

THE USE OF ACETONITRILE AS THE SOLE NITROGEN AND CARBON SOURCE BY *GEOTRICHUM* SP. JR1

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ABSTRACT

A yeast strain identified as *Geotrichum* sp. JR1 was able to use acetonitrile as the sole carbon and nitrogen source. The strain grew in 0.5 to 2M acetonitrile. Ammonia generation as enzymatic product during the strain growth indicates the presence of an acetonitrile degrading enzyme. Acetic acid and acetamide were detected during assays with the resting cells cultivated in acetonitrile, indicating the presence of nitrile and amide degrading enzymes. This paper is the first to describe the use of acetonitrile as the sole carbon and nitrogen source by a yeast.

Key words: *Geotrichum* sp., acetonitrile, degrading enzymes

INTRODUCTION

Nitriles possess the general structure R-CN, which may occur naturally or synthetically. They are used for the synthesis of plastics, cosmetics, pharmaceuticals, herbicides and other chemicals. Unfortunately, there is an increasing dissemination of acetonitrile in the environment via industrial waste waters, and toxic activity can occur (4).

Nitrile compounds are generally catabolized by microorganisms through direct conversion to carboxylic acids and ammonia using a nitrilase system (EC. 3.5.5.1) or nitrile hydratase (EC. 4.2.1.84) that form amide as an intermediate degradation product, followed by amidase (EC.3.5.1.4), that converts the amide to the corresponding carboxylic acid and ammonia. Furthermore, most of our knowledge of the biochemistry, regulation and genetics of degradative pathways of these compounds has been obtained from studies with bacteria (6,7,11).

The widespread use of nitriles has created considerable commercial interest in nitrile-hydrating enzymes, both as

biocatalysts in chemical syntheses (10) and as a means of biological detoxification of nitrile containing waste (13).

Few studies have been carried out on the use of nitriles by yeasts (2,3,4,9,12). All these studies reported the use of nitriles and corresponding amides as the only nitrogen source. The present study describes the metabolism of acetonitrile by the yeast strain *Geotrichum* sp. JR1, which is the first yeast reported to use acetonitrile as the sole nitrogen and carbon source.

MATERIALS AND METHODS

Microorganism

The yeast strain employed in this study was isolated from a cyanide treatment bioreactor in a Brazilian Gold Mine (Mineração Morro Velho, Nova Lima, Brazil), where effluents cyanide concentration ranged from 150 to 300 ppm (2). The microorganism was identified according to Kurtzman and Fell (8). The strain was maintained on GYMP agar slant medium (2% glucose, 0.5% yeast extract, 1% malt extract, 0.2 NaH₂PO₄ and

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2% agar), under a layer of mineral oil, and was maintained at 4°C or in liquid nitrogen. Acetonitrile, acetamide and acetic acid were obtained from Merck (Darmstadt, Germany).

Kinetics of the growth

The inoculum for the experimental media was obtained from a yeast cell suspension in deionized water that was submitted to shaking for 24 h to prevent endogenous growth. A 0.5 mL of yeast suspension (10^6 cell mL⁻¹) was inoculated into 125 mL Erlenmeyer flasks holding 10 mL minimum medium (0.1% K₂HPO₄, 0.02% MgSO₄·7H₂O and 0.01% NaCl) containing acetonitrile from 0.5 up to 2 M. The flasks were incubated under shaking (120 rpm) at 28°C. At intervals of 24 h, the samples were filtered through a 0.22 mm membrane (Millipore), and the supernatant was collected for analysis. Growth was estimated in terms of dry mass by drying the membrane for 48 h at 80°C.

Substrate consumption and product formation

Substrate consumption and product formation were determined during growth and enzymatic assay (using resting cells) of *Geotrichum* sp. JR1 in acetonitrile as the sole nitrogen and carbon sources and by gas chromatography using a GC 5890 (Hewlett-Packard, series II) equipped with a flame ionization detector. The column used in this experiment was an FFAP (cross linked) with 25 m x 0.20 mm internal diameter x 0.33 film thickness. The operational conditions were as follows: hydrogen as carrier gas, 15:1 split ratio, injection volume – 1 mL, injector temperature 200°C and detector temperature 230°C.

