10-oxo-*cis*-12-octadecenoic acid produced by selected lactic acid bacteria with probiotic capability

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Abstract

Probiotics with ability to produce 10-oxo-*cis*-12-octadecenoic acid (KetoA) have been considered to be beneficial for human health with its known role in activating the fatty acids oxidation and reducing the triglyceride synthesis in white adipose tissue. Through the screening of about500 strains of lactic acid bacteria, *Lactobacillus brevis*M003 was selected as a potential probiotic for KetoA production. By analyzing the cultivation and reaction conditions to obtainthe probiotic cells with high KetoA-producing activity, theM003 bacterial cells in the exponential growth phase without inducers showed the significant production of KetoA (approximately 0.03 mg/mL).—suggest to simplify the sentence if possible—too long. NAD^+ (5 mM) and $NADP^+$ (5 mM), derived from vitamin (niacin), were considered as valuable prebiotic sources for promoting the cell growth and accumulating large quantities of KetoA (around 0.14 mg/mL KetoA was generated from 1 mg/mL 10-hydroxy-*cis*-12-octadecenoic acid). Divalent metal ion such as Zn^{2+}, which is also well known as an essential nutrient mineral of exceptional biologic and public health importance, markedly enhanced KetoA productivity (about 0.25 mg/mL KetoA).

Keywords: 10-Hydroxy-*cis*-12-octadecenoic acid, Lactic acid bacteria, Linoleic acid, 10-oxo-*cis*-12-octadecenoic acid, *Lactobacillus brevis*M003

Introduction

Probiotics are considered as important components of functional foods which have several health benefit properties such as anti-inflammatory, anti-cancer activity, and so on. Polyunsaturated fatty acids (PUFAs) as well are functional components of diet and show healthy effects on lipid metabolism, cardiovascular system, etc. Therefore, the contribution of beneficial components such as potential probiotics and PUFAs to gut microbiome aiming to prevent the dysbiosis has undoubtedly become the focus of growing concern.

Oxo fatty acids which have been especially known as pharmaceuticals were generated from linoleic acid saturation metabolism by intestinal microbes. For example, one earlier study demonstrated that a certain strain of intestinal lactobacillus, *Lactobacillus plantarum* AKU 1009a, has been shown to convert linoleic acid to 10-oxo-*cis*-12-octadecenoic acid (KetoA), had ability to reduce the triglyceride synthesis and increase the fatty acids oxidation in white adipose tissue (Kishino *et al.*, 2013). Thus, introduction of probiotics with high KetoA-producing activity to gut microbiome may regulate microbial communities and prevent the lifestyle diseases related to gut–microbial lipid metabolism. However, KetoA production has been indicated in only *Lactobacillus plantarum* AKU 1009a, but its productivity hasn't been clearly reported yet. Therefore, it is desirable to develop a method for the more efficient KetoA production by a variety of food-related microorganisms such as lactic acid bacteria.

In light of this, we carried out this study aiming to analyze the KetoA production by lactic acid bacteria with potentials as probiotics.

Materials and Methods

Microorganisms and cultivation medium

Lactic acid bacteria used in this study were obtained from foods, pickles (AKU Culture Collection, Faculty of Agriculture, Kyoto University). Medium for the screening was Lactobacilli MRS broth (Difco, Detroit, MI, USA).

Screening of microorganisms using resting cells

500 strains of lactic acid bacteria were screened for KetoA production. Each strain was inoculated into 15 mL of MRS medium with 200µl LA solution in screw-capped tubes at 37^{o}C and then shaken at 120 rpm for 1–2 days. After the cultivation, the cells were harvested by centrifugation (3,000 rpm; 10 min) and washed twice with 0.85% NaCl. The reactions were carried out in the test tubes containing 1 mL of reaction mixture (washed cells, 100 mM potasium phosphate buffer pH 6.5, 1 mg/mL LA, and cofactors (5 mM NAD^{+} or 5 mM $NADP^{+}$) under anaerobic condition using Aneropack “Kenki” (Mitsubishi Gas Chemical Co., Ltd., Tokyo, Japan) in a sealed chamber and then shaken at 120 rpm, 37^{o}C for 24 hours.

