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Study on the ability of producing of conjugated linoleic acid of *lactobacillus fermentum*101 isolated from human digestive tract

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Abstract

The most abundant isomers of conjugated linoleic acid (CLA) are cis-9 trans-11 (c9t11) and trans-10 cis-12 (t10c12) that involved in a number a health aspect. In human, lactic acid bacteria was thought to produce CLA from LA. Therefore, the study was organized to check the conjugated linoleic acid (CLA) producing ability of Lactobacillus fermentum (L. fermentum) in grape seed oil and linoleic acid. The CLA was detected on thin layer chromatography and GC-MS analysis after converting to fatty acid methyl esters. L. fermentum 101 could produce CLA by TLC analysis. L. fermentum 101 cultured in MRS-LINO for 36 h could produce 9,11 di-ene of C18 at the retention time of 30.299 min with 0.67%. Moreover, there were many products produced as 9, 12; 9, 15; 11; 10; 14-C18 with higher percentage in MRS-LINO and MRS-Oil. In summary, the study was the preliminary report of L. fermentum 101 in converting LA to CLA.

Keywords: Chromatography Mass Spectrometry (GC-MS), Conjugated linoleic acid, Grape Seed Oil, Linoleic acid, Gas, thin layer chromatography.

Introduction

Lactic acid bacteria (LAB) have the major metabolite of the carbonhydrate fermentation. LAB are identified into different strains but common strains including *Lactobacilli*. This bacteria can produce anti-microbial, anti-cancer cell line, immunoregulatory metabolites. The metabolite showing these biological effects is CLA (Al-Hindi et al., 2015). Linoleic acid includes nearly 20 types of isomers with disparity position (7, 9; 8, 10; 9, 11; 10, 12 and 11,13) and geometric (cis, cis; cis, trans; trans, cis; trans, trans) combination (Christie et al., 1997). The major of biologically active CLA isomer is cis-9, trans-11 (c9, t11) and trans-10, cis-12 (t10,c12) (Bhattacharya et al., 2006). Reccently, more researches desmontrate that CLA in dairy product, ruminant meat, vegetable oil contains c9, t11 in 80%. There is no doubt that CLA play vital role in increasing metabolic rate; lowers cholesterol and triglyceride levels; boosting the immune system, enhancing fat burning process. Linoleic acid represents major unsaturated fatty acid present in practically all oils, but grape seed oil contains significant percentage of linoleic acid (more than 75%) that is thought as a source for making CLA (Al-Saman et al., 2016).

Therefore, the study is set to solve the problem of obesity and blood fat. *L. fermentum* isolated from the gastrointestinal tract as well as other sources have not been studied for this characteristic. Therefore, this research was new and essential.

Materials and methods

Culture condition

Linoleic acid (cis-9, cis-11-octadecadienoic acid) was purchased from Santa Cruz (Dallas, Texas 75220, USA). Grape seed oil was purchased from market in Ho Chi Minh city. Grape seed oil was checked for LA existence before used. All other chemicals were of analytical grade and are commercially available. *L. fermentum* used in this study was isolated form human saliva and cultured in de Man Rogosa and Sharpe (MRS) medium at 37°C for 24 - 48 h under condition (5% CO_2) and pH was maintained at 6.5. After incubation, the culture was checked for contamination before using for experiment.

Contamination check for GABA-Producing *L. fermentum*

L. fermentum was cultured with 10 mL medium and incubated from 12h to 48h in MRS broth without or with LA (grape seed oil: O) or purified LA. For the conditions using oil, oil was added to MRS culture with different concentrations: 0.1%, 0.2%, 0.3%, 0.4%, 0.5% (v/v). Incubation time was optimal in 12h, 24h, 36h, 48h. Then, each culture was checked for bacterial growth and the CLA production ability.

Extraction of lipid and FAME preparation

After incubation time, the culture was centrifuged (13000 rpm for 15 min at 4°C). The culture supernatant was collected and heated at 125°C to gain the biomass, then mixed with chloroform with an equal ratio (v/v) and shaken at room temperature for 2 minutes. The extract was transferred into new tube and then evaporated overnight and then lipid was weighed. The esterified fatty acids (EFAs) were transformed into the corresponding FAMEs to be analyzed by GC-MS that were trans-esterified through alkali-catalyzed reaction (Metcalfe et al., 1996; Bligh et al., 1959). The lipid extract was dissolved in 1M potassium methoxide (methanol solution) and the sample was homogenized, then incubated at 55°C for 1 hour.

Thin layer chromatography

Fatty acids esters were separated by thin layer chromatography (Randerath et al.,1966). The silica gel plate was soaked in AgNO3 and then dried before all the samples were placed on a silica gel plate. The following solvent system was hexane: chloroform (1:3 in v/v).

