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Short Communication

Determination of cephalosporin acylase activity by biological and colorimetric method in bacteria

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The effective production of 7-aminocephalosporanic acid (7-ACA) is a matter of concern in the pharmaceutical industry because it is a starting material for the synthesis of semi synthetic cephalosporin. Therefore screening for new source of cephalosporin acylase positive bacteria is very important. The cephalosporin acylase can be found in several *Pseudomonas* sp. and other bacteria. To facilitate the attempts of obtaining the microorganisms with higher cephalosporin acylase activity from natural environments, development of new and specific methods for screening environmental microorganisms with cephalosporin acylase activity is very important. In this study, a biological and colorimetric method was evaluated for determination of cephalosporin acylase product in bacteria. Samples were cultured in general and selective media, and the routine biochemical laboratory tests were used for diagnosis of *Pseudomonas* sp. All of the isolated strains were tested for cephalosporin acylase by a biological and colorimetric method. A total of 180 *Pseudomonas* sp. out of 350 samples were isolated. Two strains capable of producing cephalosporin acylase were identified from 180 candidates. The *Pseudomonas* bacteria isolated in this study is a source for cloning and cephalosporin acylase enzyme production.

Key words: Cephalosporin acylase, *Pseudomonas*, 7-aminocephalosporanic acid.

INTRODUCTION

The effective production of 7-aminocephalosporanic acid (7-ACA) is a matter of concern in the pharmaceutical industry because it is a starting material for the synthesis of semisynthetic cephalosporins, which are the best-sold antibiotics worldwide, with global sales of $8.3 billion of $466.3 billion of the total pharmaceutical market in 2003. Semisynthetic cephalosporins are made by the modification of the side chains of positions 3 and 7 of 7-ACA, which are commercially supplied mainly by the chemical deacylation of cephalosporin C (CPC), produced by the fungus *Acremonium chrysogenum* (Velasco et al., 2000; Oh et al., 2003). However, the chemical process requires several complicated steps using toxic compounds and produces a lot of chemical wastes. The innovation of an enzymatic process involving two enzymes has recently become the new deacylation process of CPC on an industrial scale. This enzymatic process involves no toxic raw materials, proceeds under mild reaction conditions, and reduces waste significantly. In this process, D-amino acid oxidase converts CPC to 7β-(5-carboxy-5-oxopentanamido)-cephalosporanic acid, followed by autoconversion to glutaryl-7-ACA (GL-7-ACA). Cephalosporin acylase then deacylates GL-7-ACA to 7-ACA (Kumar et al., 1993; Li et al., 2001; Battistel et al., 1998). The critical enzyme of this bioprocess is cephalosporin acylase, and extensive screening for this enzymatic activity is extremely important. However, this enzyme has been found only in a limited number of bacterial strains (Franzosi et al., 1995; Lee et al., 1998; Sonawane, 2006; Chen et al., 2002; Elander, 2003).

The cephalosporin acylase can be found in several *Pseudomonas* sp. (such as *Pseudomonas putida*, *Pseudomonas cepacia* BY21, *Pseudomonas nitroreducens*, *Pseudomonas syringae* p.SE83, p.V22, p.SY-77, p.sp.130, *Bacillus cereus*, *Achromobacter xylosooxidans*, *Bacillus* sp. and *Pseudomonas paucimobilis*) and other bacteria (Linda et al., 2002; Shi et al., 1991; Zha et al.,

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2003). Therefore isolation of cephalosporin acylase positive *Pseudomonas* sp. from clinical and environmental specimens is very important. The discovery of such an industrially useful cephalosporin acylase is dependent on the availability of a sensitive assay which allows detection of even low acylase activity in crude biological samples. Different enzymatic methods for detecting microorganisms capable of producing cephalosporin C (CPC) acylase and/or 7-(4-carboxybutanamido) cephalosporanic acid (GL-7-ACA) acylase have been developed (Zhu et al., 2003; Ishii et al., 1994; Plhácková et al., 2003; Velasco et al., 2000). For example a method is based on the degradation of 2-nitro-5-(6-bromohexanoylamino) benzoic acid (NBHAB), a chromogenic substrate, into yellow 2-nitro-5-aminobenzoic acid by the action of the CPC acylase or the GL-7-ACA acylase (Aramori et al., 1991; Akio and Keh-ichi, 1985). In this study we used a modified biological and colorimetric method for determination of cephalosporin acylase in *Pseudomonas* sp.

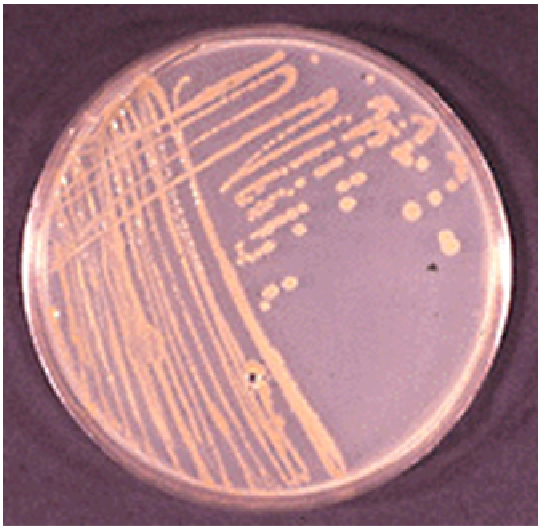

Figure 1. *Pseudomonas* sp. in tryptic soy agar.

