Bioresource Technology 101 (2010) 9734–9741

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Bioconversion of L-arabinose and other carbohydrates from plant cell walls to α-glucan by a soil bacterium, *Sporosarcina* sp. N52

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ARTICLE INFO

Article history:
Received 9 April 2010
Received in revised form 22 July 2010
Accepted 22 July 2010
Available online 3 August 2010

Keywords:
Aerobic treatment
L-Arabinose
Distillery waste
α-Glucan
Sporosarcina

ABSTRACT

A Gram-positive bacterium, N52, that produces intracellular glucan from L-arabinose, was isolated from soil and identified as *Sporosarcina* sp. according to rRNA gene sequence analysis and physiological/biochemical characterizations. Glucan production by N52 increased significantly in the exponential phase of aerobic liquid culture and was maintained at the highest level during the stationary phase, reaching 37.0% of the cell dry weight. The glucan was also produced from other tested sugars originating from plant cell walls and was composed exclusively of α-1,4- and α-1,6-glucosidic linkages. When distillery waste was treated with N52 for 72 h, the total organic carbon (TOC), chemical oxygen demand and biochemical oxygen demand were reduced by 42.6%, 45.9% and 82.5%, respectively. Bacterial cells accumulated 31.9% of glucan per cell dry weight, fixing 16.0% of the TOC in the soluble fraction. Thus, this strain could provide us with a new process for waste management, including the bioconversion of organic materials to the valuable byproduct, α-glucan.

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1. Introduction

Effluents originating from industrial fermentation processes, including bioethanol production, generally contain large amounts of organic materials that can cause extensive water pollution when they are directly discharged. Treatments to eliminate organic materials, therefore, should be performed before discharge. Such treatments can be performed by either anaerobic or aerobic processes with the aid of microbial conversion activities (Pant and Adholeya, 2007).

Anaerobic digestion is widely accepted as the first treatment step in distilleries, because this process can be operated at high organic loading rates. Anaerobic digestion also converts a significant portion (>50%) of chemical oxygen demand (COD) to biogas that can be used as fuel (Wilkie et al., 2000). However, there are certain problems with anaerobic digestion such as the requirement for long incubation periods and the generation of erosive and hazardous hydrogen sulfide. In addition, the secondary effluent after anaerobic digestion still contains a high organic content, requiring an additional aerobic digestion step for further reduction of the organic content before discharge.

Alternatively, aerobic treatments have also been adopted to efficiently reduce the organic content of effluent, since all readily degradable organic materials can be decomposed into inorganic forms under ideal conditions. Kumar and Viswanathan (1991) isolated bacterial strains from sewage and acclimatized the isolates by increasing concentrations of distillery waste. These strains were able to reduce the COD by 80% in 4–5 days; however, no valuable product could be obtained from the treatment other than carbon dioxide and volatile acids (Kumar and Viswanathan, 1991). Therefore, the simultaneous production of valuables during rapid aerobic degradation of organic materials could be a breakthrough for improving the process for distillery waste treatment.

The quality of distillery waste would vary depending on the feedstocks and ethanol production processes, which would determine the waste treatment process. Highly-nutritious distillery wastes such as corn-condensed distiller's solubles are suitable for aerobic fermentation to produce valuable products of alternan and pullulan (Leathers and Gupta, 1994). In contrast, relatively nutrient-poor distillery waste could be produced in the course of plant cell wall degradation, in which hydrolysates of cell wall polysaccharides could be the main carbohydrate sources. A widely-used ethanol-producing yeast, *Saccharomyces cerevisiae*, cannot

Abbreviations: BOD, biochemical oxygen demand; CARV, conversion after reduction of viscosity; COD, chemical oxygen demand; OD, optical density; PBS, phosphate buffered saline; TOC, total organic carbon.

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doi:10.1016/j.biortech.2010.07.092

utilize sugars originating from the polysaccharides in plant cell walls, such as D-xylose, L-arabinose, D-galacturonic acid and cellobiose, resulting in the accumulation of these sugars in distilleries. Although D-xylose-fermenting yeasts such as *Pichia stipitis* and *Candida glabrata* could produce bioethanol from D-xylose, they should overcome several problems for the practical use; such as the requirement of oxygen during fermentation, a low ethanol-tolerance and slow D-xylose consumption (Agbogbo and Coward-Kelly, 2008). Genetic engineering of microorganisms is proved to be a powerful tool to convert D-xylose and L-arabinose to bioethanol in a laboratory scale (Bera et al., 2010), whereas it would take time to achieve the commercialization mainly due to public concerns about the genetically-modified (GM) microorganisms. The cost reduction for bioethanol production from wider range of sugars with the aid of the GM microorganisms should be a trade-off with the introduction of special equipment for their containment throughout the process.

In the sugars originating from the polysaccharides in lignocellulosic feedstocks such as corn stover, rice straw and sugarcane bagasse, D-xylose should be the main target for technology development for bioethanol production due to its abundance in the feedstocks (Agbogbo and Coward-Kelly, 2008). In contrast, L-arabinose could be a minor component in various feedstocks, found not only in hemicellulose in monocotyledonous feedstocks but also in pectin in dicotyledonous feedstocks. We observed free L-arabinose at a low concentration in the distillery waste, when the conversion of sweet potato to bioethanol was performed with the CARV process (Srichuwong et al., 2009), in which enzymatic means was adopted for viscosity reduction of the mashed sweet potato (in this study). Instead of the use of GM microorganisms for ethanol production from the minor sugar, in this study we aimed at the development of a new method for its microbial conversion to valuable products and their accumulation in the microbial cells, so as to concentrate the products by gravity and to reduce the COD as well.

