Studies on Gluconate Metabolism in *Aspergillus niger* *

I. Nutritional Requirements of *Aspergillus niger* Cultivated in Gluconate Medium

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Summary. A strain of *Aspergillus niger* adapted to grow on gluconate is cultivated on a medium containing gluconic acid lactone as the sole carbon source. Nutritional requirements of the adapted mold were studied. The organism shows optimum growth at 30°C in a medium containing per litre 20 g of gluconic acid lactone, 800 mg of NH₄NO₃, 100 mg of MgSO₄·7H₂O and 270 mg of KH₂PO₄ having pH 2.0. Mycelial growth reaches its maximum on the third day. Comparative growth responses of the adapted and parent strains on gluconate, glucose and sucrose were studied.

Since the discovery of the occurrence of gluconic acid in the culture filtrates of *Aspergillus niger* by MOLLIER (1922), a good deal of information has been collected regarding its formation in fungi in general and *Aspergilli* and *Penicillia* in particular. The extensive studies carried out by MÜLLER (1929 and 1936) and FRANKE and his coworkers (1939 and 1944) with the cell free enzyme preparation of *A. niger* producing gluconic acid clearly established the role of glucose oxidase in its formation from glucose. Despite the studies on the fermentative production and the mechanism of gluconic acid formation, the utilisation of gluconic acid by *A. niger* has not been studied thoroughly. It is known that gluconic acid formed during the initial stages is reutilised at a later stage forming oxalic acid or citric acid, possibly involving certain adaptive enzymes. Contradictory views have been expressed regarding the efficacy of gluconate as a carbon source. CZAPEK (1903) and ALLSOP (1937) reported gluconic acid as a good source of carbon for *A. niger* while STEINBERG (1942) reported that the strain used by him could not utilise gluconate as a source of carbon. In order to study the problem systematically, a gluconate utilising strain of *A. niger* was obtained by serial subculturing a citric acid accumulating strain of *A. niger* in a

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medium containing gluconate as sole carbon source. Details of the procedure by which the strain was developed and the studies on the optimum conditions for the growth are presented in this communication.

**Materials and Methods**

A strain of *Aspergillus niger* (NCIM 611) was obtained from National Collection of Industrial Microorganisms, National Chemical Laboratory, Poona, (India). This strain was adapted for growth on gluconate by serial subculturing in the medium containing gluconic acid lactone as the sole carbon source. The medium used for adaptation was essentially that of Shah and Ramakrishnan (1963), excepting that citric acid was replaced by 2% gluconate. In the initial stages, the organism took considerable time for growth and sporulation. In the subsequent transfers, however, the period for growth and sporulation came down to about four days. After six subculterings in the gluconate medium the resulting strain was taken up in gluconate slants containing 2.5% agar in the liquid medium. Agar was autoclaved separately and mixed with gluconate mineral salt media after cooling down to 45–50°C to avoid hydrolysis. The spores from the above slants were transferred to a fresh set of agar slants and the spores from the resulting culture taken up in soil and stored as soil culture as described by Foster (1949). The soil culture was grown on gluconate slants and was subcultured at least twice before use in the subsequent experiments.

Unless otherwise specified, the medium employed for cultivation contained, per litre, 20 g of gluconic acid lactone, 2 g of ammonium nitrate, 0.45 g of potassium dihydrogen ortho-phosphate and 0.2 g of magnesium sulphate. The pH of this medium was found to be 2.2 and when necessary was adjusted to the desired pH by using either 1 N KOH or 1 N HCl. 50 ml of the medium was distributed in 250 ml Erlenmeyer flasks and sterilised at 10 lbs. pressure for 10 min.

The spore suspension was prepared from the agar slants by flooding each slant twice with 5 ml of sterile distilled water and gentle scrapping. The flasks were inoculated with 2 ml of this spore suspension under sterile conditions.

Whenever the effect of sodium nitrite was studied, it was added after autoclaving the medium containing the remaining ingredients.

The mycelium was removed on the desired day, washed with distilled water till free from the medium, and dried at 80°C to constant weight before determining the dry weight. The medium and the washings were pooled and analysed for titratable acidity and organic acids by chromatographic method (Sekharavarma and Ramakrishnan, 1956). Titratable acidity of the medium was estimated by titrating 10 ml of it against N/10 NaOH using phenolphthalein as indicator. Results are expressed on per flask basis and are mean ± SD of at least 3 determinations.

**Results and Discussion**

Changes in the mycelial weight and the titratable acidity of the medium, with the progress of cultivation are shown in Table 1, from which it can be seen that the mycelium formation starts on second day and increases steadily till third day whereafter there is progressive decrease in mycelial weight. Along with the progress of cultivation, chromatographic analysis of the medium revealed the presence of no other acid in appre-