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Journal of Biotechnology 121 (2006) 508–516
Journal of BIOTECHNOLOGY
www.elsevier.com/locate/jbiotec

Characterization of D-ribose biosynthesis in *Bacillus subtilis* JY200 deficient in transketolase gene

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Received 7 April 2005; received in revised form 6 July 2005; accepted 2 August 2005

Abstract

D-Ribose is a functional five-carbon sugar, which has been used for the commercial production of riboflavin. Mechanisms of D-ribose biosynthesis from xylose were investigated in the genetically engineered *Bacillus subtilis* JY200 with a deficiency in transketolase. A transketolase gene (*tkt*) disruption cassette in plasmid pUNKC was introduced into the chromosomal *tkt* gene in the wild type *B. subtilis* 168. Analysis of culture broth by thin layer chromatography confirmed that the disruption of *tkt* allowed *B. subtilis* JY200 to produce D-ribose. In a batch culture of *B. subtilis* JY200, a loss of cell viability was observed after glucose depletion. Fed-batch cultivation by feeding 400 g l^{-1} glucose solution as a co-substrate was carried out to supply energy to xylose metabolism and to maintain cell viability throughout cultivation. Fed-batch cultivation of *B. subtilis* JY200 in a complex medium containing 11 g l^{-1} xylose and 5 g l^{-1} glucose initially gave the best result of 10.1 g l^{-1} D-ribose concentration, 0.24 g g^{-1} D-ribose yield and 0.29 g l^{-1} h^{-1} productivity, corresponding to 40-, 5- and 12-fold increases compared with those in the batch culture. A kinetic study of D-ribose production in fed-batch cultivations of *B. subtilis* JY200 suggested that xylose uptake might be critical to maximize D-ribose biosynthesis from xylose.

Keywords: D-Ribose; Xylose; *Bacillus subtilis*; Transketolase; Fed-batch culture; Kinetics

1. Introduction

D-Ribose is an important aldo-pentose present as the ribosyl residue of biomolecules such as ATP, RNA, NAD, NADP, FAD and coenzyme A. In the context of commercial applications, D-ribose has long served as a starting material for the chemical synthesis of riboflavin, which can be used not only for pharmaceuticals but also for animal feed additives, cosmetics and foods (Park et al., 2004). D-Ribose itself has a cardioprotective effect on the adenine nucleotide metabolism

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doi:10.1016/j.jbiotec.2005.08.003

in the heart muscle of rat (Zimmer, 1983). Supplementation of D-ribose increased the skeletal muscle adenine salvage rate during recovery from the intense contraction (Zarzeczny et al., 2001).

Biological production of D-ribose was mediated by the dephosphorylation of D-ribose-5-phosphate, which could accumulate by the deficiency of transketolase activity in the pentose phosphate pathway (Park and Seo, 2004). Transketolase deficient strains derived from several microorganisms were screened. An *Escherichia coli* mutant needed aromatic amino acids and several ring-type vitamins or shikimic acid to grow on a minimal medium (Josephson and Frankel, 1969). An *E. coli* mutant deficient in two isoenzymes (TktA and TktB) required pyridoxine (Vitamin B6) for growth (Zhao and Winkler, 1994). But the accumulation of D-ribose was not detected either intracellularly or extracellularly. A transketolase-negative mutant of *Salmonella typhimurium* and a double deletion mutant of two transketolase genes (*TKL1* and *TKL2)* in *Saccharomyces cerevisiae* had the same auxotrophic characteristics as other organisms (Eidels and Osborn, 1971; Schaaff-gerstenschläger et al., 1993) but the production of D-ribose was not reported. A transketolase mutant of *Corynebacterium glutamicum* produced 2 g l^{-1} D-ribulose, but D-ribose synthesis was not observed (Ikeda et al., 1998). Inosine-producing *Bacillus* species characterized as transketolase mutants could secrete D-ribose into culture broth (Sasajima and Yoneda, 1971). Chemical mutation of transketolase in *Bacillus subtilis*, *B. pumilus*, *Brevibacterium thiogenitalis*, *B. ammoniagenes*, *Arthrobacter globiformis*, *Aerobacter aerogenes* and *Micrococcus denitrificans* revealed that only *Bacillus* species could accumulate D-ribose (Sasajima and Yoneda, 1989; De Wulf and Vandamme, 1997). Many attempts using the transketolase deficient mutants of *Bacillus* species have been made to develop the process of D-ribose mass production. *B. subtilis* ATCC 21915 produced D-ribose from glucose with 48% yield (De Wulf and Vandamme, 1997). Various carbon sources (glucose, sorbitol, mannitol and maltose) were useful for D-ribose biosynthesis and corn steep liquor was effective for large-scale production (Sasajima and Yoneda, 1971). Supplementation of aromatic amino acids increased the yield of D-ribose synthesis and suppressed the formation of gluconic acid (Kishimoto et al., 1990). When a D-ribose producing mutant was grown on glucose plus a second substrate (D-gluconate, D-xylose, L-arabinose and xylitol), catabolite repression on the utilization of the second substrate was not found (De Wulf and Vandamme, 1997). Our group isolated a transketolase deficient *B. subtilis* strain, which was able to produce 23 g l^{-1} D-ribose from xylose, and optimized a biological process of D-ribose production in batch and fed-batch cultivations (Park and Seo, 2004; Park et al., 2004).

This study aimed to develop a transketolase negative system by genetic engineering of the wild type *B. subtilis* 168 strain and to characterize the modified *B. subtilis* JY200 system for D-ribose production from xylose in batch and fed-batch cultivations.

2. Materials and methods

2.1. Bacterial strains and plasmids

All bacterial strains and plasmids used and constructed in this study are described in Table 1. *E. coli* DH5α was used as a host strain for cloning and *B. subtilis* 168 (ATCC 23857) was purchased from American Type Culture Collection (ATCC, Manassas, U.S.A.) and used as a parental strain to construct the transketolase negative system.

2.2. Culture conditions

E. coli and *B. subtilis* strains were grown in LB medium (10 g l^{-1} Bacto-tryptone (BD, Sparks, MD, U.S.A.), 5 g l^{-1} yeast extract (BD, U.S.A.), 10 g l^{-1} NaCl) at 37 °C. Transformants were selected against 50 mg l^{-1} ampicillin for *E. coli* or 50 mg l^{-1} kanamycin for *B. subtilis*. MY medium containing 10 g l^{-1} yeast extract, 5.0 g l^{-1} $(NH_4)_2SO_4$, 6.4 g l^{-1} KH_2PO_4, 0.5 g l^{-1} $MgSO_4{\cdot}7H_2O$, 0.1 g l^{-1} citric acid, 5 mg l^{-1} thiamine HCl, 40 mg l^{-1} tryptophan, 10 ml l^{-1} trace element solution (5 g l^{-1} $FeCl_3{\cdot}6H_2O$, 0.55 g l^{-1} $CaCl_2{\cdot}2H_2O$, 0.4 g l^{-1} $MnCl_2{\cdot}4H_2O$, 0.25 g l^{-1} $ZnCl_2$, 0.3 g l^{-1} H_3BO_4, 0.25 g l^{-1} $Na_2MoO_4{\cdot}2H_2O$, 0.25 g l^{-1} $CoCl_2{\cdot}6H_2O$, 0.15 g l^{-1} $CuCl_2{\cdot}2H_2O$ and 0.84 g l^{-1} EDTA·disodium salt·$2H_2O$) and carbon sources (xylose and glucose) were used for D-ribose production. Batch culture was carried out in a 3.7 l jar bioreactor (Type ALF, Bioengineering AG, Switzerland) with 1 l of MY medium