# PRODUCTION OF D-TAGATOSE, A NOVEL LOW CALORIES SWEETENER BY A NEW ACETIC ACID BACTERIUM, *Asaia bogorensis* NRIC 0311$^{T}$

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## Abstract

The D-tagatose sugar is a ketohexose monosaccharide sweetener, which is an isomer of D-galactose. D-tagatose is rarely found in nature and it can be utilized in many ways particular in prebiotic property. It has been reported that the D-tagatose can be produced from D-galactitol (dulcitol) via the oxidation reaction by the acetic acid bacteria such as *Acetobacter globiformis, Gluconobacter oxydans*. *Asaia bogoresis* NRIC 0311$^{T}$ is belonging to acetic acid bacterial genera that has ability to grow in D-galactitol (dulcitol) and produce acid, but it has never been clarified for its oxidation product. Therefore, this study aimed to clarify the oxidation product and the optimum conditions for D-tagatose production by *A. bogoresis* NRIC 0311$^{T}$. Detection of oxidation product from the culture medium by thin layer chromatography was performed using triphenyl tetrazolium chloride as the detection reagent in which highly specific to ketose. It was clearly detected the single spot which corresponded with the authentic D-tagatose. The optimum conditions for D-tagatose production was obtained in high aerated flask (baffled-flask) by inoculation the cells into culture medium containing 0.5% D-galactitol and incubation at 30°C with vigorous shaking at 150 rpm for 12 h. D-Tagatose obtained from the optimum conditions gave the highest relative yield of 68.84%.

**Key words:** D-tagatose, D-galactitol, *Asaia bogorensis* NRIC 0311$^{T}$, oxidative product

## Introduction

The marketing of synthetic sweetener has expanded rapidly such as aspartame, acesulfame and saccharin. Since the people turn to health care more. Especially who is diabetes, obesity and tooth decay patients are caused by nutrients of carbohydrate turn to attention it too. As synthetic sweetener is low calories sweetener, resulting in less energy and friendly in dental health (O'Brien Nabors et al., 1991). For this reason, synthesis sweetener is an alternative sugar for healthy. Later, the discovery a new kind of sweetener is interesting is called "tagatose". The tagatose is a rare natural ketohexose C-4 fructose epimer that be considered as a low calories bulk sweetener, with 92% of the sweetness of sucrose but less than half the calories (Levin et al., 1995). Besides, the one properties of this sugar that makes it interesting is prebiotic. The D-tagatose can be absorbed only 15-20% in the small intestine and not-digestible or fermented in the colon by microflora

resulting in production of short-chain fatty acid (SCFA) and butyrate. The benefit of the above products are selectively stimulating the growth and/or activity of bacteria for improving host health (Bertelsen et al., 1999) and it has important role in colon epithelium protection (Johnson et al., 1995).

D-tagatose production can be produced two ways; Chemical method, tagatose is produced from D-galactose by using a calcium catalyst (Beadle et al., 1991) and biological method is used biocatalysts from bacteria. At the same time, D-tagatose production can be produced from D-galactiol (dulcitol) by using microorganism directly which the microorganism must be consisting of enzymes involved. *Arthrobacter globiformis* (Izumori et al., 1984), *Mycobacterium smegmatis* strain SMDU (Izumori and Tsuzaki et al., 1988), *Enterobacter agglomerans* strain 221e (Muniruzzaman et al., 1994), *Klebsiella pneumoniae* strain 40b (Shimonishi et al., 1995) and *Gluconobacter oxydans* (Rollini and Manzoni et al., 2005) have been reported to convert D-galactitol into D-tagatose. But there are limits to those pathogens (except *Gluconobacter oxydans*) that can be dangerous if the D-tagatose production was used while it has impurity. In the meantime, *Asaia bogorensis* NRIC 0311$^T$ that it is classified in acetic acid bacteria like *Gluconobacter oxydans* can be also oxidized D-galactiol (Yamada et al., 2008). However, *A. bogorensis* NRIC 0311$^T$ had been isolated just a few years ago and there are no reports about D-galactitol oxidation with this isolate. Therefore, this study aims to clarify the oxidation product from dulcitol and the optimum conditions for D-tagatose production by *Asaia bogorensis* NRIC 0311$^T$ was also conducted.

