Food Chemistry 232 (2017) 203–209

Contents lists available at ScienceDirect

Food Chemistry

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

The physico-chemical alteration of lovastatin and enhanced antioxidant effect of *Bacillus subtilis* fermented-red yeast rice product

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ARTICLE INFO

Article history:
Received 5 October 2016
Received in revised form 10 March 2017
Accepted 4 April 2017
Available online 6 April 2017

Keywords:
Bacillus subtilis
Red yeast rice
Fermentation
3(2H)-benzofuranone
Antioxidant activity

ABSTRACT

Red yeast rice product (RYP) has been used as a food supplement because of its lipid lowering, and in food additives as a natural colorant. Lovastatin of RYP is a hypolipidemic commercial drug. To enhance the beneficial effects of RYP, we performed a bioconversion with *Bacillus subtilis*. This *B. subtilis*-fermentation process of RYP increased the ratio of the active open-hydroxyl acid form and the prodrug lactone form of lovastatin, which is a potent cholesterol synthesis inhibitor. 3(2H)-benzofuranone was newly produced in the fermented red yeast rice product (FRYP) as analyzed by GC–MS. FRYP increased the free radical scavenging activity compared with RYP. FRYP blocked xanthine oxidase (XO)-induced oxidative cytotoxicity and inhibited the H_2O_2-induced intracellular ROS in cells. This is the first study to illustrate that *B. subtilis*-fermented FRYP is useful for facilitating the alteration in the physico-chemical property of lovastatin and enhancing antioxidant activity, which may have greater pharmacological activity.

1. Introduction

Red yeast rice fermented by *Monascus* species is believed to ameliorate indigestion and hyperlipidemia as a traditional therapy in Asia (Ma et al., 2000; Xiong, Wang, Li, Zhang, & Li, 2015). This microbial product is widely used for the coloring of foodstuffs (Ma et al., 2000). Most researches on red yeast rice has focused on the production of monacolin K, which is a cholesterol synthesis inhibitor leading to the upregulation of low-density lipoprotein (LDL) receptors in the liver and thus decreasing LDL content in the blood (Pan et al., 1990). Monacolin K produced by fermentation was the first pharmaceutical product in the market and then synthetic statin drugs has developed. Monacolin K, also known as lovastatin, is administered as an inactive lactone closed ring form, which must be metabolized to a β-hydroxyl acid active open ring form that acts as a reversible competitive inhibitor of HMG-CoA reductase in the liver (Hamelin & Turgeon, 1998; Pan et al., 1990). Lovastatin and other statins have been further studied for their pleiotropic effects independent of their HMG-CoA reductase (Michalik et al., 2013; Mira et al., 2013).

The cellular dysfunction caused by reactive oxygen species (ROS) plays a pivotal role in the pathogenesis of liver injury, cardiovascular disease and cancer (Cesaratto, Vascotto, Calligaris, & Tell, 2004; Soory, 2009). A variety of antioxidants against oxidative damage have been studied to maintain cellular redox-status homeostasis (Cesaratto et al., 2004; Trueba, Sanchez, & Giuliani, 2004). Diverse antioxidants protect against acetaminophen-induced hepatotoxicity, which is caused by the reactive metabolic intermediate NAPQI (Gum & Cho, 2013; Oz et al., 2004). The intake of antioxidants such as polyphenol and flavonoid compounds found in food products has been suggested as a strategy to reduce oxidative damage (Soory, 2009). Fermented products have been reported to significantly increase the contents of bioactive phenolic compounds with a decrease in the amount of glycoside forms in natural foods (Martins et al., 2011).

Fermentation using microorganisms is known as a core technique to produce enhanced value in the field of the food, pharmaceutical and cosmetic industries. *Bacillus subtilis* has a GRAS (generally regarded as safe) status and produces a variety of proteases, β-glucosidase and other enzymes, which are able to degrade a variety of natural substrates producing secondary metabolites dependent on these substrates (Westers, Westers, & Quax, 2004). *B. subtilis* is the main species of fermentation to produce Natto, Korean Chungkookjang, Indian Kinema and fermented soybean products (Ashiuchi et al., 2001; Yin, Lin, & Jiang, 2010). These

Abbreviations: FRYP, fermented red yeast rice product; ROS, reactive oxygen species; RYP, red yeast rice product; XO, xanthine oxidase.

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http://dx.doi.org/10.1016/j.foodchem.2017.04.023
0308-8146/

B. subtilis-fermented products improve the antioxidant effect that is associated with a reduced risk of disease (Chou, Chung, Peng, & Hsu, 2013). *B. subtilis* is believed to be a highly efficient producer of secondary metabolites (Moayedi et al., 2016). In the present study, for the first time we evaluated the beneficial effect of *B. subtilis*-fermented RYP conversion into the active form of lovastatin and protection against chemical-induced ROS generation.

2. Materials and methods

2.1. Materials

SD-350, red yeast rice product (RYP) using *Monascus anka*, was obtained from MSC. CO., LTD (Yangsan, Korea). Xanthine, xanthine oxidase, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), lovastatin, vitamin C and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from sigma (St. Louis, MO, USA). 2′,7′-Dichlorofluorescin diacetate (DCF-DA) was purchased from molecular Probes (Eugene, OR, USA), Tryptone, NaCl and yeast were purchased from BD Biosciences (San Jose, CA, USA).

