EVALUASI KEMAMPUAN BAKTERI ASAM LAKTAT *INDIGENOUS* ASAL INDONESIA BERPOTENSI PROBIOTIK DALAM MENGHASILKAN URIKASE

Intisari

Urikase adalah enzim yang mengkatalisis oksidasi asam urat menjadi allantoin, hidrogen peroksida dan karbondioksida. Beberapa isolat asam laktat telah diketahui berpotensi sebagai probiotik dan mampu memproduksi urikase serta menurunkan asam urat pada tikus percobaan. Bakteri asam laktat *indigenous* yang diisolasi dari kulit markisa dan manggis yang berasal dari Medan dan Brastagi juga diketahui memproduksi urikase. Bakteri asam laktat *indigenous* berpotensi probiotik telah diisolasi dari gatot, growol, dadih, susu, nira, teri dan feces bayi yang berasal Yogyakarta, Sumatra Barat dan Banyumas. Namun potensinya untuk memproduksi urikase belum diketahui. Agar dapat digunakan untuk menurunkan asam urat dalam saluran pencernaan maka urikase harus stabil dalam saluran pencernaan. Upaya meningkatkan produksi urikase pada beberapa mikrobia telah dilakukan dengan melakukan optimasi waktu fermentasi, jenis dan konsentrasi induser, suhu fermentasi serta komposisi media pertumbuhan. Namun pengaruh kondisi lingkungan terhadap produksi urikase oleh bakteri asam laktat *indigenous* belum dilakukan. Bakteri asam laktat juga telah digunakan sebagai kultur starter untuk menghasilkan komponen bioaktif selama fermentasi susu namun kemampuan bakteri asam laktat indigenous untuk memproduksi urikase selama fermentasi susu belum dilakukan. Kebaruan dari penelitian ditinjau dari evaluasi stabilitas urikase yang dihasilkan bakteri asam laktat indigenous dalam model saluran pencernaan serta kemampuan bakteri asam laktat memproduksi urikase selama fermentasi susu.

Tujuan umum dari penelitian ini adalah mengevaluasi potensi bakteri asam laktat berpotensi probiotik *indigenous* penghasil urikase untuk penurunan asam urat. Tujuan khususnya adalah (1). Mengevaluasi potensi bakteri asam laktat untuk memproduksi urikase serta stabilitas urikase yang dihasilkan dalam model lambung dan usus halus. (2). Mengevaluasi pengaruh waktu fermentasi, konsentrasi asam urat, suhu fermentasi, jenis induser dan komposisi media terhadap produksi urikase oleh bakteri asam laktat (3). Mengevaluasi kemampuan bakteri asam laktat untuk memproduksi urikase selama fermentasi susu skim. Untuk mencapai tujuan tersebut, penelitian dilaksanakan dalam 3 tahap yaitu (1) *Screening* bakteri asam laktat penghasil urikase (2). Evaluasi pengaruh lingkungan terhadap produksi urikase oleh bakteri asam laktat (3). Produksi urikase selama fermentasi susu skim.

Hasil penelitian menunjukkan 13 isolat bakteri asam laktat yang digunakan mampu memproduksi urikase ekstraseluler, intraseluler dan terikat membran dengan aktivitas yang rendah. Tiga isolat yaitu *L.brevis* OL-5, *Lactobacillus plantarum* Dad-13 dan *L. plantarum* Mut 7 menghasilkan urikase intraseluler lebih tinggi dibandingkan isolat lain. Hasil pengujian stabilitas urikase terhadap model saluran pencernaan menunjukkan urikase ekstraseluler dan terikat membran yang diproduksi *L. plantarum* Dad-13, *L. plantarum* Mut 7 dan *L.brevis* OL-5 serta urikase intraseluler yang diproduksi *L. plantarum* Mut 7 kehilangan seluruh aktivitasnya dalam model lambung dan usus halus. Pada *L. plantarum* Dad-13 dan *L. brevis* OL-5 penambahan *gastrik juice* dan *intestinal juice* menyebabkan kehilangan aktivitas urikase intraseluler sebesar 91-94% dan menyisakan residu aktivitas yang sangat rendah yaitu 58 dan 44 mU/mL kultur dari aktivitas semula sebesar 715 dan 743 mU/mL kultur. Oleh karena itu urikase yang diproduksi bakteri asam laktat *indigenous* tidak stabil dalam model saluran pencernaan. *L. plantarum* Dad-13 mampu mengeluarkan urikase intraseluler sehingga berpotensi menurunkan asam urat yang ada di luar sel. Hasil penelitian optimasi produksi urikase yang dilakukan terhadap bakteri asam laktat indigenous menunjukkan urikase yang diproduksi *L. plantarum* Dad-13 bersifat *indusible,* memerlukan asam urat sebagai induser,

sedangkan adenin dan guanin tidak mampu menginduksi produksi urikase. Produksi urikase tinggi oleh *L. plantarum* Dad-13 diperoleh dengan fermentasi menggunakan media PGY pada suhu 37ºC selama 22 jam, menggunakan asam urat sebagai induser pada konsentrasi 0,15%. Produksi urikase oleh *L. plantarum* Dad-13 dan dihambat oleh allantoin. Hasil penelitian juga menunjukkan urikase tidak dapat diproduksi oleh *L. plantarum* Dad-13 dalam susu selama fermentasi. Asam orotat yang terdapat dalam susu bersifat menghambat transport asam urat masuk kedalam sel, sehingga sel tidak dapat memproduksi urikase.