Enzymatic assay

The resting cells, obtained from culture in acetonitrile as the sole carbon source, were investigated for enzymatic activity by measuring the production of ammonia (5). The yeast cells were harvested by centrifugation (10 min., 5000 rpm), washed twice in a 10 mM phosphate buffer (pH 7.0). The assay was prepared with 6 mg of the resting cells (dry weight) in 10 mL solution (10 mM phosphate buffer at pH 7.0) containing acetonitrile or acetamide. After assay, the yeast cells were harvested by centrifugation and ammonia was assayed in the supernatant (5). The supernatant was assayed in tubes containing 1.0 mL solution of acetonitrile or acetamide in 10 mM sodium phosphate buffer at pH 7.0 and 1.0 mL of supernatant at 30°C. As a control process, the presence of ammonia was measured in the growth supernatant. To determine whether the enzyme system for nitrile metabolism was inducible or constitutive, the strain was subcultured ten times in YCB (Yeast Carbon Base) medium containing 0.1% ammonium sulfate. Enzymatic assays were made by varying pH from 3 to 5 in citrate buffer, from 6 to 8 in sodium phosphate buffer, and from 7 to 8 in Tris HCl buffer, and at pH 9 in glycine buffer and at pH 10 carbonate buffer. All buffer solutions were at 10 mM.

RESULTS AND DISCUSSION

Although *Geotrichum* sp. JR1 was able to grow under high acetonitrile concentrations (1; 1.5 and 2M), the best growth rate was observed when the strain was grown in 0.5 M acetonitrile (Fig. 1). The yeast *Candida guilliermondii* UFMG-Y 65 strain (4) used a maximum of 2.2 M acetonitrile as nitrogen source, but was not able to use this compound as carbon source. Between 48 and 72 h, the growth varied according to acetonitrile concentration (Fig. 1). An inhibitory effect was observed in the first hours of growth, probably due to the toxic effect of acetonitrile and to the fact that the yeast was not adapted to this substrate. This fact has not been observed for *Candida famata* (9) when grown in high nitrile concentrations, because the strain was let to previously adapt to increasing concentrations of acetonitrile.

The enzyme systems were found to be inducible because the washed cells (intact) obtained after ten subcultures in Yeast Carbon Base (YCB) with 0.1% ammonia failed to hydrolyse acetonitrile or acetamide. The same result was reported by Dias *et al.* (4).

The activity of the nitrile degrading enzyme increased with the increase of incubation time and reached a maximum at 72 h of growth in 0.5 M acetonitrile medium (Fig. 2). These results suggest that after 96 h, even at high acetonitrile concentrations, the strain is adapted to use nitrile as sole nitrogen and carbon source (Fig. 2). A decrease (Fig. 3) in consumption of acetonitrile was observed with an increase in ammonia concentration, showing that acetonitrile degradation had occurred.

Assays with intact cells indicated that acetonitrile breakdown yielded acetic acid, acetamide as indicated by gas chromatography (Fig. 4) and ammonia. Acetic acid and ammonia were found in the reaction mixture when acetamide was used as substrate for the enzyme system (data not shown). None of

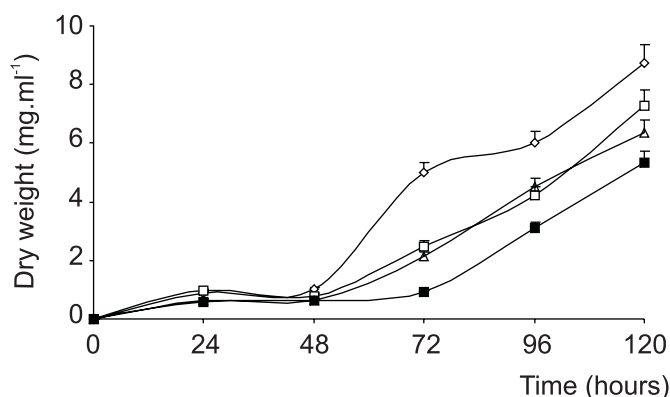


Figure 1. Kinetics of growth of *Geotrichum* sp. JR1 using different acetonitrile concentrations (■ 2 M, △ 1.5 M, □ 1 M, ◇ 0.5 M).

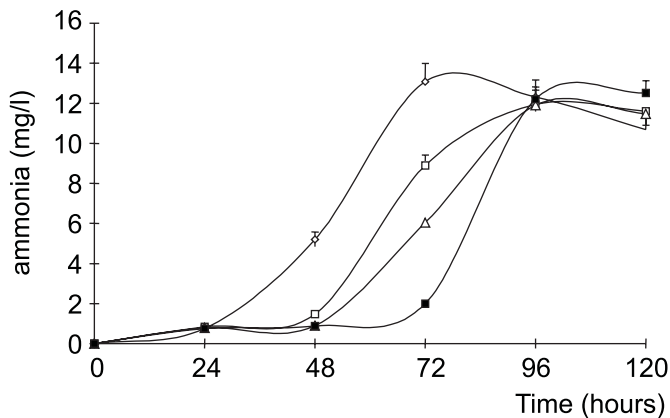


Figure 2. Kinetics of ammonia generation (5) during growth of *Geotrichum* sp. JR1 on different acetonitrile concentrations (■ 2 M, △ 1.5 M, □ 1 M, ◇ 0.5 M).