**Methodology**

Microorganism and culture conditions

D-Tagatose production was carried out employing *Asaia bogorensis* NRIC 0311$^T$ (The isolate was kindly supported by Department of Biological chemistry, Faculty of Agriculture, Yamaguchi University, Japan). The culture was maintained on a potato medium containing 1% peptone, 1% yeast extract, 0.5% glucose, 2% glycerol, 10% potato extract and 0.5% calcium carbonate. The medium was sterilized at 121°C for 15 min. The strain was streaked onto agar slant and incubated at room temperature for 24-48 h. The stock culture was kept in 50% glycerol at -80°C.

Chemicals and reagents

D-galactitol (Dulcitol) and D-tagatose were supplied by Sigma. Thin Layer Chromatography (Silica gel 60 $F_{254}$) Aluminum sheets were supplied by Merck. The other reagent grade chemicals were obtained from commercial sources.

D-Tagatose production

D-Tagatose production was carried out with growing cells of *A. bogorensis* NRIC 0311$^T$ by incubating in D-galactitol medium containing 0.5-3.0% D-galactitol, 0.3% yeast extract and 0.3% peptone. The production process was carried out in 500 ml flasks both Erlenmeyer-flask and baffled-flask in which each flask was contained 100 ml of the culture medium. The culture was incubated at room temperature with shaking at 150 rpm. In addition effects of temperature and substrate concentration were also performed. During cultivation, samples were collected for every 6 h until 48 h.

Determination of D-tagatose from culture medium

Culture broths were collected and centrifuged at 10,000 rpm for 3 min in order to remove the cells. Then culture supernatant was spotted onto thin layer chromatography plate (silica). After drying, the TLC plate was sprayed with detection reagent (freshly prepared of 2,3,5-triphenyl tetrazolium chloride solution) and then incubated at 80°C for 2 min. Ketose detected in TLC plate had orange-red spot while D-galactitol could not be detected. DNS method (Burner et al., 1964) was used for quantitative analysis of D-tagatose in culture supernatant using authentic D-tagatose as the standard.

**Results and Discussion**

Detection of the oxidation product from culture medium containing D-galactitol

*Asaia bogorensis* NRIC 0311$^{T}$ was inoculated into the culture medium containing 1% D-galactitol as the carbon source. After centrifugation to remove the intact cells, culture broth of every 6 h incubation was spotted onto thin layer chromatography plate compared to the authentic D-tagatose. As shown in figure 1, one single spot was detected in each lane suggesting that the only one oxidation product was found in the culture medium. Measuring Rf value of the spot detected in the culture supernatant was corresponded to that of the authentic D-tagatose. Thus, it can be concluded that the oxidation product found in culture supernatant was D-tagatose.