Lipid extraction and analysis

Lipids were extracted with 5 mL of chloroform/methanol/1.5% KCl in H_2O (2:2:1, by volume), following the procedure of Bligh–Dyer (Bligh, Dyer, 1959) and then concentrated by evaporation under reduced pressure.

The resulting lipids were analyzed by gas-liquid chromatography (GC) using a Shimizu (Kyoto, Japan) GC-1700 gas chromatograph equipped with a flame ionization detector and a split injection system, fitted with a capillary column (SPB-1, 30 m x 0.25 mm I.D., SUPELCO, PA, USA).

Results and discussion

Screening of high KetoA-producing lactic acid bacteria

Through the screening of about 500 strains of lactic acid bacteria, the results showed that LA saturation metabolism by lactic acid bacteria generated characteristic fatty acids. However, only 21 strains had ability to produce KetoA. Among of them, *Lactobacillus brevis* (*L. brevis*) M003 was selected as a potential strain with the highest KetoA-producing activity.

Analysis of cultivation conditions for obtaining L. brevis M003 probiotic cells with high KetoA-producing activity

Effect of inducers

The findings showed that the KetoA-producing activity of the M003 strain cells tended to decrease with inducers (Fig 1A, 1B). This may be due to the inhibitory effect of LA and HYA on bacterial growth that has been reported by many authors who have demonstrated that there are different tolerances according to strains (Jiang *et al.*, 1998; Lin *et al.*, 1999).

