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Reduction of antibiotics using microorganisms containing glutathione S-transferases under immobilized conditions

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ABSTRACT

The degradation of several antibiotics (tetracycline, sulfathiazole, ampicillin) was performed with immobilized bacterial cells containing the glutathione S-transferases (GSTs). Antibiotics in animal feed contaminated wastewater usually inhibit the growth of microorganisms that treat the wastewater, so a bio-friendly treatment method is required. Therefore, we have shown that the inhibitory effects of antibiotics on bacteria were reduced by microorganisms containing detoxifying enzyme GSTs by using a cell immobilizing method in a bioreactor. The initial concentrations of tetracycline, sulfathiazole and ampicillin were 100 mg/L, 100 mg/L and 50 mg/L respectively, which are typical of the range detected in pig feed in Korea. In the results, we observed the removal efficiency of tetracycline to be almost 70% with *Staphylococcus epidermidis* in the bioreactor, suggesting that this method of antibiotic removal is worthy of further study.

1. Introduction

Traditional biological treatment processes can destroy a large fraction of biodegradable organic compounds that exist in wastewater. Moreover, the biological treatment cost is much lower than physical and chemical methods. However, many hazardous compounds are not properly removed in conventional biological processes due to their toxicity or recalcitrance (Dainelli et al., 2002). Furthermore, they also have an adverse impact on the composition and activities of microorganism communities in activated sludge flocs, thus reducing the overall performance of these facilities. The removal of these compounds is a real challenge for waste treatment engineers.

Among the problematic agents in sewage, antibiotics, sourced from animal feeds are plentiful and they retain their bacteriostatic and bactericidal activities in the waste (Walsh, 2003). Antibiotics often leave treatment plants untreated and this has resulted not only in significant environmental contamination but has also inhibited microorganisms found in the biological waste treatment processes. In particular, the concentration of antibiotics in manure wastewater treatment plants is higher than other sites because they are used as growth promoters or for medicinal purposes. Knowledge of antibiotic fate in the environment has not kept pace with their increased usage (Halling et al., 1998; Kummerer, 2001).

Although there are many reports on antibiotics' effect on the environment and microorganisms, there are few reports on antibiotics' biological degradation. Elimination in the environment by other mechanisms may occur, but will not completely destroy the active compounds (Kim et al., 2007; Lindberg et al., 2007). Of course, toxic pharmaceuticals such as antibiotics are resistant to biodegrading microorganisms, as they are so effective in disrupting their growth and metabolism.

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GSTs (glutathione S-transferases) are a family of heterogeneous, multifunctional dimeric proteins, which are found in animals, plants and microorganisms. Eukaryotic GSTs are believed to be involved in the cellular detoxification of substrates of xenobiotic origin, forming glutathione adducts which are then further metabolized or even transported out of the cell (Hays and Pulford, 1995). These enzymes usually act as dimers and can be induced by a wide range of compounds, including the xenobiotics. Enzyme GSTs commonly react with electrophilic compounds to yield stable glutathione conjugates (Chesney et al., 1996).

Theoretically, enzymatic conversion of xenobiotics to nontoxic products may decrease the toxic effects, but information about the microbial conversion of these compounds is scarce. And, in contrast to the well-studied eukaryotic GSTs, bacterial GSTs have only recently attracted the interest of microbiologists. The high sequence variability found among bacterial GSTs is probably linked to their functional versatility. Even though the involvement of GSTs in many different processes has been reported, the best investigated examples of GSTs using GSH (glutathione) as co-enzyme and substrate are those concerning degradation reactions (Santos et al., 2002) such as epoxide hydrolase (van Hylckama Vlieg et al., 1998, 1999) dehalogenase (Chesney et al., 1996; McCarthy et al., 1996) and lignin etherase (Masai et al., 1993).

On previous research, we have studied the biological reduction of antibiotics by using detoxifying enzyme GSTs. With that enzyme assay *in vitro*, we have observed the feasibility of antibiotics' reduction by detoxifying enzyme GSTs (mammalian GSTs from rat) and have observed the lowering the inhibitory effect of antibiotics on microorganisms (Park and Choung, 2007). And then, through batch testing *in vivo*, we examined the reduction of antibiotics by microorganisms expressing GSTs and compared their reduction performance with bacteria that have function of susceptibility to antibiotics. We have observed the possibility of antibiotics' reduction by bacterial enzyme GSTs (Park and Choung, 2010).

