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Effect of Dithiocarbamates on Citric Acid Production by *Aspergillus niger*

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ABSTRACT. Dithiocarbamates were found to enhance the production of citric acid under solid-state fermentation conditions by *Aspergillus niger*. Maximum increase was observed with tetramethylthiuram disulfide (TMTD). Percent increases observed were 74.2 % with 2.5 μg/mL of TMTD, 19.6 % with 2.5 μg/mL of sodium dimethyldithiocarbamate and 33.1 % with 0.6 μg/mL of zinc dimethyldithiocarbamate.

The commercial importance and versatile utility of citric acid is well documented. This industry has been revolutionized so much by fermentation that citric acid is now principally produced through fermentation of sugars by *Aspergillus niger*. In spite of fair knowledge of this process the industry is affected from time to time by heavy losses due to low production of citric acid. This has been attributed to a variety of causes, of which metal ion impurities, such as those of manganeese, play an important role (Kisser *et al.* 1980; Kubicek and Rohr 1982). Metal-chelating agents, such as hexacyanoferrate (HCF) (Leopold and Valtr 1964), ethylenediaminetetraacetic acid (EDTA) and trans-1,2-diaminocyclohexanetetraacetic acid (CDTA) have been used to obtain high citric acid yields (Chaudhary and Pirt 1966; Quadeer and Jaffer 1971). Sulfur-containing fungicides (dithiocarbamates), such as tetramethylthiuram disulfide (TMTD), sodium dimethyldithiocarbamate (NaDDC) and zinc dimethyldithocarbamate (ZnDDC), having metal-chelating properties (Delepine 1962), are known to inhibit spore germination of molds at low concentrations. They are known not to markedly interfere with mycelial growth at these low concentrations, in spite of their inhibitory effect at higher concentrations (Kaars Sijpesteijn and Janssen 1959). Few data are available about the effect of these compounds on the production of citric acid by *A. niger* under solid-state fermentation conditions (Cahn 1935; Lakshminarayana *et al.* 1975; Chaudhary *et al.* 1978).

In the present study the effect of different concentrations of TMTD, NaDDC and ZnDDC on the production of citric acid by *A. niger* was examined under solid-state fermentation conditions.

MATERIALS AND METHODS

Organism. Aspergillus niger MULDER procured from the *Division of Mycology and Plant Pathology, IARI*, New Delhi, was used. The stock culture was

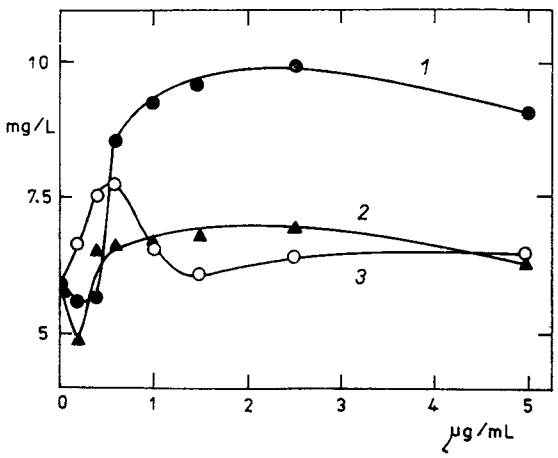

FIG. 1. Effect of dithiocarbamates (µg/mL) on citric acid production (mg/mL) by *A. niger*, under solid-state fermentation conditions; *1* — TMTD, *2* — NaDDC, *3* — ZnDDC.

maintained on potato-dextrose-agar (PDA) slopes by repeated subculturing at regular intervals of 30 d. The same medium was used for harvesting spores for the preparation of inoculum.

Production medium. Shu and Johnson (1947) medium with an initial sugar concentration of 15 % at pH 2.0 was used. The solid inert material was prepared from sugar-cane bagasse obtained from a nearby village. It was washed in distilled water, dried in the sun and then in an oven at 60 °C for a day. Dried bagasse was chopped up into small bits and pulverized in an electric pulverizer.

Dithiocarbamates. TMTD and ZnDDC were obtained from *Fluka* (Switzerland), NaDDC from *British Drug House* (India) and dimethyl sulfoxide for solubilizing the pesticides was from *Reidel* (USA).

Preparation of inoculum. Spores of *A. niger* were harvested from 5-d-old cultures on PDA, using sterile Tween-80 (0.1 %) in distilled water. The spore load was adjusted to 10^5 to 10^6 per mL of the production medium.

