Biocatalysis and Agricultural Biotechnology ∎ (∎∎∎∎) ∎∎∎–∎∎∎

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

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

Original Research Paper

Production of polyol oils from soybean oil by bioprocess: results of microbial screening and identification of positive cultures ☆

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ARTICLE INFO

Article history:
Received 28 July 2014
Accepted 20 August 2014

Keywords:
Soypolyol oils
Soybean oil
Bioconversion
Hydroxyl triacylglycerides
Hydroxyl diacylglycerides
Microbial identification

ABSTRACT

Recently we reported methods for microbial screening and production of polyol oils from soybean oil through bioprocessing (Hou and Lin, 2013). Soy-polyol oils (oxygenated acylglycerols) are important starting materials for the manufacture of polymers such as polyurethane. Currently, they are produced by a two-step chemical process involving epoxidation and subsequent opening of the oxirane ring. A total of 650 cultures isolated from soil samples collected from a biodiesel plant in Iowa and from soil and water samples collected from Peoria, IL were screened for the production of polyol oil from soybean oil. Out of these 650 cultures screened, 50 cultures were positive for converting soybean oil to two product groups: diacylglycerol polyol oil (polyol DAGs) and diacylglycerol (DAD) with normal fatty acids. These two product groups can be separated by a two-solvent system TLC. In this study, we identified the 11 most active of these positive cultures using 16S rRNA gene analysis. The 11 strains investigated were found to be bacterial and no active yeast strains were found in the present study. Phylogenetic analysis identified two strains A01-35 and E03-25 as *Acinetobacter haemolyticus*, a single strain was identified as *Pseudomonas aeruginosa*, the other active strains were identified as *Pseudomonas protegens.*

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1. Introduction

Triacylglycerols (TAG) containing hydroxy fatty acids, e.g., castor oil, have many industrial uses such as the manufacture of aviation lubricant, plastic, paint, nylons and cosmetics, because of the hydroxyl groups on the fatty acid (FA) constituents. Castor oil is the only commercial source of TAG containing hydroxy FA. Diacylglycerols (DAG) containing hydroxy FA can also be used in the above mentioned industries. DAG are the intermediate molecules in the biosynthesis of TAG and phospholipids. In the 2002–2003 growing season, 30.6 million tons of soybean oil were produced worldwide, constituting about half of worldwide edible vegetable oil production, and 30% of all fats and oils produced, including animal fats and oils derived from tropical plants (USDA, 2004). The major use of this oil is for food products such as shortenings, salad and cooking oils, and margarines. Soy-polyols (oxygenated TAG) are important starting materials for the manufacture of polymers such as polyurethane. Currently, they are produced by a two-step chemical process involving epoxidation and then the subsequent opening of the oxirane ring (Hamdy, 2006; Demosthenes, 2009).

We have been performing research to discover new uses and novel materials from soybean oil through bioprocesses. Our previous research established that microbial systems can convert FA to ricinoleic acid-type oxygenated FA, including many bioactive FA such as monohydroxy-, dihydroxy- and trihydroxy-unsaturated FA, tetrahydrofuranyl unsaturated FA, and diepoxy bicyclic unsaturated FA (Hou et al., 1993, 1998, 2001; Hou, 1994, 1995, 1997; Gardner et al., 2000; Iwasaki et al., 2002; Hosokawa et al., 2003a, 2003b, 2003c; Hou and Hosokawa, 2005; Chang et al., 2007; Suh et al., 2011; Bae et al., 2010). The bio-based polymer industry, however, requires acylglycerol (soybean oil) polyols and not FA polyols. Recently we developed a new method to screen microorganisms for the direct production of polyol oils from soybean oil (Lin et al., 2012; Hou and Lin, 2013). We found that TLC with two step solvent system development or HPLC with a C18 reverse phase column and a linear gradient of 100% methanol to 100% 2-propanol could separate the polyol products from the soybean oil substrate. We also discovered new DAGs with normal fatty acids among our soybean oil bioconversion products (Lin et al., 2012). A total of 650 microbial cultures isolated from soil samples collected from a biodiesel plant in Iowa and from soil and water samples collected in the area around Peoria, IL were screened for

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http://dx.doi.org/10.1016/j.bcab.2014.08.004
1878-8181/Published by Elsevier Ltd.

potential bioconversion activity. Of the 650 cultures screened, 50 cultures were observed to convert soybean oil to polyol oil products. In this study, we report the results of the microbial screening experiments and the identification of the 11 most active microbial strains using 16S rRNA gene sequence analysis.

2. Materials and methods

The FA used in this study were purchased from NU-Check-Prep Inc. (Elysian, MN, USA). Soybean oil, castor oil, yeast extract, Silica gel 60A 70–230 mesh, all solvents and chemicals were purchased from Sigma (St. Louis, MO). Thin-layer precoated Kieselgel 60F_{254} plates were obtained from EM Science (Cherry Hill, NJ).

Microorganisms were isolated from soil and water samples collected from the vicinities of a biodiesel manufacturing plant (Rooney et al., 2009a) in Ralston, Iowa, and Peoria, Illinois, U.S.A. Each culture was selected from a single colony on a TGY agar plate and was judged to be a pure culture based on microscopic observations. The cultures were grown aerobically at 28 °C with orbital shaking at 200 rpm in the culture medium used in our previous research (Hou and Lin, 2013).

Bioconversion experiments and products extraction were conducted as described in our previous paper (Hou and Lin 2013).