Kata kunci: Bakteri asam laktat, probiotik *indigenous*, urikase, optimasi produksi, susu skim.

EVALUATION OF PROBIOTIC POTENTIAL OF INDONESIAN INDIGENOUS LACTIC ACID BACTERIA TO PRODUCE URICASE

Abstract

Uricase is an enzyme that catalyses the oxidation of uric acid to allantoin, hydrogen peroxide and carbon dioxide. There are several lactic acid isolates that have been known exhibit capabilities as a probiotic as well as produce uricase and reduce uric acid in mice serum. It has been identified that indigenous lactic acid bacteria which are isolated from passion fruit and mangosteen peel from Medan and Brastagi have a capability to produce uricase. In addition, indigenous lactic acid bacteria that have a potency as probiotics have been isolated from gatot, growol, dadih, milk, coconut sap, anchovies, and infant faeces from Yogyakarta, West Sumatera and Banyumas Regency. In spite of their capability as probiotics, their potency to produce uricase has not been identified yet. In order to reduce uric acid orally, uricase should be stable in the gastrointestinal system. The stability of uricase produced by indigenous lactate acid bacteria in the digestive system, however, has not been known yet. A number of studies have been conducted to increase uricase in some microbes by optimizing the fermentation time, type and concentration of inducer, fermentation temperature, and concentration of growing medium. However, the influence of fermentation condition on uricase production by lactic acid bacteria has not been done yet. The lactic acid bacteria have also been utilized as a culture starter to yield bioactive components during skim milk fermentation. However the capability of indigenous lactic acid bacteria in uricase production during milk fermentation has not been known yet. The novelty of the present study was evaluated from the evaluation of uricase stability produced by lactic acid bacteria in the gastrointestinal model and the ability of lactic acid bacteria to produce uricase during skim milk fermentation.

In general, this research focuses on the evaluation of indigenous lactic acid bacteria in uricase production to reduce uric acid. The specific objectives of this research are as follows: (1) To evaluate the potency of lactate acid bacteria in uricase production and the stability of uricase produced in stomach and small intestine models, (2) To evaluate the fermentation condition i.e fermentation time, type and concentration of the inducer, fermentation temperature, and the composition of the medium of high uricase production by lactate acid bacteria, and (3) To evaluate the capability of lactic acid bacteria to produce uricase in skim milk during fermentation. In order to achieve the aforementioned objectives, this research was conducted in 3 stages, i.e. (1) screening of the uricase producing lactate acid bacteria, (2) evaluation the fermentation condition on uricase production by lactic acid bacteria and (3) uricase production in skim milk during fermentation.

The results show that 13 isolates of lactate acid bacteria used in this research can yield extracellular, intracellular and membrane-bound uricase with minor activities. Three of the isolates, which are *L.brevis* OL-5, *Lactobacillus plantarum* Dad-13 dan *L. plantarum* Mut 7, produced a higher amount of intracellular uricase than the other isolates. The results of the stability test of uricase in digestive system show that both extracellular and membrane-bound uricase which were produced by *L. plantarum* Dad-13, *L. plantarum* Mut 7 and *L.brevis* OL-5 lacked all their activities in the stomach and small intestine models. Such condition also occurred on intracellular uricase produced by *L. plantarum* Mut 7. The addition of gastric juice and intestinal juice on *L. plantarum* Dad-13 and *L.brevis* OL-5 led to a lack of intracellular uricase activity by 91-94% and left very small residue of activities, which were 58 and 44 mU/mL culture, respectively, from the initial activities of 715 and 743 mU/mL culture, respectively. Uricase produced by indigenous lactate acid bacteria, therefore, was not stable in the digestive system model. Test results also revealed that *L. plantarum* Dad-13 had the ability to excreted intracellular uricase into the media. Furthermore, the results of the optimization of

uricase production by the indigenous lactate acid bacteria show that uricase yielded by *L. plantarum* Dad-13 was inducible and required uric acid as the inducer substrate, while adenine and guanine were not able to induce uricase production. The optimum uricase production by *L. plantarum* Dad-13 was achieved by fermentation using PGY medium at temperature of 37 ºC for 22 hours and using uric acid as the inducer on concentration of 0.15%. Uricase was produced by *L. plantarum* Dad-13 and was inhibited by allantoin. Moreover, the results show that uricase cannot be produced by *L. plantarum* Dad-13 in milk during fermentation. Orotic acid in milk inhibited the transport of uric acid into cells so that the cells cannot produce uricase.

Keywords: Lactic acid bacteria, probiotic indigenous, uricase, optimation production, skim milk