2.2. Microorganisms and growth conditions

Bacillus subtilis was isolated from soil samples and identified as *B. subtilis* which has 100% similarity with *Bacillus subtilis* subsp. *subtilis* NCDO 1769T (X60646) based on phylogenetic analysis using 16S rRNA gene sequencing (Fig. 1)(Chun et al., 2007). The complete genome sequences of the different members of the *Bacillus* family were obtained from GenBank using BLAST (http://www.ncbi.nlm.nih.gov/BLAST). Sequence analysis and phylogenetic tree construction were conducted using MEGA version 3.1 (http://www.megasoftware.net). A subculture of *B. subtilis* was grown in LB (Luria Bertani) medium containing 1% tryptone, 0.5% yeast, and 1% NaCl.

2.3. Fermentation of RYP

RYP was inoculated with *B. subtilis* (1% v/v) into 10 ml of RYP base media (2% RYP in LB media). All broth culture were incubated for 7 days at 37 °C with constant shaking at 120 rpm and then filtered through a 0.2 μm syringe filter (Advantec, Tokyo, Japan).

2.4. GC–MS analysis

RYP and FRYP were analyzed using an Agilent 6890 gas chromatograph equipped with a 5975 GC/MSD mass selective detector (Agilent Technologies, Santa Clara, CA, USA). Lovastatin was used as a reference standard. Separation was performed in a 30 m length × 0.25 mm i.d. and 0.25 μm film thickness fused silica capillary column HP-5MS (Agilent Technologies, Santa Clara, CA, USA). The carrier gas was ultrapure helium with a flow of 1 ml/min and the splitless injector temperature was set as 280 °C. The column temperature program was as follows: an initial temperature of 70 °C for 4 min, and increased by 2 °C/min 70 to 100 °C (held 2 min). Then, the temperature was varied from 100 to 200 °C at 5 °C/min (held 20 min) and increased to 280 °C (held 5 min) at 10 °C/min, for a total run time of 73 min. Mass spectral analyses were performed using the NIST05 library resident in the computer. The percentage composition was calculated using the area normalization method.

2.5. Free radical scavenging activity

The DPPH assay was conducted as previously described (Wang & Moreno, 2005). Briefly, the freshly prepared DPPH solution (25 μM) was mixed with RYP or FRYP at the indicated dose. The reaction mixtures were maintained in the dark for 30 min. The absorbance of the resulting solutions was measured in 96-well plates using a microplate reader (Sunrise, Tecan Co. Ltd., Australia)

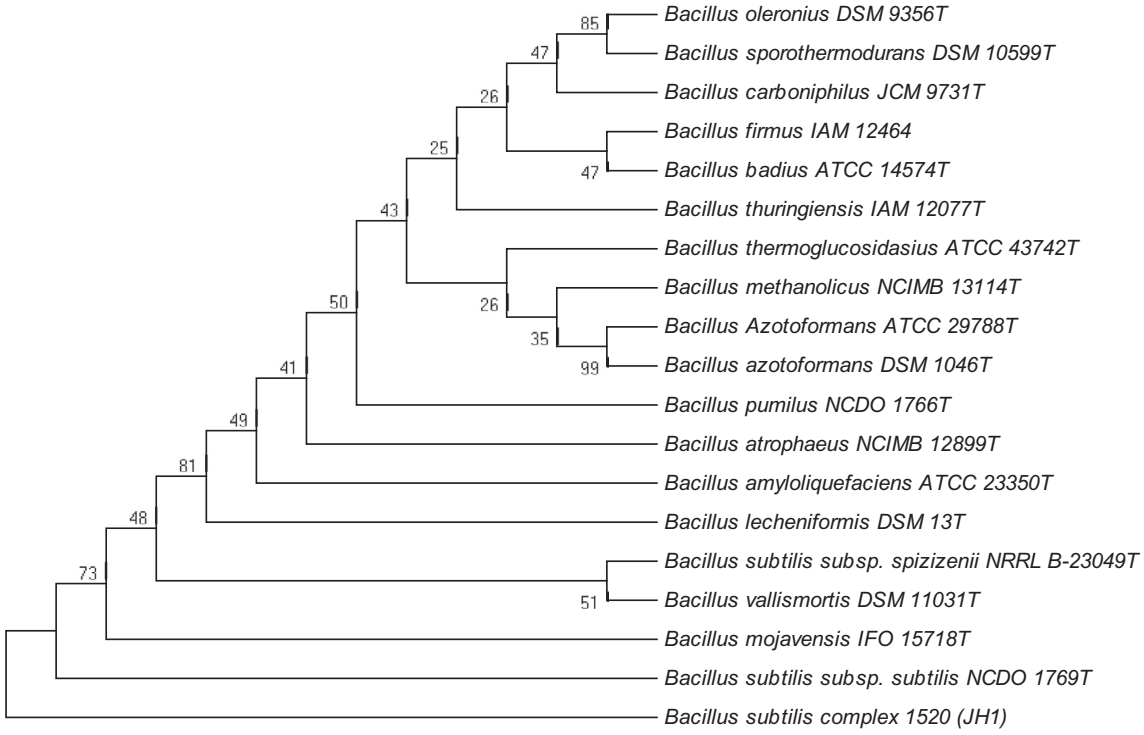

Fig. 1. The identification of the isolated *Bacillus subtilis* strain with a phylogenetic tree using 16S rRNA gene sequencing.