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Overexpression and characterization of a new organic solvent-tolerant esterase derived from soil metagenomic DNA

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ABSTRACT

In this study, an esterase, designated EstC23, was isolated from a soil metagenomic library. The protein was amenable to overexpression in *Escherichia coli* under control of the T7 promoter, resulting in expression of the active, soluble protein that constituted 30% of the total cell protein content. This enzyme showed optimal activity at 40 °C and retained about 50% maximal activity at 5–10 °C. EstC23 showed remarkable stability in up to 50% (v/v) benzene and alkanes (high log*P* solvents). When incubated for 7 days in the presence of 50% benzene or alkanes, the enzyme maintained its 2–3 fold elevated activity. The purified enzyme also cleaved sterically hindered esters of tertiary alcohols. These results indicate that EstC23 has potential for use in industrial processes.

1. Introduction

Enzymes are highly efficient biocatalysts for the development of environmentally friendly industrial processes (Alcalde et al., 2006). Modern industry has an increasing demand for novel biocatalysts, which has prompted the development of novel approaches to isolate biocatalyst-encoding genes. Metagenomics is an alternative approach to conventional microbial screening methods (Daniel, 2005; Lorenz and Eck, 2005; Streit and Schmitz, 2004). Numerous enzymes with biotechnological importance, such as lipase, epoxide hydrolase, α-amylase, 2,5-diketo-D-gluconic acid reductases and nitrile hydratases, have been identified by metagenomic approaches (Kotik, 2009).

Esterase (EC 3.1.1.1) catalyzes ester hydrolysis, ester synthesis, transesterification, and other reactions. It is useful in synthesizing optically pure compounds, perfumes, and antioxidants (Panda and Gowrishankar, 2005). Most of these reactions occur in non-aqueous environments, but the high enzyme deactivation rates in organic solvents limits the use of enzymes (Dordick, 1989). To overcome this problem, several novel esterases have been isolated that display resistance to organic solvents, including esterases from *Pyrobaculum calidifontis*, *Bacillus licheniformis S-86*, *Burkholderia cepacia strain ST-200*, *Arthrobacter nitroguajacolicus*, and *Bacillus* sp. 4 (Ateslier and Metin, 2006; Hotta et al., 2002; Schütte and Fetzner, 2007; Takeda et al., 2006; Torres et al., 2009). These enzymes show remarkable stability and activity in up to 50% (v/v) polar organic solvents (log*P* < 2), such as dimethylsulfoxide, methanol, acetonitrile, acetone, and propanol. There are no other reports of esterases that satisfactorily maintain their activity in the presence of high log*P* (>2.0) organic solvents. In this article, we report the cloning, overexpression, and biochemical enzymatic characterization of a novel organic solvent-resistant esterase, EstC23, from a soil-derived metagenomic library. The enzyme can be overexpressed in *Escherichia coli*, shows high stability in the presence of *n*-hexane (log*P* = 3.1), highly active in low temperature, and is able to cleave esters of tertiary alcohols.

2. Methods

2.1. Bacterial strains, plasmids, and growth conditions

The *E. coli* strains DH5α and pBluescript SK(+) (Stratagene, La Jolla, CA, USA) were used as the host and vector, respectively, for cloning the esterase, and the plasmid pBluescript SK(+) was used for gene cloning and sequencing. *E. coli* strain BL21 (DE3) (Stratagene, La Jolla, CA, USA) and pET-21a(+) (Novagen, Inc., Madison, WI) were used as the host and vector, respectively, for heterologous expression of the esterase. *E. coli* cells were grown in Luria–Bertani (LB) medium at 37 °C, supplemented with 100 μg/ml ampicillin.

2.2. Soil metagenomic library construction and screening

The soil was sampled at a depth of 30 cm on Mount Fanjing in the city of Tongren, Guizhou province, China. The soil's metagenomic DNA was isolated by Mo Bio Power Soil DNA isolation kit (MO BIO Laboratories, Inc., Carlsbad, CA). In order to remove any

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