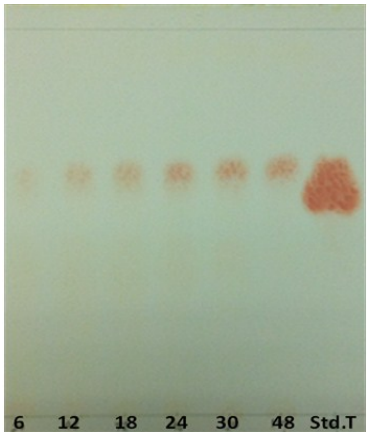


**Figure 1** Thin Layer Chromatogram of culture supernatants compared to the authentic D-tagatose as the standard.

Effects of aeration on D-tagatose production

Acetic acid bacteria are aerobic bacteria which able to oxidize sugar alcohol to corresponding ketose. In this experiment, baffled-flask, a high aerated flask, was used in order to compare D-tagatose production to that of Erlenmeyer-flask which is ordinary used in the laboratory. As shown in figure 2, D-tagatose production from D-galactitol by *A. bogorensis* NRIC 0311$^{T}$ with baffled-flask was three-time higher than that of Erlenmeyer-flask in which the yield of D-tagatose produced in baffled-flask and Erlenmeyer-flask was 3.5 and 1 mg/ml or it had relative conversion of 34.46% and 9.81%, respectively. By the ways, the production of the highest D-tagatose in baffled-flask was obtained at 12 h while production with Erlenmeyer-flask was obtained at 6 h. The results were corresponded with Ruetaikongthaworn (2003) reported that baffled-flask had affected to dissolve oxygen in liquid medium.

Effects of temperature on D-tagatose production

Production of D-tagatose from D-galactitol by *A. bogorensis* NRIC 0311$^{T}$ both in a high aerated flask (baffled-flask) and in Erlenmeyer-flask at 30°C and 37°C was performed. D-Tagatose produced in Erlenmeyer-flask in both at 30°C and 37°C was lower than that of baffled-flask in both temperatures indicating that aeration had much affected on D-tagatose production. The results in figure 3 revealed that *A. bogorensis* NRIC 0311$^{T}$ was well oxidized D-tagatose from D-galactitol in the present of high aeration. The yield of D-tagatose obtained when cultured in baffled-flask at 30°C and 37°C was 3.44 and 3.40 mg/ml (the relative yield; 34.46% and 34.02%), respectively. The results obtained in this experiment was similar to the kinetic of L-arabinose isomerase (AI) enzyme that it has efficiency in production of the product at high temperature more rapidly which affect to shift toward ketose (Ibrahim and Spradlin et al., 2000).

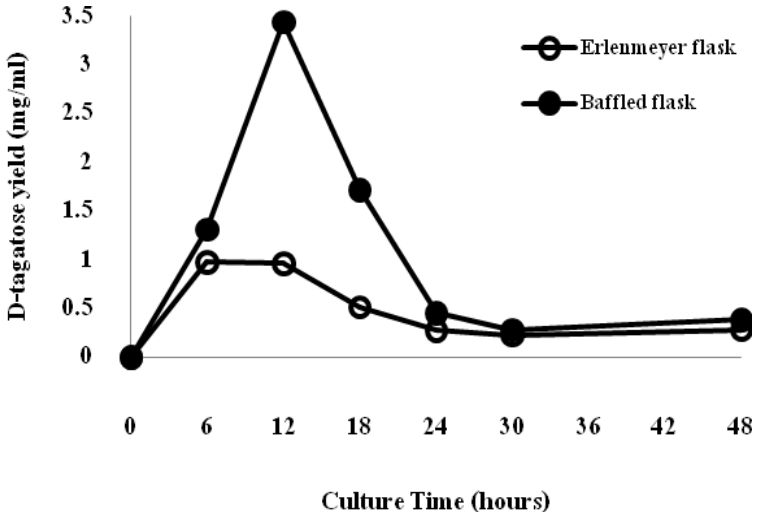


**Figure 2** Time course of D-tagatose production by *Asaia bogorensis* NRIC 0311$^{T}$ in the medium containing 1.0% D-galactitol at 30°C.

