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BIOCONVERSION OF POULTRY WASTES I – FACTORS INFLUENCING THE ASSAY AND PRODUCTIVITY OF CRUDE URICASE BY THREE URICOLYTIC FILAMENTOUS FUNGI

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The optimum temperature for biomass yield and uricase production by uricolytic fungi, *Aspergillus terreus*, *A. flavus* and *Trichoderma* sp. was at 30 °C. The time required for maximum production of uricase and biomass yield was 4 days for two *Aspergillus* species and 6 days for *Trichoderma* sp. The optimum pH was at 6.4 for *A. terreus* and pH 6.6 for *A. flavus* and *Trichoderma* sp.

The maximum fungal biomass yield was achieved in medium supplemented with 4% poultry waste. The best carbon sources for the production of uricase and mycelia yield were glycerol, sucrose and maltose by *A. terreus*, *A. flavus* and *Trichoderma* sp., respectively.

Uric acid was found to be the best nitrogen source for production and activity of uricase by the three tested fungi. The addition of some vitamins to the culture media increased the maximum biomass yield of all the isolates, although no significantly increased uricase production was found.

Keywords: bioconversion, filamentous fungi, poultry wastes, uricase

Introduction

In Egypt, a great deal of poultry wastes (ten metric ton/day) is produced. These wastes are rich in nitrogenous compounds, particularly uric acid in addition to several other secondary metabolites. These compounds usually create many environmental

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problems if they are left to accumulate in the environment and become a dangerous source of pollution. Moreover, these wastes are considered as a source of many infectious diseases. For these reasons, many attempts have been conducted to eradicate this danger source of pollution. One of these attempts is to use these wastes to fertilize the proclaimed lands but on the long run these wastes would lead to increase the alkalinity of land. Sometimes these wastes may be used as a fish feed.

Fungi and bacteria have been used for the treatment and conversion of wastes into useful forms [1–2]. Several attempts were made to utilize the animal and poultry wastes as sources for the production of some biogas, particularly methane as the major biogas from anaerobic fermentation process [3–4].

Uricase catalyzes the oxidation of uric acid to allantoin, carbon dioxide and hydrogen peroxide [5]. Allantoinase is the only enzyme known to attack allantoin to form allantoic acid [6]. Many species and strains possess allantoinase that catalyzes the conversion of allantoate into (-)-ureidoglycolate and urea and the conversion of (+)-ureidoglycolate into glyoxylate and urea [7]. Then urease enzyme splits urea into ammonia and carbon dioxide [8]. All of these enzymes are responsible for the conversion of uric acid into ammonia and carbon dioxide. This series of enzymes is known as the uricolytic enzyme system [9–10]. Several fungi can utilize uric acid as a sole source of nitrogen or to satisfy their requirements of nitrogen and carbon [11]. The ability of many bacterial species to produce uricases was reported by several authors [12–13].

The effect of various carbon sources on the formation of uricase by microorganisms was studied by several authors [14–16]. The utilization of uric acid as a nitrogenous inductive agent for the production of uricase by microorganisms had been intensively studied [17–18]. Several investigators [13, 19–20] studied the optimal temperature and pH for the production of uricase by microorganisms. The present investigation was conducted to study the potentiality of some fungi for the production of uricase on poultry waste as a sole substrate for fungal growth. Special emphasis was laid upon the determination of the optimum conditions for uricase production by some selected uricolytic fungi.

Materials and methods

Organisms and media

Three selected uricolytic fungi (*Aspergillus terreus* Thom, *Aspergillus flavus* Link and *Aspergillus* sp.) were isolated from poultry farms in Dakahlia Governorate, Egypt. These fungi were grown in Czapeks medium at 30 °C for 5 days for identification by the authors. Stock cultures of these purified isolates were stored on

uric acid agar medium (2 g; uric acid, 0.5 g; K_2HPO_4, 0.5 g; KH_2PO_4, 0.5g; $MgSO_2$, 0.5 g; NaCl, 0.01g; $FeSO_4$ and 15 g Difco purified agar made up to 1 l with distilled water and the pH was adjusted to 6.6) under sterile mineral oil at 4 °C.