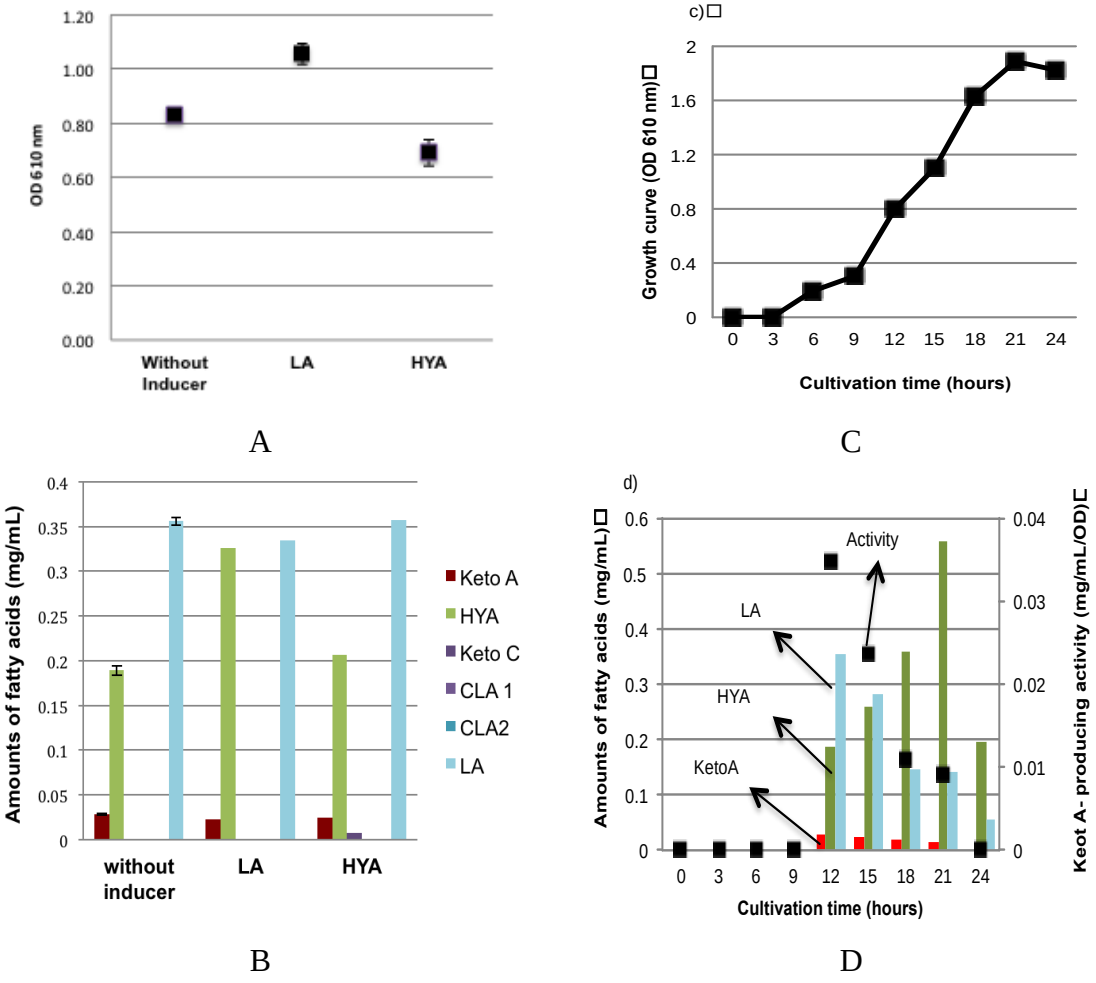

Figure 1 Effect of inducers and cultivation time on the high KetoA-producing activity of *L. brevis* M003. A) The OD value of the M003 strain cells with different inducers. B) Fatty acids production of the M003 strain cells with the different inducers. C) The growth curve of the M003 strain cells. D) The KetoA-producing activity at the different cultivation times. The data were presented by means and standard deviation error.

Effect of cultivation time

The results showed that the resting cells obtained from cultivation without inducer for 12 hours produced more KetoA than those cells in other cultivation times (Fig. 1D). The optimal density in the exponential phase (12 incubation hours) was around 0.8 (Fig. 1C).

Analysis of reaction conditions for obtaining the information of prebiotic compounds

Effect of cofactors and substrates

Among of tested cofactors, NAD^+ (5 mM) and $NADP^+$ (5 mM) have been indicated as efficient cofactors for KetoA accumulation (Fig. 2). This means that niacin should be considered as a good compound for prebiotics.

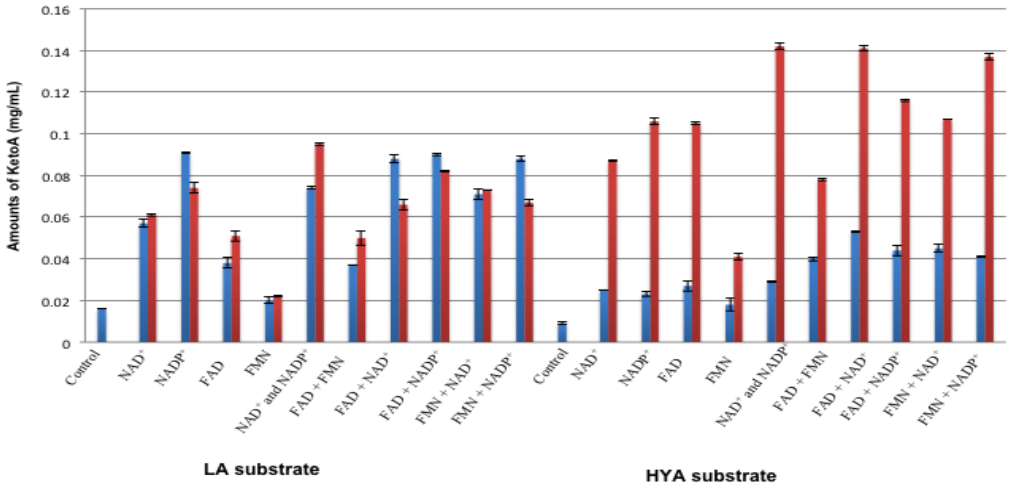

Figure 2 Effect of cofactors and substrates on the high KetoA-producing acitivity. Blue bars: KetoA production by the M003 strain cells with substrates (LA/HYA, 1 mg/mL), 100 mM KPB pH 6.5, and the different cofactors (NAD^+/NADH/$NADP^+$/NADPH, 5 mM; FAD/FMN, 20 mM). Red bars: KetoA production by the M003 strain cells with the different cofactors, substrates (LA/HYA, 1 mg/mL), and 100 mM Tris-HCl pH 8.0.