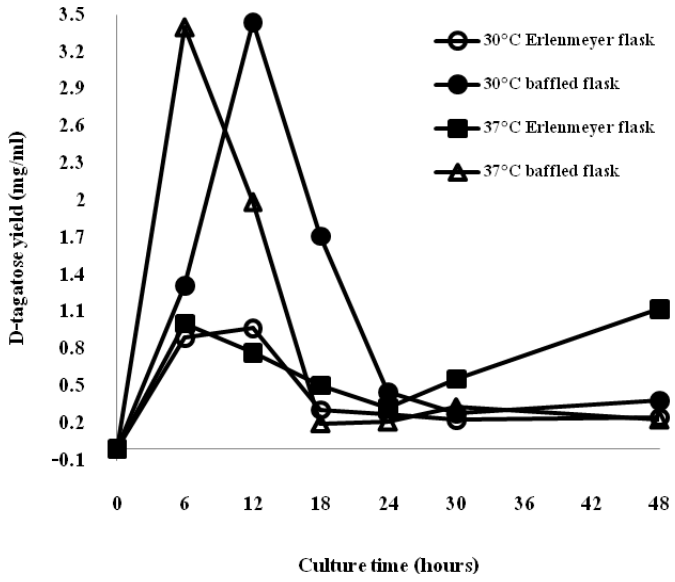


**Figure 3** Time course of D-tagatose production by *Asaia bogorensis* NRIC 0311$^{T}$ in the medium containing 1.0% D-galactitol with high aerated flask and smooth-flask at 30°C and 37°C.

Effects of D-galactitol concentration on D-tagatose production

D-Galactitol concentration was varied in the culture medium in order to determine whether D-tagatose is produced depending on substrate concentration or not. The experiment was performed by inoculation of *A. bogorensis* NRIC 0311$^{T}$ into the culture medium containing D-galactitol varying from 0.5-3% at 30°C in baffled-flask. As shown in figure 4, D-Tagatose was produced the highest at 12 h incubation at the concentration of 0.5% D-galactitol. Even more high concentration of D-galactitol as the substrate, D-tagatose was decreased in production. The yield of D-tagatose obtained from 0.5% D-galactitol as the substrate was 3.44 mg/ml or as the relative yield of 68.84%. The results were coincided with Giridhar et al. (2002) reported that the more substrate increase the lesser it produces because of substrate inhibition.

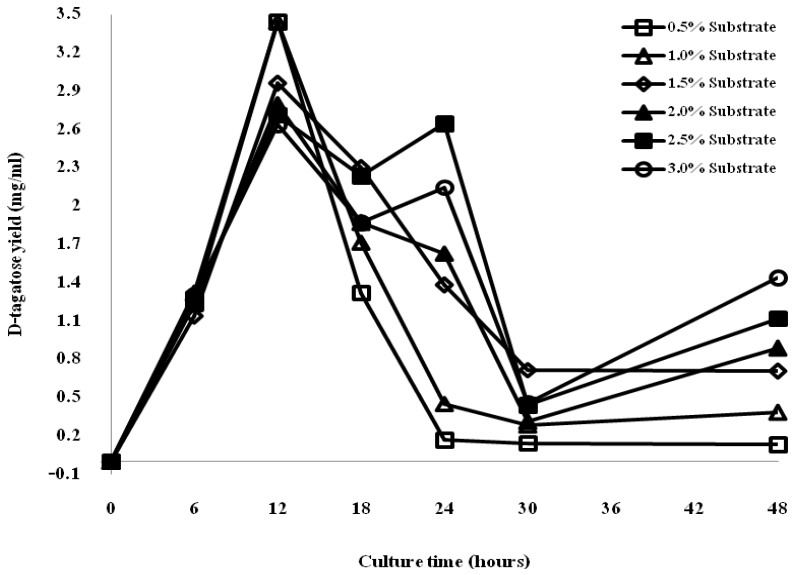


**Figure 4** Time course of D-tagatose production by *Asaia bogorensis* NRIC 0311$^{T}$ in the medium containing various D-galactitol concentrations.

## Conclusion

A potent acetic acid bacteria isolate belongs to the genus *Asaia bogorensis* NRIC 0311$^{T}$ that can produce high amount of D-tagatose from D-galactitol as precusor. The isolate produced the highest yield of D-tagatose when cultured in the medium containing 0.5% D-galactitol, 0.3% yeast extract and 0.3% peptone. The culture was put into baffled-flask and incubated at 30°C with shaking at 150 rpm for 12 h. The highest relative yield of D-tagatose obtained from the conditions was 68.84%.

## Acknowledgements

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