Uricase production

Uricase activity was measured according to the procedure described by Arima and Nose [21]. Twenty-ml aliquots of uric acid medium were dispensed in 250-ml conical flasks. One ml of each uricolytic fungal (*A. terreus, A. flavus, Trichoderma* sp.) spore suspension containing about 1×10^6 spores was injected into the media. The flasks were incubated for 4 days at 30 °C after which the mycelium of each isolate was collected by filtration then ground in a cooled mortar at 4 °C. This preparation was centrifuged at 3000 rpm for 15 min at 4 °C to get rid of all insoluble particles. One ml of the clear crude enzyme preparation was mixed with 0.5 ml of uric acid (10 μg/ml) solution as substrate in test tube containing 1 ml of the 0.2 M borate buffer (pH 8.6). The mixture was incubated at 30 °C for 30 min. The mixture was boiled for 5 min to stop the enzyme activity. To the reaction mixture, 0.5 ml of sodium carbonate (10%) was added, mixed well and allowed to stand for 5 min at room temperature. Thereafter, 0.5 ml phosphotungstic acid (color reagent) was added and incubated for 30 min at room temperature and the intensity was read at 700 nm according the method of Caraway [22]. One unit of uricase enzyme was equal to the amount of enzyme which convert 1 μmole of uric acid to allantoin per min. at 30 °C.

To evaluate maximum production, catalytic activity and stability of crude uricase produced by the tested organisms the following criteria were studied.

(a) Effect of different incubation periods

The mycelia of each isolate were harvested at different incubation periods (2, 4, 6, 8, 10 and 12 days). At the required interval, both the production of the enzyme and the biomass were determined.

(b) Effect of different concentrations of poultry waste (1, 2, 4, 8, 16 and 23 %)

This experiment was carried out to detect the suitable concentration of poultry waste.

(c) Effect of pH

The mycelia of each isolate were harvested at different pH values (6, 6.2, 6.4, 6.6, 6.8, 7). The uricase activity was estimated as described above. In the same time, the collected mycelia were dried at 80 °C for 24 h and the dry weight was recorded as fungal biomass.

(d) Effect of different temperatures

The fungal cultures were incubated at 22, 24, 26, 28, 30 and 32 °C. The pH of the culture medium was adjusted to 6.4, 6.6 and 6.8 for *A. terreus, A. flavus* and *Trichoderma* sp., respectively. At the required interval, both production of the enzyme and the biomass were determined.

(e) Effect of different carbon sources

The liquid medium containing the poultry waste was supplied with 1% of glycerol, fructose, maltose, lactose, starch, cellulose and carboxymethyl cellulose.

(f) Effect of addition of different nitrogen sources

Addition of 1% of KNO_3, NH_4Cl, $NH_4H_2PO_4$, $(NH_4)_3PO_4$, $(NH_4)_2SO_4$, peptone, beef extract, soybean meal, fish meal and casein to the culture of the tested fungi were studied.

(g) Effect of different vitamins

One hundred mg of yeast extract, vitamins B1, B6 and B12, Vitamin C and riboflavin were added to the fungal cultures. Vitamins were sterilized by filtration.

Results and discussion

Effect of incubation periods. The incubation period required for uricase production by *A. terreus, A. flavus* was 4 days and 6 days for *Trichoderma* sp. (Fig. 1). In support of these results, also Ammar et al. [15] found that 4 days was the best incubation period for the production of uricase by *A. flavus*. Five days incubation period was shown to be the best for Hyphomyces [23]. In case of *Streptomyces*

alboniger or *Streptomyces corchorusii*, three days incubation period was recorded by El-Arini [24].

