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Degradation of piperazine by *Paracoccus* sp. TOH isolated from activated sludge

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HIGHLIGHTS

- ▶ A novel bacterium was isolated from activated sludge for piperazine biodegradation.
- ▶ The optimal pH and temperature for degradation were 8.0 and 30 °C, respectively.
- ▶ Glucose as co-substrate plays a role in enhancement of degradation.
- ▶ The metabolic pathway for piperazine was proposed by the first time.

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ABSTRACT

Piperazine is widely used as an intermediate in the manufacture of insecticides, rubber chemicals, corrosion inhibitors, and urethane. In this study, a highly effective piperazine-degrading bacteria strain, TOH, was isolated from the acclimated activated sludge of a pharmaceutical plant. This strain, identified as *Paracoccus* sp., utilises piperazine as the sole source of carbon, nitrogen and energy for growth. The optimal pH and temperature for the growth of TOH were 8.0 and 30 °C, respectively. The effects of co-substrates and heavy metals on the degradation efficiency of piperazine were investigated. The results indicated that exogenously supplied glucose promoted the degradation of piperazine, while the addition of ammonium chloride slightly inhibited piperazine degradation. Metal ions such as Ni^{2+} and Cd^{2+} inhibited the degradation of piperazine, whereas Mg^{2+} increased it. In addition, metabolic intermediates were identified by mass spectrometry, allowing a degradation pathway for piperazine to be proposed for the first time.

1. Introduction

Alicyclic amines, also known as *N*-heterocyclic alkanes, are frequently detected in the environment (Kim et al., 2006). Piperazine (diethylenediamine) is an important alicyclic amine that is commonly used in the pharmaceutical industry in the synthesis of methyl and hydroxyl derivatives and as an intermediate in the synthetic chemical industry (Gift and Jeremy, 1994; Renata, 2005). The widespread use of piperazine has resulted in the discharge of large amounts of the compound into the environment, which eventually reach the biosphere. Several studies have demonstrated that piperazine and its derivatives are toxic and carcinogenic to humans and other living organisms (Gift and Jeremy, 1994). Therefore, the degradation of piperazine in the environment is of great concern.

The biodegradation of heterocyclic amines has drawn particular interest from an environmental point of view (Bae et al., 2009). While some amines are readily biodegradable, others (often structurally similar) are not (Bae et al., 2002). The biodegradation of secondary amines (pyrrolidine, piperidine, piperazine and morpholine) is extremely important due to their propensity for conversion (either chemically or microbiologically) to *N*-nitrosamines, many of which are potent carcinogens (Gift and Jeremy, 1994). Heterocyclic amines can be degraded by several species of bacteria belonging to the genera *Pseudomonas*, *Mycobacterium* (Kim et al., 2006; Adjei et al., 2007) and *Arthrobacter* (Bae et al., 2009). Pyrrolidine and piperidine are readily degradable over a long period of time, but morpholine and piperazine are considered recalcitrant to biodegradation (Poupin et al., 1999). Bae et al. (2009) isolated a total of 30 strains capable of degrading pyrrolidine and piperidine under denitrifying conditions; however, no isolate was able to degrade piperazine or morpholine. Kim et al. (2006) reported that *Mycobacterium* sp. strain THO100, which was isolated from a morpholine-containing culture, was able to utilise pyrrolidine, morpholine, piperidine, and piperazine as sole sources of carbon and nitrogen, but the degradation of piperazine was much lower than

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