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Production of citric acid by Aspergillus Niger cultivated on Deproteinised Leaf Juice (DPJ) of Lucerne (Medicago Sativa L)

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ABSTRACT

The deproteinised leaf juice (DPJ) of Lucerne (Medicago sativa L.) was found to be suitable as a medium for the cultivation of Aspergillus niger. The fungus produced citric acid, when cultivated on DPJ. The production of citric acid increased with the supplementation of glucose to the DPJ. Thus the DPJ, left after preparation of leaf protein concentrate, was found suitable in microbial technology to produce citric acid and other useful microbial metabolites.

Keywords: Deproteinised leaf juice, Fungal growth, Microbial metabolites.

INTRODUCTION

The use of leaf protein concentrate (LPC) in human and animal nutrition as a source of protein and vitamin A has long been recommended by Pirie[1]. For this purpose fresh green leaves from suitable species are pulped and subsequently presses. The leaf juice released due to pressing, is then heated to boil, as a result of which the proteins in juice coagulate to a curd referred as LPC. After isolating the LPC from heated juice, the deproteinised leaf juice (DPJ) is released as a by-product. As its random disposal may cause environmental bio-pollution, its proper utilization is necessary.

Earlier reports from this laboratory Ajay Kumar and Mungikar [2,3] suggested its use as a medium for cultivating useful micro-organisms for the production of microbial metabolites. Present investigation was undertaken to investigate the potential of DPJ for the cultivation of *Aspergillus niger* to produce citric acid.

MATERIALS AND METHODS

Fresh green foliage of Lucerne (*Medicago sativa* L.) was harvested early in the morning and immediately brought into the laboratory. The foliage was washed with water and pulped Davys et al[4]. The pulp was pressed [5] and the released juice was heated till boiling in stainless steel container. It was then filtered through cotton cloth and the leaf protein concentrate (LPC) was recovered. The deproteinised leaf juice (DPJ) released during filtration in the form of brown liquor was collected and dried in hot air oven to a fine powder for further use.

The dry DPJ was dissolved in distilled water to prepare 2 % (w/v) solution. Twenty five ml of the 2 % DPJ was distributed in conical flasks. Either 0.00, 0.25, 0.50, 0.75, 1.00 or 1.25 g glucose was added to 100 ml DPJ and distributed in conical flasks. Simultaneously, glucose- nitrate (GN) medium was also taken in conical flasks for comparison. The conical flasks were autoclaved, cooled and inoculated with spore suspension of *Aspergillus niger*. After inoculating the flasks at room temperature for 7 days the fungal mat was filtered through Whatman filter paper, dried in incubator and the yield of dry fungal biomass was recorded. The culture filtrate was employed for the determination of citric acid as described by Kapoor, et al[6].The amount of citric acid was then calculated following by Heding and Gupta [7]. The data were statistically analyzed following by Mungikar [8] for the calculation of critical difference (C.D.)

RESULTS AND DISCUSSION

Aspergillus niger is a fungus which is recommended in microbial biotechnology for the production of various microbial metabolites [9]. Citric acid is one of the organic acid synthesized and released by this fungus, when cultivated on synthetic growth medium. It is widely used for preparation and preservation of various food products, apart from its industrial uses. During present investigation attempts were made to cultivate *Aspergillus niger* on DPJ of Lucerne (*Medicago sativa* L.).

The DPJ is a by-product of leaf protein production unit. It contains several soluble cell nutrients like sugars, minerals, nitrates, free amino acids etc., and hence it is suitable as a medium for growing fungi. Table 1 gives an account on the growth of *Aspergillus niger* on DPJ of Lucerne, wherein the DPJ was supplemented with glucose as a source of carbon for increased microbial growth. The microbial biomass production on 2 % DPJ was 0.786 g against that on GN medium (0.665g). Thus The performance of DPJ, as a medium for fungal growth, was at par with that on GN medium, suggesting its suitability for this purpose. Addition of glucose significantly increased the production of fungal biomass, which was 2.246 g with 1.25 g glucose, as supplement. The DPJ can thus be considered as a basal medium for growing fungi.

The GN medium and DPJ alone produced 2.24 and 2.30 g citric acid per 100 ml culture filtrate, showing their similarity. Addition of glucose to the DPJ significantly increased citric acid production to the extent of 3.43 g per 100 ml. Thus the DPJ can be employed for cultivating *Aspergillus niger* and production of citric acid.

Table 1. Production of citric acid by *Aspergillus niger* grown on DPJ (2 %) of Lucerne

Medium	Fungal biomass (g / 100 ml)	Citric acid (g / 100 ml)
GN (Control)	0.665	2.24
DPJ (Alone)	0.786	2.30
DPJ + 0.25 g glucose	0.926	2.9
DPJ + 0.50 g glucose	1.268	3.04
DPJ + 0.75 g glucose	1.704	3.17
DPJ + 1.00 g glucose	1.992	3.30
DPJ + 1.25 g glucose	2.246	3.43
C. D. (p = 0.05)	0.532	0.40

CONCLUSIONS

The overall results obtained during present study confirmed suitability of DPJ as a medium for cultivating fungi. The growth of *Aspergillus niger* on DPJ was at par to that obtained on GN medium. The fungus produced citric acid while growing on DPJ, the production of which can be scaled up by adding glucose. Further investigations on possibilities of citric acid production on commercial scale are needed to exploit full potential of the deproteinised leaf juice left after leaf protein production.

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