GC-MS analysis

The organic phases containing FAMEs were injected (0.5 μL) into a GC apparatus (HP5890 Series II, Agilent, Waldbronn, Germany) equipped with the column (100 mm × 0.25 mm, 0.25 μm) CP-Select FAME (Varian, Palo Alto, CA, USA). The injector was kept at 270◦C. To determine the FA profile, the oven temperature ramped from 135◦C to 250◦C (2.5◦C/min) and was maintained at 250◦C for 23 min. Elution was performed with high-purity helium, with constant column head pressure of 200kPa. Qualitative analyses of FAMEs were performed with a MS quadrupole detector (HP5972, Agilent). Analysis was identified by comparison with standards (O5632, Sigma Aldrich) and by analysis of their fragmentation patterns (EI, 70eV) with the mass spectrum library NIST 2005 (Gatesburg, USA). Quantitative analyses were performed with a flame ionization detector held at 300◦C (Kathryn et al., 2012).

Statistical analysis

Statistical Package for the Social Sciences (SPSS) software version 20.0 and Microsoft Excel was employed. Values of $P < 0.05$ refer to significant differences among experiments.

Results and discussion

Identification of LA in grape seed oil

In order to use grape seed oil for the study, LA content was detected by TLC and GC-MS. The spot in grape seed oil had similar R_f (0.26) of LA on TLC (Figure 1). By GC-MS analysis, LA gave also existed in grape seed oil and occupied 70% grape seed oil equaling to 7 mg/ml oil.

Fig. 1: The present of linoleic in A) MRS B) Oil C) LINO

Influence of LA on growth

Although free LA serves as the direct precursor in CLA biosynthesis, it also inhibits bacterial growth, and the tolerance to LA varies among different strains. Therefore, screening bacterial strains with high LA tolerance might be a shortcut to obtain strains that can produce more CLAs. After incubation time, the optical density (OD) of cultures was measured, ranging between 1.1 and 2.2 that illustrated all bacterial strains able to grow in presence of LA at the optimal concentrations. *L. fermentum* survival was not influenced by LA after 12h up to 48h of incubation.

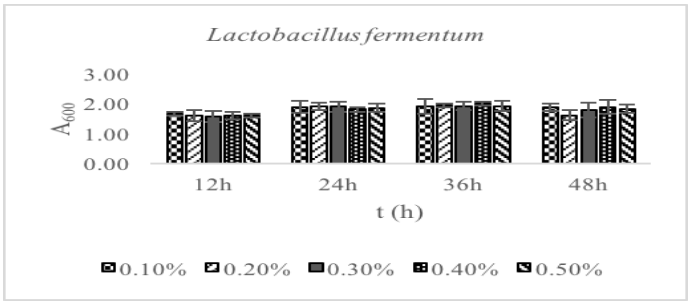

Fig. 2: Optical density of bacteria in MRS-Broth with different concentration of *Lactobacillus fermentum* ranging between 0.1% and 0.5% for 12h, 24h, 36h, 48h incubation.

Screening CLA production by thin layer chromatography

After extraction of lipid and preparation of FAME processes, the samples were detected on TLC to check the quality of sample before analyzed by GC-MS. Based on the results of TLC, the clearly and darker region showed the potential samples in CLA production. As seeing in Figure 3, *L. fermentum* could give the LA isomers more highly in 36-48h when using oil containing 0.5% LA.

Identification of different CLA isomers with GC-MS

In case of culture in MRS-LINO for 36 h, *L. fermentum* 101 could produce 9,11 di-ene of C18 at the retention time of 30.299 min with 0.67% (Figure 4).

Fig. 3: The presence of LA after FAMEs of sample that cultured in MRS-LA

The *L. fermentum* 101 could convert LA into CLA. It was reported that 9 cis 11 trans CLA was the master regulator of adipocyte differentiation that is a mechanism for explaining the antihypertensive, antihyperlipidemic, antidiabetic effects mediated by this CLA. In the contrast, trans 10 cis 12 CLA has been proved that it increases lipolysis, leading to rise insulin resistance, inflammation.

In the study, *L. fermentum* 101 produced a little of 9,11-C18. Normally, free polyunsaturated fatty acids inhibit the growth of anaerobic bacteria, therefore, the saturation reactions are surmised to be detoxification mechanisms.

In *L. fermentum* 101, 10-octadecenoic acid and 12-octadecenoic acid were produced mostly, suggesting that the biological CLA production processes are isomer-selective. However, the factors affecting the isomer ratio in CLA of lactic acid bacteria should be done to understand the fatty acid transformation reactions, such as isomerization, hydration.

Conclusion

There are more and more positive effects through a series of disparity mechanism of human gut microbial which are one of the most relevant health promoting, typically lactic acid bacteria. From the obtained result, it is concluded that there are significant differences between the concentration of LA and time for incubation on the CLA production. The effect of LA may be due to the composition of its fatty acids and the biotransformation of linoleic fatty acid into CLA. Depending on the result, we can conclude that there are positive effects for oils on the production CLA of *L. fermentum*. Moreover, the details of the metabolism, characteristics of the enzymes involved, and their gene organization have not been delineated clearly.

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