We, therefore, screened microorganisms that can aerobically convert L-arabinose and other sugars from plant cell walls to intracellular glucans. Glucans in the cells could readily be converted to glucose, an energy form suitable for use as feed or nutrients, by enzymatic- or chemical means after cell wall disruption. We also performed an aerobic treatment of a distillery waste after bioethanol production from sweet potato, using the screened bacterium, *Sporosarcina* sp. N52.

2. Methods

2.1. Isolation of microorganisms

Soil samples (0.1 g, collected from Tsukuba, Ibaraki, Japan) were suspended in 1 ml of 10 mM phosphate buffered saline (PBS, pH 7.3) and incubated at 30 °C for 15 min. After serial dilution of the suspension with the buffer, the aliquots (50 µl) were spread onto plates containing M1 medium (0.4% tryptone, 0.2% yeast extract and 1% L-arabinose, pH 6.8) with agar (2%), and the plates were incubated at 30 °C for 24 h. Morphologically different colonies were selected, inoculated into 5 ml of M1 medium for cultivation at 30 °C for 24 h with agitation at 250 rpm. The cell growth was monitored by measuring the optical density at 600 nm (OD 600) using a spectrophotometer (Spectra max plus, Molecular Devices, Sunnyvale, CA).

For evaluation of glucan production, cells from an aliquot (1 ml) of the culture after centrifugation at 8000*g* for 15 min were rinsed twice with 1 ml PBS. The cells were suspended in 0.1 ml of 9% (v/v) sulfuric acid for hydrolysis at 100 °C for 1 h. Then, the samples were neutralized at room temperature with 1 M NaOH (0.3 ml), and centrifuged at 15,000*g* for 5 min. The glucose concentration of the supernatant was determined with a Glucose C-II Test Wako (Wako Pure Chemical Industries, Osaka, Japan). The amount of glucan was estimated as milligrams of produced glucose per milliliter of culture.

L-Arabinose was analyzed by high-performance liquid chromatography with a LC-20 AD instrument (Shimadzu Corporation, Kyoto, Japan) equipped with an refractive index detector (RID-10A, Shimadzu Corporation) and an Aminex HPX87P column (Bio-Rad Laboratories, Hercules, CA) operated at 60 °C with Milli-Q water at a flow rate of 0.6 ml min^{-1}.

2.2. Growth conditions

A single colony of the N52 was inoculated into 10 ml of M1 medium. Preculture was performed at 30 °C for 24 h with agitation at 250 rpm. Then, the cells were collected by centrifugation at 8000*g* for 15 min, washed twice with PBS, and resuspended in 10 ml of PBS. A portion of the suspension was inoculated into 10 ml of M1 medium to yield an initial OD 600 of 0.1, and the cells were cultivated at a defined temperature and time with agitation at 250 rpm.

The pH of M1 medium was adjusted to 4–10 by addition of either 1 M NaOH or 1 M HCl, in order to evaluate the effect of medium pH on the growth of N52. Another series of M1 media supplemented with NaCl (1%, 3%, 5%, 8%, 10% and 15%) was prepared to determine the strain's salt tolerance. In both experiments, bacteria were cultivated at 30 °C for 72 h. The effect of temperature on growth of N52 was evaluated by cultivation in M1 medium at 4, 10, 20, 30, 40 or 50 °C for 72 h.

For analysis of substrate specificity, the precultured N52 cells were inoculated into 10 ml of M9 medium (42 mM Na_2HPO_4, 22 mM KH_2PO_4, 8 mM NaCl, 18 mM NH_4Cl, 1 mM $MgSO_4$, 0.1 mM $CaCl_2$ and 0.001% thiamine, pH 6.8) supplemented with 1% of D-glucose, D-mannose, D-fructose, D-galactose, L-rhamnose, D-galacturonic acid, D-glucuronic acid, L-arabinose, D-xylose, cellobiose or sucrose, as sole carbon sources, to yield an initial OD 600 of 1. Cultivation was performed at 30 °C for 72 h. Then, N52 cells were collected to determine cell biomass and glucan production. Cell biomass was determined after drying the cells at 75 °C for 24 h, and glucan production was determined after sulfuric-acid treatment as described above.

For glucan extraction, precultured N52 cells were inoculated into 200 ml of M1 medium (initial OD 600 of 0.1) in a 500 ml flask and cultivated at 30 °C for 72 h. Cells were collected, washed and freeze-dried at −80 °C. The cells were stored at room temperature prior to glucan determination.

2.3. Identification of the microorganism

The isolated strain was identified using 16S rRNA gene sequence analysis. A 16S rRNA gene was amplified by polymerase chain reaction using bacterial universal primers 27f (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1522r (5′-AAGGAGGTGATCCANCCRCA-3′). Genomic DNA was extracted from N52 cells by a ZR Funfal/Bacterial DNA Kit (Zymo Research Corporation, Orange, CA) according to the instruction manual and used as template for 16S rRNA gene amplification. PCR thermal conditions were as follows: 94 °C for 3 min, 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 1 min, extension at 72 °C for 2 min, and a final extension at 72 °C for 7 min. An amplification product of about 1.5-kb was separated by agarose gel electrophoresis, purified and sequenced. The 16S rRNA gene sequence was analyzed by the dideoxy chain termination method using the BigDye® Terminators v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster, CA) and an ABI Prism 3100 Genetic Analyzer (Applied Biosystems). The 16S rRNA

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