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Bioconversion of ginsenoside Rd into compound K by *Lactobacillus pentosus* LH6 isolated from Kimchi

Korean Ginseng Center Most Valuable product and Ginseng Genetic Resource Bank, Kyung Hee University

Quan Lin-Hu, Ho-Bin Kim, Se-Hwa Kim and Deok Chun Yang*

Objectives

Therefore, this study investigated the optimum conditions for better growth of the isolated microbes. Additionally, the conditions for efficient ginsenosides bioconversion were optimized using an active β-glucosidasesLH6 lactobacillus strain that was isolated from Kimchi.

Materials and Methods

Materials

The MRS broth was purchased from Difco (Miller, Becton Dickinson and Co., MD, U.S.A), while the Crude ginseng leaf saponin was acquired from FusongCity, China. On the other hand, the standard ginsenosides, 20(S)-Rb1, 20(S)-Rd, 20(S)-Rg3, 20(S)-Rh2, 20(S)-F2, and compound K were obtained from KT&G in Daejeon,Korea.

Methods

The LH6 strain was grown in the MRS broth at the same temperature of 37°C, until absorbance at 600 nm reached 1.0. The crude enzymes from each culture broth were dissolved in a 20 mM glycine-NaOH buffer (pH 10.0), and mixed with 0.2 mM ginsenoside Rd that was dissolved in distilled water in a 1:4 ratio(v/v).Afterwards, the mixture was incubated at 30°C and 190 rpm for 72 h. During the reaction period, a 1.25 ml aliquot was taken every 12 h time interval. This was extracted via n-butanol that is saturated with H_2O, and then analyzed by both TLCand HPLC.

Results

In this study, ginsenosides Rd was converted into compound K by using the enzyme secreted by the β-glucosidaseproducing bacteria *L. pentosus* LH6, which is isolated from kimchi. As such, enzymes secreted by microbes such as *Lactobacillusbifidus*(Bae et al., 2003), *Aspergillus*(Chi and Ji, 2005) and soil bacteria (Cheng et al., 2006) have been used conventionally to transform ginsenosides Rd into compound K. Basically, the *L. pentosus* LH6 strain was an aerobic and edible lactic acid bacteria. The enzyme activity of this strain was found highest at 30°C and decreased beyond 35°C. According to this result, the optimum temperature was slightly lower than those reported by others, such as 45°C for *Rhizopusjaponicas*derivative β-glucosidase (Kim and Seu, 1989), 40°C for*Aspergillusniger*48gand*A.niger*848gderivativeβ-glucosidase(Zhang et al., 2003), and 40~50°C for general β-glucosidase(Sano et al.,

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(Corresponding author) : 양덕춘 E-mail : dcyang@khu.co.kr Tel : 031-201-2688

1975; Yoshioka and Hayashida, 1980). In addition, the crude enzyme showed a high activity rate at pH 8.0 - 12.0, while the maximum hydrolysis activity was found at pH 10.0. Generally, these pH values were much higher than those reported by others, such as pH 5.0 for *A. niger 48g and A. niger* 848g derivative *β*-glucosidase(Zhang et al., 2003), pH4.8-5.0 for *R. japonicas* derivative *β*-glucosidase(Kim and Seu, 1989), pH6.0 for *Fusobacterium*K-60derivative*β*-glucosidase(Park et al., 2001), and pH5.0 for ginseng derivative *β*-glucosidase(Zhang et al., 2001). This is an efficient conversion system where the major ginsenosides are converted into minor ones. In conclusion, more than 100 microbes from Kimchi were isolated and tested for the effect of conversion ability. It was identified that the *L. pentosus* LH6 showed strong *β* -glucosidase activity, making it one of the best species for ginsenosides conversion. Furthermore, the growth of bacteria and the optimum conditions for better conversion of ginsenosides were stabilized. Thus, this stain is capable of transforming 87% of the ginsenosides Rd into compound K after 72 hrs post reaction.

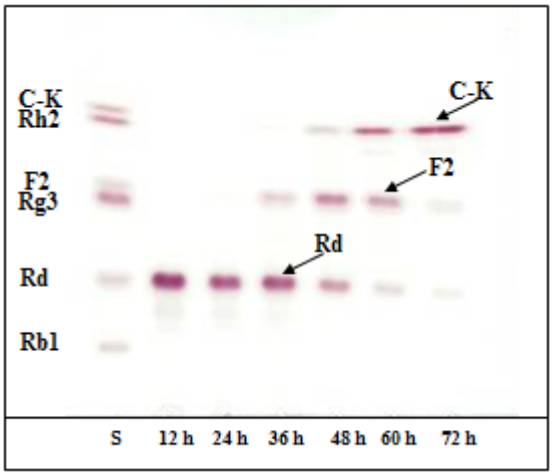

Fig. 1. The TLC analysis of ginsenosides bioconversion at various reaction times.
S indicates the mixture of ginseng standards.