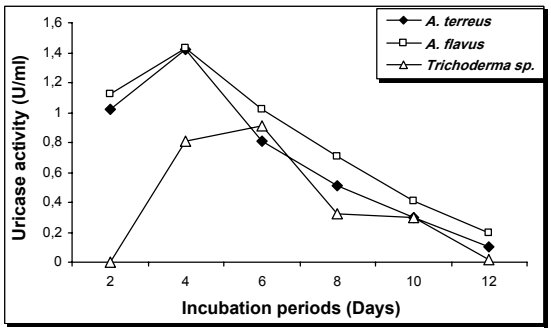

Fig. 1. Effect of different icubation periods on uricase production of *A. terreus, A. flavus* and *Trichoderma* sp.

Effect of different concentrations of poultry waste. The most suitable concentration of chicken waste for the production of uricase was 4% in case of *A. terreus and A. flavus* and 8% for *Trichoderma* sp. (Fig. 2). The highest biomass yield of the three tested fungi was obtained in medium supplemented with 4% poultry waste. Higher concentrations seemed to create unfavorable conditions for fungal growth, where at 32% concentration, a very low biomass yield was noticed. At poultry manure concentrations greater than 16%, lower uricolytic activities could be detected in the Aspergilli. However, poultry manure higher than 8% sharply dropped the uricolytic activity of *Trichoderma* sp. These results are in accordance with those of obtained by Chen [25].

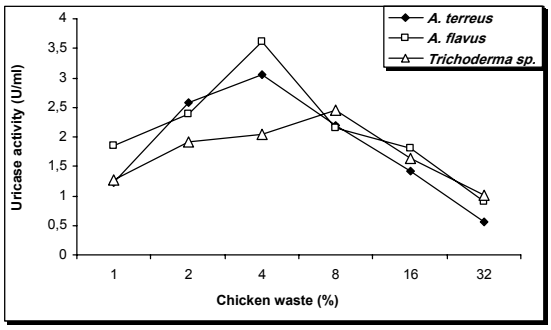

Fig. 2. Effect of different chicken waste concentrations on uricase production of *A. terreus, A. flavus* and *Trichoderma* sp.

Effect of different initial pH values. The maximum production of uricase was recorded at pH 6.4 for *A. terreus* and pH 6.6 for *A. flavus* and *Trichoderma* sp. (Fig. 3). Thapar *et al*. [26] showed that the highest production of uricase of *A. wentii* was obtained at pH 10. In this connection, the optimum pH for uricase production by *A. flavus* S.79 was reported at pH 9.2 [15].

The present results also showed that the fungal biomass increased with the decreasing of initial pH, where it reached maximum at pH 6.8 for *A. flavus*, pH 6.6 for *Trichoderma* sp. and pH 6.0 for *A. terreus*. The biomass yield declined at pH 7.0 in all the fungi tested.

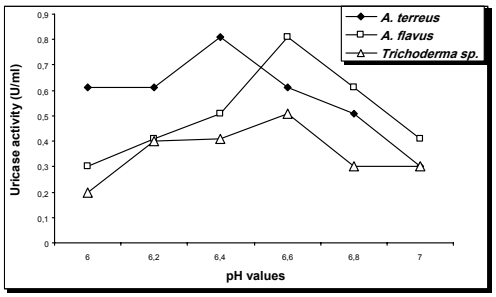

Fig. 3. Effect of pH values on uricase production of *A. terreus, A. flavus* and *Trichoderma* sp.

Effect of different temperatures. The optimum temperature for uricase production was 30 ºC for *A. flavus, A. terreus* and *Trichoderma* sp. (Fig. 4). The highest biomass yield was obtained at 30 ºC and it appeared to be the optimum temperature for growth of the three isolates. Ammar et al. [15] reported that 30 ºC was optimum for growth of *A. flavus* S.79. Optimum temperature for uricase production by *Streptomyces aureomonopochiales* and *Bacillus megaterium* were 35 ºC and 30 ºC, respectively [13].