Preparation of trays containing bagasse. Pulverized bagasse was dispensed at the rate of 150 g per enamel tray (300×350×50 mm) and covered with thick brown paper. Trays containing bagasse were autoclaved at 103 kPa for 20 min.

Production of citric acid under solid state conditions. One litre of the production medium was dispensed per 2-L flask and autoclaved at 69 kPa for 30 min. Pesticide solutions were prepared in Me_2SO, and filter-sterilized through G-5 sintered glass filters. Sterile TMTD, NaDDC and ZnDDC were aseptically dispensed in the medium, to the requisite concentration (0.2—5 µg/mL) and mixed thoroughly. The medium in each flask was inoculated with 10 mL of spore suspension and mixed thoroughly. The control contained on equal amount of Me_2SO solution.

Aseptically, the contents of each flask were dispensed uniformly per tray of bagasse, in a laminar-flow chamber. The trays were immediately covered again. Incubation was done at 28 ± 2 °C for 7 d. The fermented material was then extracted repeatedly with distilled water and the final volume was made up to 5 L. The extract was filtered through Whatman filter paper no. 1.

TABLE I. Percentage change in citric acid yield by *A. niger* under solid-state fermentation conditions, in the presence of TMTD, NaDDC and ZnDDC[a]

Dithiocarbamate μg/mL	Citric acid change, % TMTD	NaDDC	ZnDDC
0	—	—	—
0.2	−06.6	−19.6	+13.3
0.4	−05.1	+12.3	+30.2
0.6	+45.1	+12.8	+33.1
1.0	+61.7	+14.4	+12.0
1.5	+67.6	+16.5	+02.8
2.5	+74.2	+19.6	+08.9
5.0	+58.3	+08.0	+10.4

[a] Me_2SO *per se* had no effect on citric acid production, at the concentrations used. Me_2SO, TMTD, NaDDC and ZnDDC did not interfere with the estimation of citric acid by the Marrier and Boulet (1958) method.

Citric acid estimation. Citric acid content was estimated following the method of Marrier and Boulet (1958).

RESULTS

The data showing the effect of various concentrations of TMTD, NaDDC and ZnDDC on citric acid production is shown in Fig. 1 and Table I. In general, all the three agents increased citric acid yield. TMTD stimulated citric acid production at higher concentrations (0.6—5 μg/mL), a maximum increase being found at 2.5 μg/mL (74.2 %). However, lower concentrations were inhibitory to citric acid yield.

The effect of NaDDC on citric acid production was similar to that of TMTD but less pronounced.

ZnDDC caused an increase in citric acid production at low doses only (0.2—1.0 μg/mL), its maximum effect (32.1 %) being at 0.6 μg/mL.

The dry mass of the mycelium was 16 g per tray of fermented material.

DISCUSSION

Dithiocarbamates have been known to affect biological systems (Thorn and Ludwig 1962). Their exploitation in industry, research, medicine and agriculture has an extensive range due to their metal-binding capacity (Delepine 1907; Eckert 1957); also they act as enzyme inhibitors (Chefurka 1957). Hence they were used in the present study for investigating their stimulatory and/or inhibitory effect on solid-state production of citric acid by *A. niger*.

Keeping in view the above information and other reports available in the literature, these compounds should exert an inhibitory effect on citric acid production, as they inhibit spore germination and growth of *A. niger* and *P. italicum* because of their interaction with an essential enzyme or enzyme system (Kaars Sijpesteijn and Van der Kerk 1956): this reportedly resulted in the accumulation of pyruvic acid by these two strains. Inhibition has also been proposed to be due to complex formation with metal-containing enzymes or to interference with electron shifts between mercapto or amino groups of the enzyme and substrate molecules (Horsfall 1945; Owens 1953). In contrast,

we found all the three compounds to stimulate citric acid yield although the degree of stimulation varied with respect to the concentration used. This was different for different compounds and could either be attributed to their chelating properties (Rohr and Kubicek 1981; Chaudhary and Pirt 1966; Quadeer and Jaffer 1971; Dhankar 1972), or the metal part of the compound could be acting as the actual mediator in some of the enzymes involved in biosynthesis of citric acid or modifying the enzyme system because of involvement of allosteric enzymes (Kaars Sijpesteijn and Van der Kerk 1956; Horsfall 1945; Owens 1953). This could result in an accumulation of pyruvic acid which, as acetylCoA (Reed 1974), would then be easily available to the citric acid cycle (Krebs 1970), causing an increased yield of citric acid, as compared to the control.

Further studies are being pursued to substantiate the findings and the reason for increased production of citric acid by *A. niger* in the presence of dithiocarbamates under solid-state fermentation conditions.

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