MATERIAL AND METHODS

A total of 350 clinical (patient samples) and environmental (water, soil and hospital environment) samples were screened for *Pseudomonas* sp. The samples were cultured in general and selective media, soil isolates were obtained by plating diluted soil suspension onto medium, and the routine biochemical laboratory tests (such as oxidase test, oxidation-fermentation test, etc) were used for diagnosis of *Pseudomonas* sp. All of the isolated *Pseudomonas* sp were tested for cephalosporin acylase by partially modification biological and colorimetric method (Akio and Keh-ichi, 1985). For detection of microorganisms with cephalosporin acylase activity, bacteria were grown for 3 day; then each colony on the plate was exposed to chloroform vapor for 15 min, scraped with a toothpick, and suspended in 100 ul of GL-7ACA (1 mg of GL-7ACA or cephalosporin C per ml of 0.1 M phosphate buffer [pH 7.0]) in a well of a microtiter dish. The mixture was incubated at 37°C for 60 min, and the reaction was terminated by addition of 120 µl of acetic acid : 4.25 M NaOH (2:1), followed by addition of 40 µl of p-dimethylaminobenzaldehyde (0.5% in methanol). P-dimethylaminobenzaldehyde makes a yellow condensation product with 7ACA or cephalosporin C. The absorbance was measured at a wavelength of 414 nm using the buffer solution of the respective samples as reference in spectrophotometric assay.

RESULTS AND DISCUSSION

In this study, a total of 180 *Pseudomonas* sp. (105 isolates from clinical samples, 50 from hospital environment samples, 17 from water samples, and 8 from soil samples) were isolated (Figure 1). All of isolated *Pseudomonas* sp. were tested for cephalosporin acylase by biological and colorimetric method. Two strains capable of producing cephalosporin acylase were selected from 180 of candidates by this method.

Medically useful semisynthetic cephalosporin antibiotics are made from precursor 7-aminocephalosporanic acid (7-ACA). Cephalosporin acylase (CA), which catalyzes hydrolysis of both glutaryl-7-aminocephalosporanic acid (GL-7ACA) and cephalosporin C (CPC) to 7-ACA, is thus a very important enzyme for producing semi-synthetic beta-lactam antibiotics (Shi et al., 1991). To facilitate the attempts of obtaining the microorganisms with higher CA activity from natural environments, a new and specific method for screening environmental microorganisms with cephalosporin acylase activity was developed. The core part of cephalosporin was replaced by 6-amino penicillinic acid (6-APA) to generate new substrates glutaryl-6-APA and adipoyl-6-APA for screening. *Serratia marcescens* that is sensitive to 6-APA and resistant to penicillin G, glutaryl-6-APA and adipoyl-6-APA was used as an indicator strain in an overlaid-agar screening system. Others enzymatic methods is based on the degradation of 2-nitro-5-(6-bromohexanoylamino)benzoic acid (NBHAB), a chromogenic substrate, into yellow 2-nitro-5-aminobenzoic acid by the action of the CPC acylase or the GL-7-ACA acylase (Aramori et al., 1991; Akio and Keh-ichi, 1985). In this study we used a modified bBiological and colorimetric method for determination of cephalosporin acylase in *Pseudomonas* sp. The results indicated that this method is a useful and easy for detecting microorganisms capable of producing cephalosporin acylase.

The pseudomonas are the largest group of gram negative aerobic heterotrophic bacteria found in soil; several species have been described as aquatic, and many are associated with the rhizosphere of plants. They have been widely studied because of the amazing metabolic diversity within this genus. The pseudomonades have been heavily used in biological disease control and bioremediation. The literature on degradation of organic compounds probably contains more citations on pseudomonas than any other bacterial genus (Yong and Jian, 1999; Pollegioni et al., 2005). Ten genes encoding cephalosporin acylases from different sources have been cloned (Li et al., 1998; Wang and Zheng, 2002;

Rajendhran and Gunasekaran, 2007; Mao et al., 2002).

The gene encoding a cephalosporin acylase from *Pseudomonas* sp. 130 (CA-130) has been expressed in *Escherichia coli* and some properties of the enzyme studied. We isolated 2 cephalosporin acylase positive *Pseudomonas* sp. from clinical samples by biological and colorimetric; therefore, these are new gene source for cloning.

Conclusion

To facilitate the attempts of obtaining the microorganisms with higher cephalosporin acylase activity from natural environments, a new and specific method for screening environmental microorganisms with cephalosporin acylase activity is very important. The results of our study showed that the biological and colorimetric method developed in this study is a useful and simple method for determination of cephalosporin acylase activity in bacteria

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