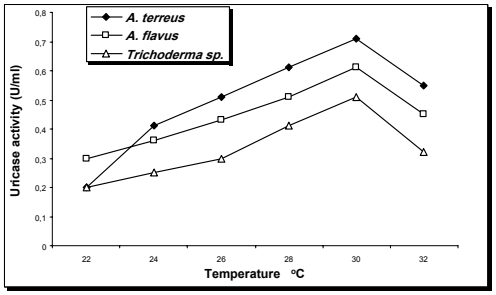

Fig. 4. Effect of temperature on uricase production *of A. terreus, A. flavus* and *Trichoderma* sp.

Effect of different carbon sources. The results presented in (Fig. 5) clearly indicated that the most efficient carbon sources for uricase production were glycerol, sucrose and maltose at the same concentration (1%) for *A. terreus, A. flavus* and *Trichoderma* sp. On the other hand, cellulose, lactose and mannitol induced an inhibitory effect on the production of uricase by the three tested isolates. These results agree with those of Zenjiro et al. [27] who recorded that uricase production increased by glycerol as a carbon source and Liu and Li [16] who found that sucrose, glucose, D-mannose and D-fructose were suitable carbon sources for uricase formation by *Candida utilis.* Glycerol (30g/l) was shown to be the best carbon source in case of *Streptomyces alboniger* and *Streptomyces corchorusii* [24] and for *Streptomyces aureomonopodiales* [13].

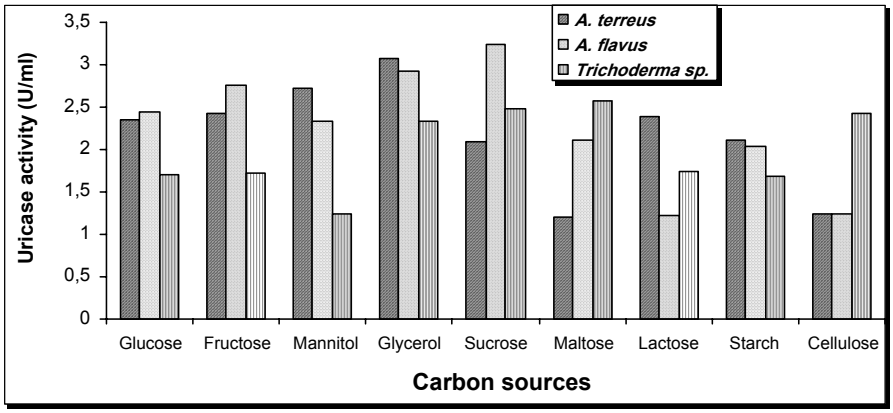

Fig. 5. Effect of different carbon sources on uricase production of *A. terreus, A. flavus* and *Trichoderma* sp.

The biomass yield of the investigated isolates was increased when the poultry waste medium was supplemented with different carbon sources. The maximum yield was achieved in the presence of the same sugars, which resulted in the highest yield of uricase, indicating that the uricase yield was proportionally related to the fungal biomass.

Effect of different nitrogen sources. It is shown in Fig. 6 that when peptone and casein were added separately to the basal medium containing poultry waste, they caused complete inhibition for uricase induction in the three tested isolates. On the other hand, the addition of KNO_3 and $(NH_4)_2SO_4$ induced a slight uricase activity in case of *Trichoderma* sp., while $NaNO_3$ caused complete depression of uricase production by *A. terreus* and *A. flavus*. However, maximum biomass yield was

obtained by the addition of KNO_3, NH_4Cl and NH_4NO_3 for *A. Terreus, A. flavus* and *Trichoderma* sp., respectively. These are in conformity with others who used *Streptomyces alboniger, Streptomyces corchorusii* and *Streptomyces aureomonopodiales* and found that addition of similar nitrogen sources inhibited uricase production [13, 24]. From these results it is concluded that uricase is an inducible enzyme and it could not be induced unless uric acid is present. On the other hand, the addition of nitrogen sources to the poultry manure medium enhanced the mycelial yield. The addition of organic sources such as peptone, beef extract, casein, soybean and fish meal increased the biomass yield. This may be essentially due to the fact that the complex nitrogen sources yield a variety of amino acids, which might be better for initiating growth, and satisfy the fungal metabolic activities. The utilization of proteins by the tested fungi indicated the presence of proteases. This finding agrees with that obtained by Abo-Hamed *et al.* [28] who found that addition of peptone, soybean and casein enhanced the biomass yield of *Penicillium notatum, Fusarium oxysporum, A. niger, A. flavus* and *A. oryza.*

