INDUCTION OF IRON AND COBALT DEPENDENT ACRYLONITRILE HYDRATASES IN ARTHROBACTER SP. IPCB-3

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SUMMARY

ACN-hydratase in Arthrobacter sp. IPCB-3 has been found to be induced by acetonitrile and urea and repressed by glucose. When acetonitrile was used as an inducer the synthesis of enzyme increased to about 2 folds and 4.5 folds on addition of iron and cobalt to the medium, respectively. However, when urea was used as an inducer only cobalt stimulated the enzyme synthesis and gave maximum activity (70 units/mg dry cells). In contrast to the stimulation of iron containing ACN-hydratase, yeast extract failed to stimulate further the synthesis of cobalt containing enzyme irrespective of the inducer present in the medium.

INTRODUCTION

Nitriles are degraded by a wide variety of microorganisms through two enzymatic pathways. Nitrilase catalyses the cleavage of nitriles to the corresponding acids and ammonia and nitrile hydratase catalyses the hydration of nitriles to amides (Jallageas et al, 1980; Asano et al, 1982; Nagasawa and Yamada, 1989). These enzymes have recently attracted increasing attention due to their potential application in production of industrially important amides and acids (Nagasawa and Yamada, 1989, 1990; Yamada and Nagasawa 1990; Yamamoto et al 1990, 1991). Recently, the use of Pseudomonas chlororaphis-B-23 nitrile hydratase for the production of acrylamide, an important commodity chemical, has been reported (Nagasawa and Yamada 1990; Ryuno et al, 1988). Moreover, nitrile hydratase from different sources have shown different characteristics (Nagasawa et al, 1988; Yamada and Nagasawa, 1990). In order to look for a novel source of nitrile hydratase, we carried out a screening of large number of bacterial strains capable of growing on acrylonitrile (ACN) and acetonitrile. Among these Arthrobacter sp. IPCB-3 was found to possess appreciable ACN-hydratase activity (Ramakrishna and Desai, 1992). In the present communication we wish to report the synthesis of two types of nitrile hydratases in Arthrobacter sp. IPCB-3.
MATERIALS AND METHODS

Arthrobacter sp. IPCB-3 was isolated from soil by enrichment with acetonitrile containing medium as described earlier (Ramakrishna and Desai, 1992). The organism was grown on medium described by Watanabe et al (1987) from which glucose and malt extract were omitted. The organism was grown in the above medium at 30 °C on a rotary shaker (300 rpm) for 22 h. Cells were harvested by centrifugation (6000 x g for 10 min.), washed and ACN hydratase activity was measured as described by Watanabe et al (1987). The assay system containing 0.5 mmol Potassium phosphate buffer (pH 7.7); 4.72 mmol acrylonitrile, and cell suspension in the final volume of 10 mL. After 10 min. of incubation at 5°C on a shaking water bath, cells were removed by centrifugation and acrylamide formed was estimated on Shimadzu 15 A gas chromatograph with flame ionization detector as described earlier (Ramakrishna and Desai, 1992).

A unit of enzyme is defined as the amount of bacterial cells which catalysed the formation of 1 μmol acrylamide per min. under the given assay condition.

RESULTS AND DISCUSSION

Indian Petrochemicals Corporation Limited, Baroda has an ACN manufacturing plant with an installed capacity of 30,000 tpa. While developing a biotechnological process for the treatment of wastewater generated from this plant (Ramakrishna et al, 1989) about 200 bacterial strains capable of degrading ACN and acetonitrile were isolated. Among them Arthrobacter sp. IPCB-3 was found to possess the highest (2.4 units/mg dry cells) ACN-hydratase activity (Ramakrishna and Desai, 1992). Among different nitriles, amides and acids tested, aliphatic saturated and unsaturated nitrile amides and acids supported the growth of organism, while aromatic nitriles failed to support the growth. It was interesting to note that compared to acrylonitrile, acrylamide and acrylic acid grown cells, acetonitrile, propionitrile and aetamide grown cells exhibited about 10 times higher ACN-hydratase activity and acetonitrile was found to be the best inducer (data not shown). Nitrile hydratase of Pseudomonas chlororaphis B-23 has been reported to be induced by various aliphatic nitriles, amides and acids (Yamada et al, 1986).

In P. chlororaphis B-23 (Yamada et al, 1986) and Brevibacterium sp. R312 (Nagasawa et al, 1986) nitrile hydratase has been found to be an iron containing enzyme and the addition of ferric or ferrous ions to the medium stimulated its activity. The occurrence of cobalt containing nitrile hydratase which acts upon both aromatic and aliphatic nitriles has also been demonstrated in Rhodococcus rhodochrous J1 by Nagasawa et al (1988). During our efforts to optimise a culture medium for the formation of ACN-hydratase, we observed that enzyme is induced by acetonitrile and