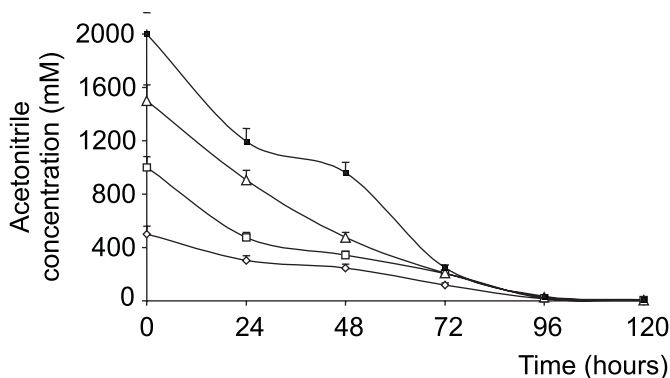


Figure 3. Kinetics of acetonitrile consumption (by gas chromatography analysis) during growth of *Geotrichum* sp. JR1 on different acetonitrile concentrations (■ 2 M, △ 1.5 M, □ 1 M, ◇ 0.5 M).

these metabolites was detected during growth, suggesting their rapid utilization as carbon and nitrogen source. Moreover, ammonia was always detected, showing acetonitrile breakdown. These facts strongly suggest that acetonitrile was the sole carbon and nitrogen source.

The acetonitrile degrading enzyme system by *Geotrichum* sp. JR1 suggests the presence of nitrile hydratase and amidase. Generally, it has been suggested that simple aliphatic nitriles are metabolized in a two-step process by nitrile hydratase and amidase (1). Rezende *et al.* (12) reported a nitrile degrading enzyme that shows the presence of nitrile hydratase and amidase and demonstrated acid and amide formation during propionitrile degradation by *Cryptococcus* sp UFMG-Y28. The ability of *Geotrichum* sp. JR1 to produce ammonia over a wide range of pH values (3 to 10) at high acetonitrile concentration (data not

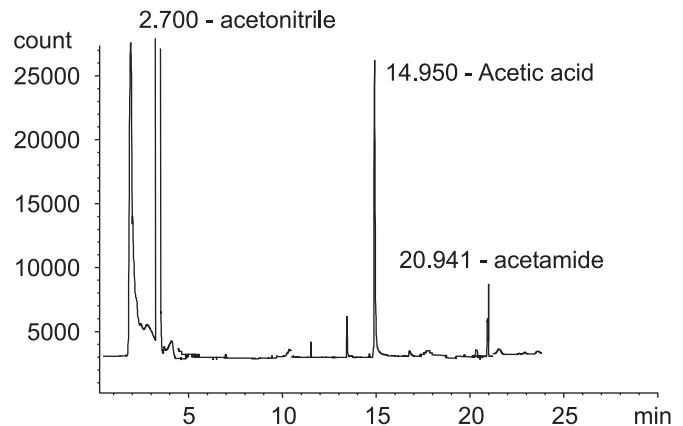


Figure 4. Enzymatic assay with intact cells of *Geotrichum* sp. JR1 showing acetonitrile breakdown (retention time 2.700) yielding acetic acid (retention time 14.950) and acetamide (retention time 20.941), suggesting the presence of nitrile hydratase – amidase system.

shown) indicates the potential use of this organism in the treatment of toxic wastes containing nitrile. It could also be used for acid and amide bioproduction.

Others yeast strains have been studied for nitrile degradation. Some yeast cultures have the capacity to use nitriles as nitrogen sources (9). Studies on yeast immobilization for nitrile degradation have been made (3, 4), but this work is the first to report on a yeast able to use nitrile at high concentrations as its sole nitrogen and carbon source.

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RESUMO

Uso de acetonitrila como única fonte de carbono e nitrogênio por *Geotrichum* sp. JR1

Uma linhagem de levedura identificada como *Geotrichum* sp. JR1 foi capaz de utilizar acetonitrila, em concentrações de 0,5 a 2M, como única fonte de carbono e de nitrogênio. A geração de amônia durante o crescimento do microrganismo indica a presença de sistema enzimático capaz de degradar acetonitrila. Durante os ensaios enzimáticos, com células

cultivadas em acetonitrila, foram detectados ácido acético e acetamida como produtos indicando a presença de sistema enzimático capaz de degradar acetonitrila e acetamida. Este trabalho é o primeiro a descrever a utilização de acetonitrila como única fonte de carbono e de nitrogênio por uma levedura.

Palavras-chave: *Geotrichum* sp., acetonitrila, enzimas de degradação

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