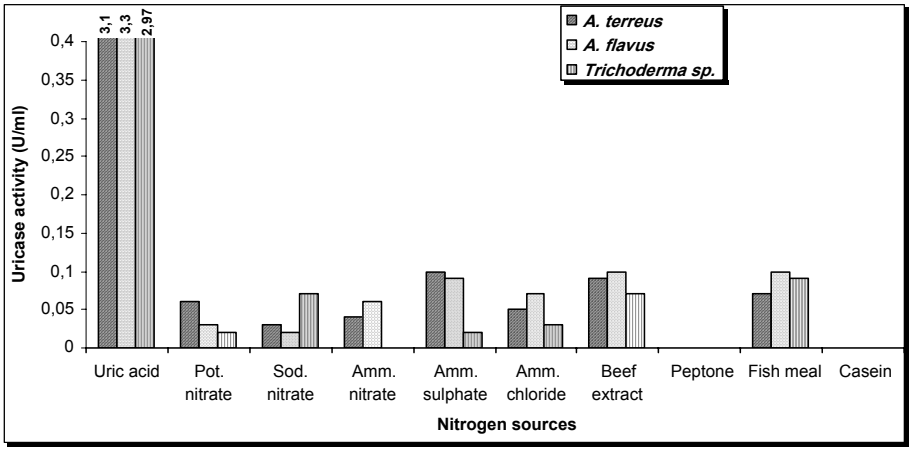

Fig. 6. Effect of different nitrogen sources on uricase production of *A. terreus, A. flavus* and *Trichoderma* sp.

Effect of different vitamin sources. As shown in Fig. 7, the production of uricase was hardly affected by the incorporation of most studied vitamins irrespective of the fungus. However, the addition of vitamin C, molasses and vitamin B12 slightly stimulated this process in *A. terreus, A. flavus* and *Trichoderma* sp., respectively. In this connection, the addition of yeast extract was shown to have no stimulatory effect on uricase production by *Streptomyces albogrisedus*, while ascorbic acid, thiamin and pyridoxine exerted a slight stimulatory effect [17]. They also demonstrated that

addition of nicotinic acid, folic acid and riboflavin exhibited various inhibitory effects against uricase production. The present results revealed also that the addition of molasses, vitamin B12 and yeast extract to the poultry waste medium increased the mycelial yield of the tested fungi.

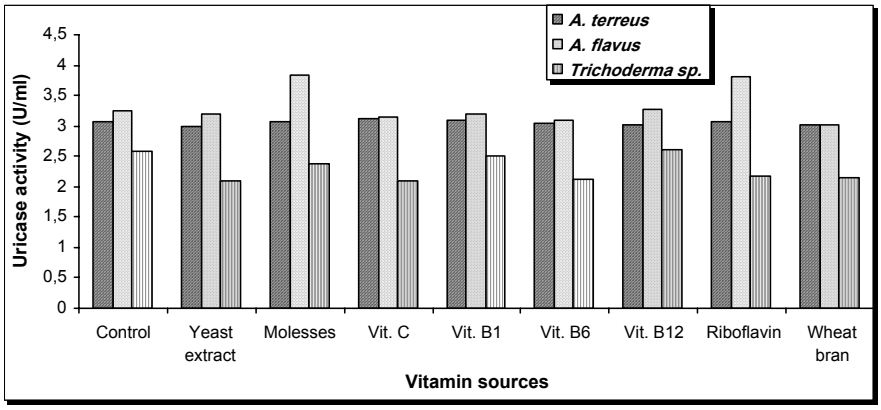

Fig. 7. Effect of different vitanim sources on uricase production of *A. terreus, A. flavus* and *Trichoderma* sp.

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