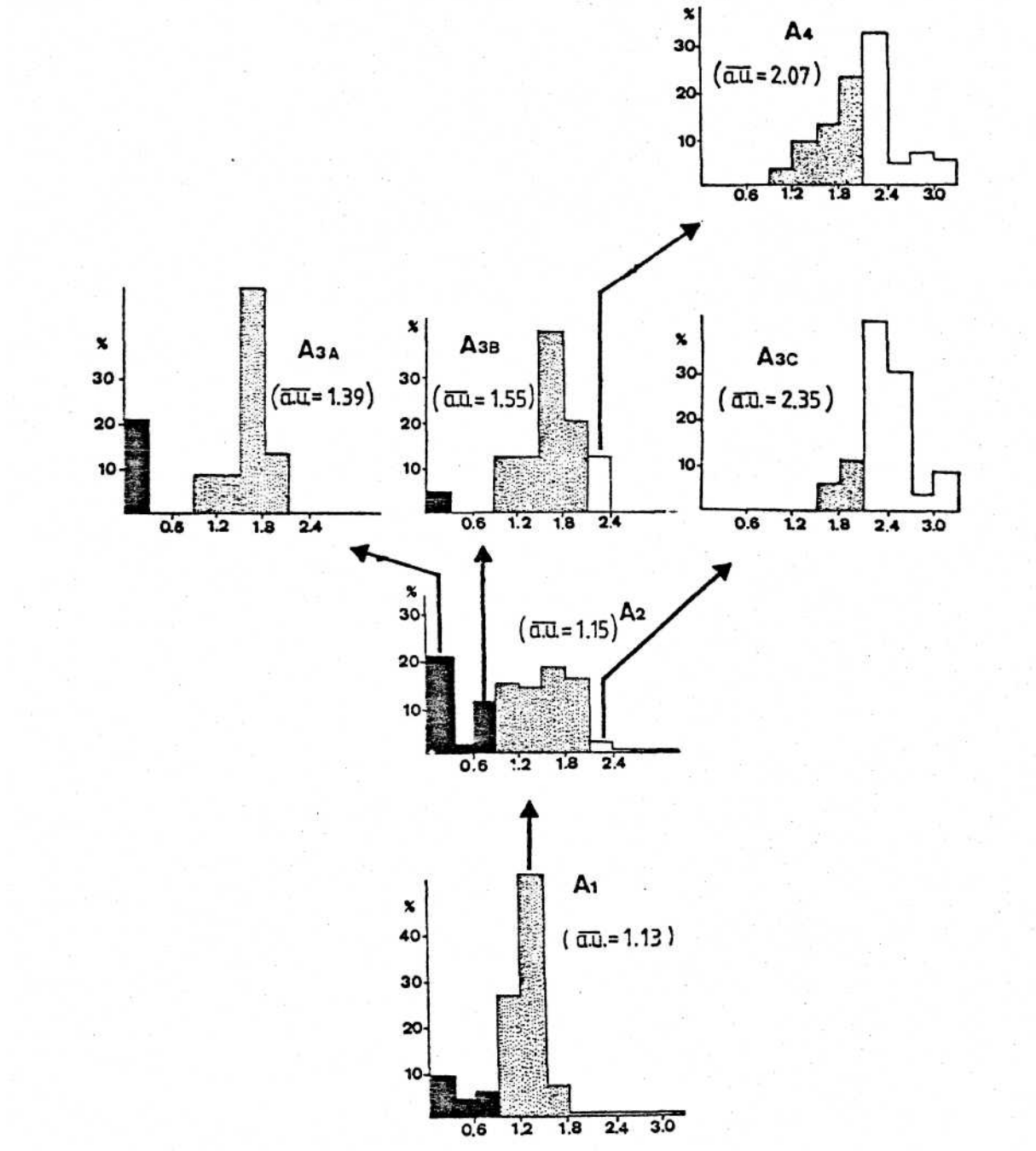

Figure 1

Selection experiment showing the respective distribution of acid unitages within a population of conidia. The origins of arrows indicate the AU-range of the cultures used as an inoculum for the next population to be characterized.Values in brackets indicate the mean AU-value of the population.