Analysis of other reaction conditions related to gut lipid metabolism

Effect of metal ions, chelating agents, and inhibitors

The result showed that there was a considerable increase in KetoA production of the strain M003 cells with divalent metal ion Zn^{2+} (about 0.25 mg/mL KetoA was produced from 1 mg/mL HYA) (Fig. 3).

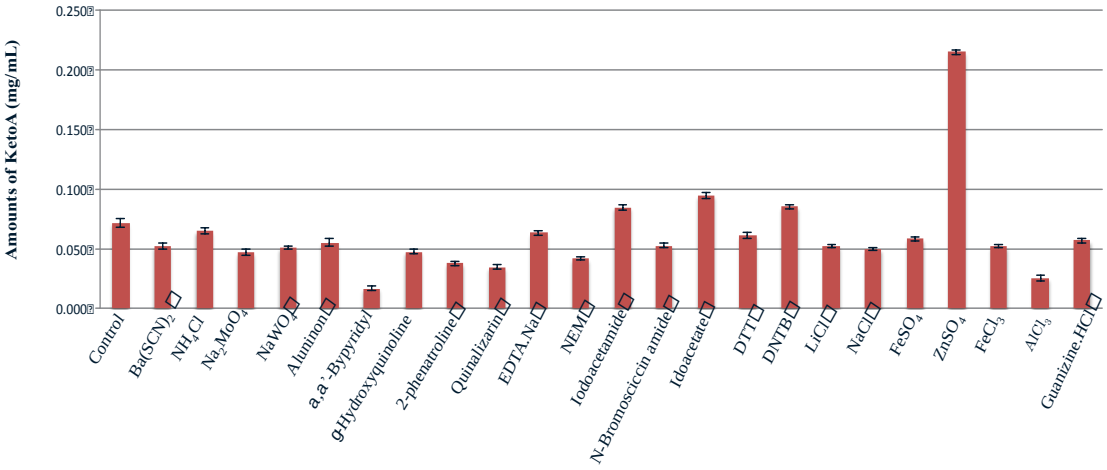

Figure 3 Effect of metal ions, chelating agents, and inhibitors.

Time course of KetoA production at the cell reaction

The amount of KetoA reached a peak of about 0.14 mg/mL at 24 hours. The further incubation of 24 hours decreased the KetoA-producing activity. This result illustrated the relation of KetoA production by probiotic cells and residence time of dietary fatty acids in the intestine (Fig. 4).

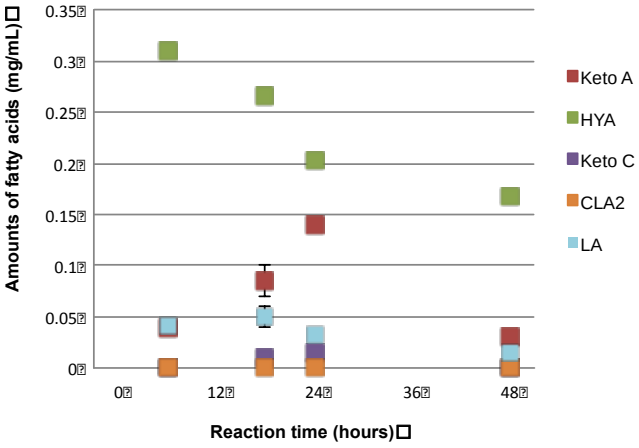

Figure 4 Time course of KetoA production at the cell reaction.

Conclusion

In this study, we found that *L. brevis* M003 had a possibility to convert LA to characteristic fatty acids such as KetoA, KetoC, HYA, and CLA. However, the amounts of other produced fatty acids, exception KetoA, were insignificant. As the results of analysis of KetoA

production, the M003 probiotic cells required cofactors (NAD^+ and $NADP^+$) as catalysts for accelerating reactions to produce KetoA. This means that prebiotics (niacin) plays a vital role in promoting the probiotic cells. HYA and LA were appropriate substrates for increased conversion of HYA (LA) to KetoA.

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