So in this research, we have tried to find the effective biological transformation of antibiotics by using bacteria that are presented below. As antibiotics can disturb microorganisms' treatment in livestock farms' wastewater treatment system, lowering the inhibitory effect from the antibiotics on microorganisms in manure wastewater treatment system is an important step to enhancing the effectiveness of the manure treatment system (Andreozzi et al., 2004; Drilla et al., 2005). In this research, we have shown that the inhibitory effect of antibiotics on bacteria was reduced by immobilized microorganisms containing detoxifying enzyme GSTs. These enzymes were capable of degrading the antibiotics that act as toxic chemicals to the microorganisms to less toxic materials (Park and Choung, 2007). Therefore, to enhance the activity of microorganisms in this research we used the immobilization method that could show better performance of cells by supporting micro-environments (*i.e.* concentration gradient) which facilitate the degradation of toxic materials (Shuler and Kargi, 2002).

By immbilizing the microorganisms on alginate beads, cells are less likely to be inhibited by toxic chemicals to be processed, whilst continuous enzymatic biologic catalytic activity is supported and promoted by the benefits of the presence of the whole organism; in addition, potentially damaging enzyme purification and recovery processes are sidestepped using this approach (Shuler and Kargi, 2002; Zaborsky, 1974; Trevan, 1980; Song and Shin, 1999). Alginate was preferred over other materials due to its various advantages, such as biodegradability, hydrophilicity, rapidity of micro-sphere fabrication, low cost, and natural origin (Park and Choung, 2007). In this study, *Staphylococcus epidermidis* (ATCC: 31874) was selected to be immobilized on the alginate beads, as it is capable of degrading antibiotics with enzyme of GSTs. *Bifidobacterium thermophilum* (ATCC: 25525) was also used as a comparison, as it is susceptible to antibiotics when immobilized (Leahy et al., 2005; Simpson et al., 2004; Vuilleumier, 1997). The use of immobilized microbial cells has received increasing interest in the field of wastewater treatment, particularly in terms of their application for the degradation of numerous toxic compounds.

The present study investigates the use of immobilized bacteria in the GST-mediated detoxification of antibiotic concentrations routinely detected in pig feed slurry in Korea (National Veterinary Research Quarantine Service Official 2000–10, Assorted Feed Artificial Veterinary Pharmaceutical Additive Standard).

2. Materials and methods

2.1. Substances and reagents

Tetracycline hydrochloride min 95%, sulfathiazole, and ampicillin were supplied by Sigma. The sodium alginate and calcium chloride used were of analytical grade. Difco LB Broth (Miller, Luria-Bertani; constituents: tryptone 10 g/L, NaCl 10 g/L, yeast extract 5 g/L) was used. Solutions were freshly prepared in distilled water (Millipore, Biocel from Millipore).

2.2. HPLC analysis

The disappearance of the antibiotics was determined for all substances for which an analytical method was available by means of HPLC (mg/L conc.). HPLC analysis was performed with an HP-1100 high performance liquid chromatography system (Hewlett Packard (Agilent) Co. Waldbronn, Germany). Quantification of samples was based on peak areas. The UV detector was set at 254 nm, and C18 (250 mm × 4.6 mm, 5 um) column was used in this experiment. A Gradient of 100% acetonitrile (A) and 0.1% Acetic acid in water (B) (0–25 min-11% A, 29 min-14.5% A, 49 min-22% A, 50 min-0% A) was employed.

2.3. Culturing, immobilization and transformation batch test

Bifidobacterium thermophilum (ATCC: 25525), and *Staphylococcus epidermidis* (ATCC: 31874) that came from the KCCM (Korean Culture Center of Microorganism), were cultured at 30 °C with LB broth for 48 h under the same condition. After cells were centrifuged (1300 rpm, 10 min), clear solution was discarded and cells were washed with sterilized 0.9% NaCl. After being washed twice with 0.9% saline, the immobilized cells were incorporated into a little medium and deposited in the