TLC plates were developed with a two stage development procedure (Hou, 1997; Hou and Lin, 2013) except that benzene was replaced by toluene: (i) toluene/ether/ethyl acetate/acetic acid (80:10:10:1 v/v) was developed with the solvent was 8.5 cm above the origin, and then (ii) hexane/ether/formic acid (80:20:2 v/v) developed in the same direction to the top of the TLC plate. The plate was air-dried before the second development. After development, products on the plate were identified first by exposure to iodine vapor and then by spraying with 60% aqueous sulfuric acid and charring.

The crude extracts were analyzed on a Shimadzu model SCL-10A HPLC equipped with a SIL-10AF Auto Injector (Colombia MO). The HPLC method used a linear gradient starting with 100% methanol going to 100% 2-propanol over 60 min at 1 mL/min flow rate on a Supelco 25 cm × 4.5 mm, 5 μ C18 reverse phase column (Hou and Lin, 2013). Detection was monitored by Evaporative Light Scattering Detector, Alltech Model 500 ELSD (Deerfield, IL USA). The drift tube temperature of the ELSD was set at 80 °C. The nitrogen gas flow of the nebulizer of the ELSD was set at 1.0 L/min. The nitrogen pressure on the regulator of the nitrogen tank was set at about 65 p.s.i.

Genomic DNA was isolated from all strains using UltraClean® microbial DNA isolation kits (MoBio Labs, Carlsbad, CA) following the instructions of the manufacturer. The 16S ribosomal RNA gene from all strains was amplified and sequenced using primers and protocols as described in Rooney et al. (2005, 2009b, 2011). Amplified products were purified using ExoSAP-IT (Affymetrix, Santa Clara, CA) and sequenced using BigDye 3.1 on an ABI model 3730 sequencer in the NCAUR core sequencing facility. Raw sequences were assembled using Sequencher 5.0 (Gene Codes Corp., Ann Arbor, MI) and sequences were deposited with GenBank under accession numbers KC847480 to KC847492, KF185109, and KF185110.

Table 1
Screening results, top producers of polyol oil from soybean oil.

Species	Percent Products based on HPLC Chromatogram[a]	
	Polyol oil (%)	**DAGs** (%)
Pseudomonas aeruginosa E03-12 (NRRL B-59991)	42.12	22.38
Acinetobacter haemolyticus E03-25 (NRRL B-59992)	7.42	30.94
Acinetobacter haemolyticus A01-35 (NRRL B-59985)	5.64	40.97
Pseudomonas protegens CHA0T YLMS09 (NRRL B-59980)	0.86	48.56
Pseudomonas protegens CHA0T YKCS37 (NRRL B-59984)	30.65	48.81
Pseudomonas protegens CHA0T YLMS12 (NRRL B-59978)	1.09	38.51
Pseudomonas protegens CHA0T YBPS27 (NRRL B-59975)	0.44	15.96
Pseudomonas protegens CHA0T YLMS17 (NRRL B-59983)	1.16	40.44
Pseudomonas protegens CHA0T YLMS16 (NRRL B-59982)	0.85	22.44
Pseudomonas protegens CHA0T YLMS04 (NRRL B-59979)	0.37	0.41
Pseudomonas protegens CHA0T YLMS14 (NRRL B-59981)	0.77	33.26

[a] % Polyol oil product = Polyol oil/(polyol oil + DAGs + Remaining substrate, soybean oil). % DAGs = DAGs/(polyol oil + DAGs + Remaining substrate, soybean oil).

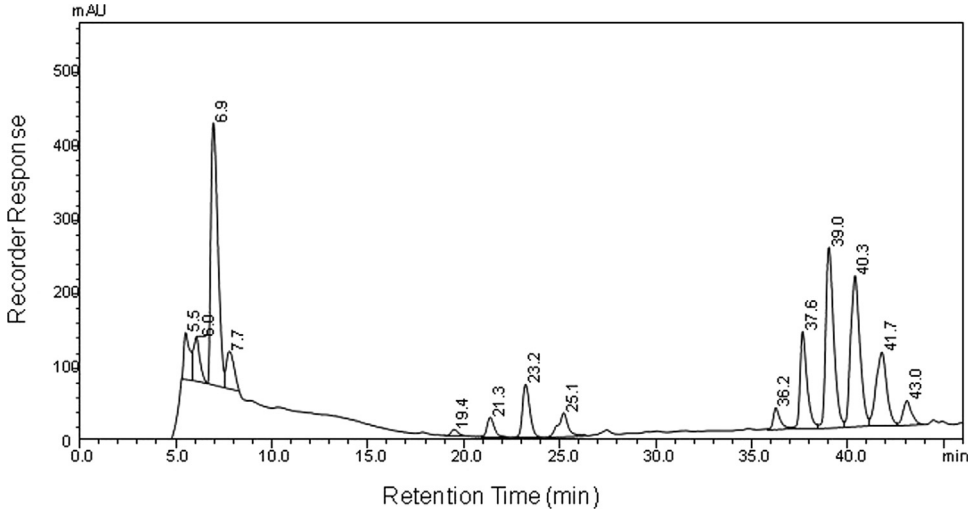

Fig. 1. HPLC chromatogram of crude extracts produced from soybean oil by *Pseudomonas aeruginosa* E03-12 B-59991. Retention time 5–15 min free fatty acids and polyol oils; 15–28 min DAG containing two normal FA; 36–45 min remaining soybean oil.

Please cite this article as: Hou, C.T., et al., Production of polyol oils from soybean oil by bioprocess: results of microbial screening and identification of positive cultures. Biocatal. Agric. Biotechnol. (2014), http://dx.doi.org/10.1016/j.bcab.2014.08.004