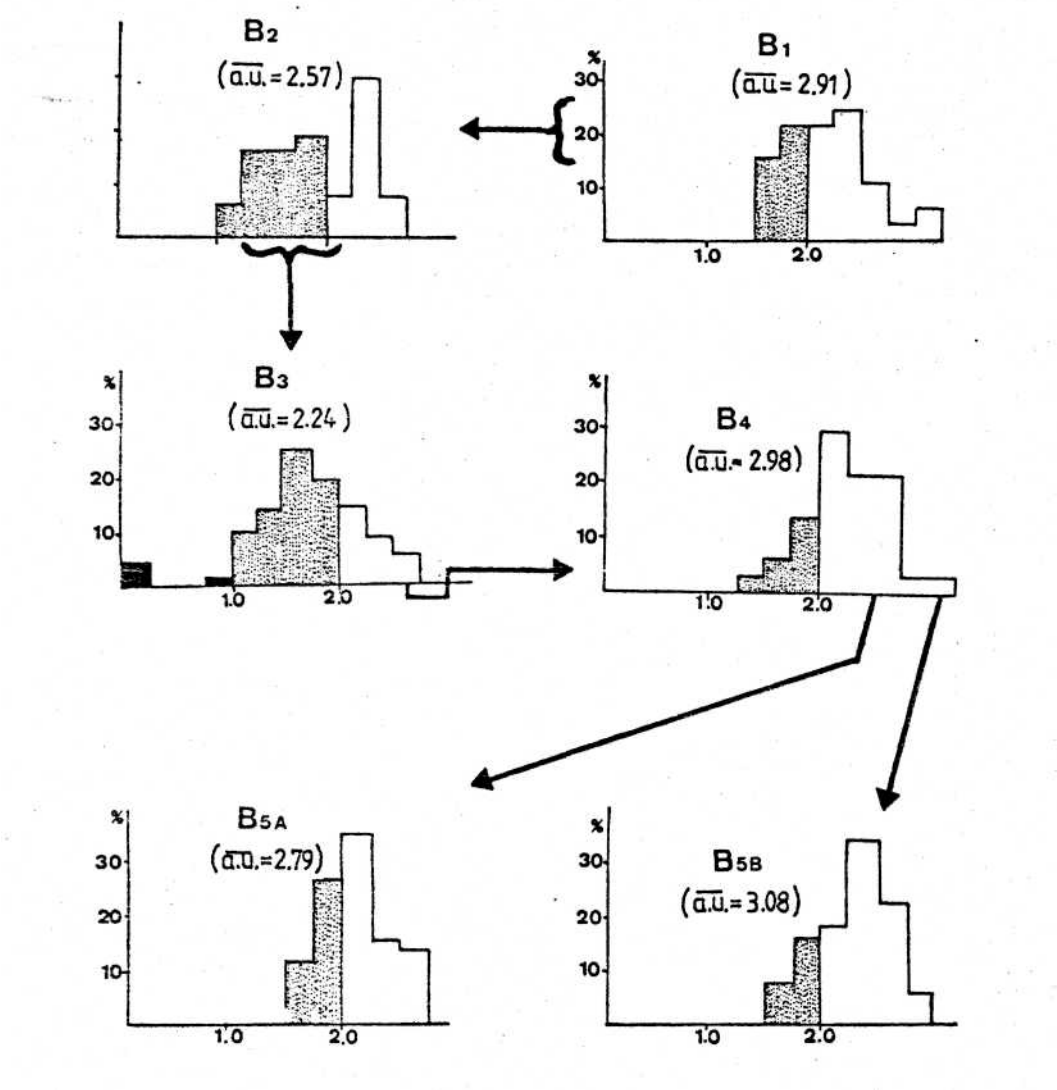

Figure 2

Experiment showing the distribution of acid unitages during the course of successively transferring a producing culture. B1 - B3 indicates that the decay in productivity is due to an increase in the percentuai distribution of populations with lower AU— values.The restoration of the original capacity is possible when conidia of high AU-values only are transferred as described in legend to Figure 1. The origin of the arrows refers to **the** AU-range of the culture used for the next cultivation.

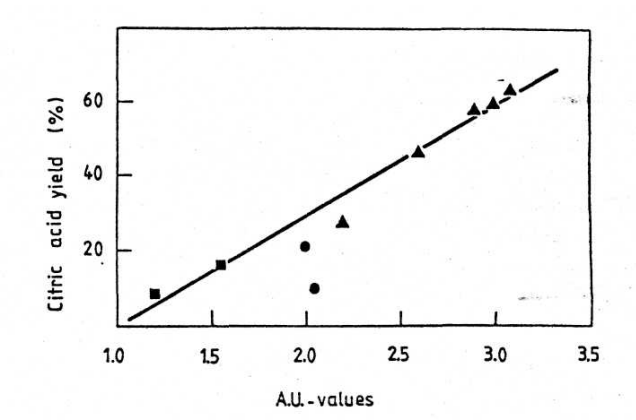

Figure 3

Correlation between AU-values in the range of 1.0 - 3.5 and citric acid yields in submerged culture { given as grams of acid produced per 1oo grams of sugar consumed) . Methods for submerged cultivation have been described [3] .Different symbols are related to different experiments: # Aspergillus niger W, 3 data from the selection experiment, ▲ data from the degeneration experiment.

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