

ISOLASI, SELEKSI, DAN IDENTIFIKASI YEAST ASAL KEFIR SERTA EVALUASI KINETIKA FERMENTASI DENGAN SISTEM *BATCH* UNTUK MENDAPATKAN STARTER TUNGGAL

INTISARI

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Kefir mengandung berbagai macam mikrobia, salah satunya yeast. Yeast merupakan organisme eukariotik yang berperan sebagai kultur starter dalam proses fermentasi kefir. Dalam proses fermentasi kefir, diperlukan mikroorganisme yang dapat mendegradasi laktosa dan memfermentasi alkohol, sehingga diperlukan yeast yang tidak hanya mampu menghasilkan alkohol tetapi juga memiliki aktivitas laktase yang baik. Penelitian ini bertujuan untuk mengisolasi dan mengidentifikasi yeast asal kefir dan menguji kinetika fermentasi secara batch. Isolat yeast yang terpilih berpotensi diaplikasikan dalam pembuatan susu fermentasi sebagai starter tunggal. Sampel untuk isolasi yeast berasal dari kefir. Isolat yang didapatkan dilakukan identifikasi melalui pengamatan makroskopis dan mikroskopis, pengujian biokimia, dan molekuler. Isolasi DNA dilakukan dari isolat yeast yang tumbuh pada media laktosa, diikuti dengan visualisasi hasil PCR dengan agarose gel elektroforesis dan UV transilluminator, kemudian dilakukan sekuensing. Isolat DNA diamplifikasi dengan PCR menggunakan primer ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') sebagai *forward primer* dan ITS4 (5'-TCCTCCGCTTATTGATATGC-3') sebagai *reverse primer*. Hasil penelitian menunjukkan bahwa identifikasi makroskopis dan biokimia menghasilkan 10 isolat yang memiliki karakter yeast yang mampu mendegradasi laktosa. Dari 10 isolat, selanjutnya dipilih 7 isolat untuk pengujian molekuler. Berdasarkan analisis sekuens nukleotida, 5 isolat yaitu KFA 3, KFA 7, KFA 9, dan KFB 1 diklasifikasikan sebagai *Kluyveromyces marxianus*, sedangkan KFA 4 diklasifikasikan sebagai *Pichia kudriavzevii*. Data kinetika fermentasi sistem batch dari kelima isolat diplotkan pada model logistik untuk mendapatkan koefisien daya dukungnya dalam membandingkan kinerja strain. Hasil koefisien daya dukung dari isolat KFA 3, KFA 4, KFA 7, KFA 9, dan KFB 1 masing-masing adalah 0,382; 0,259; 0,389; 0,478; dan 0,415 h⁻¹. Hasil ini mempersempit strain prospektif menjadi isolat KFA 3, KFA 7, KFA 9 dan KFB 1 yang semuanya serupa dalam laju pertumbuhan seperti yang ditunjukkan oleh koefisien daya dukungnya. Dari hasil pemodelan kinetika, strain KFB 1 dan KFA 3 digunakan sebagai starter tunggal dan dibandingkan dengan kefir grain dengan konsentrasi inokulasi masing-masing 5% (v/v) ke dalam susu kambing. Semua perlakuan diinkubasi pada suhu 30°C selama 18 jam. Hasil inokulasi menggunakan starter tunggal KFA 3 dan KFB 1 serta kefir grain tidak berpengaruh nyata terhadap total yeast, kadar air, total solid, viskositas, dan sineresis namun berpengaruh terhadap pH dan kadar alkohol. Kesimpulan yang diperoleh adalah starter KFA 3 dan KFB 1 sebagai starter tunggal mampu memfermentasi susu kambing.

Kata kunci: *Kluyveromyces marxianus*, kefir *starter culture*, kinetika fermentasi, kualitas susu fermentasi

ISOLATION, SELECTION, AND IDENTIFICATION OF YEAST FROM KEFIR AND EVALUATION OF FERMENTATION KINETICS USING BATCH SYSTEM TO OBTAIN A SINGLE STARTER

ABSTRACT

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Kefir contains various kinds of microbes, one of which is yeast. Yeast is a eukaryotic organism that acts as a starter culture in the kefir fermentation process. In the kefir fermentation process, microorganisms are needed that can degrade lactose and ferment alcohol. Yeast is needed that is not only able to produce alcohol but also has good lactase activity. This study aims to isolate and identify yeast from kefir and to test the kinetics of batch fermentation. The selected yeast isolate has the potential to be applied in the manufacture of fermented milk as a single starter. The samples for yeast isolation were derived from kefir and the isolates obtained were identified through macroscopic and microscopic observations, biochemical and molecular tests. DNA isolation was carried out from yeast isolates grown on lactose media, followed by visualization of PCR results with agarose gel electrophoresis and UV transilluminator, then sequencing was performed. DNA isolates were amplified by PCR using primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') as a forward primer and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') as a reverse primer. The results showed that macroscopic and biochemical identification resulted in 10 isolates that had yeast characteristics capable of degrading lactose. From 10 isolates, 7 isolates were selected for molecular testing. Based on nucleotide sequence analysis, 5 isolates namely KFA 3, KFA 7, KFA 9, and KFB 1 were classified as *Kluyveromyces marxianus*, while KFA 4 was classified as *Pichia kudriavzevii*. The batch system fermentation kinetics data from the five isolates were plotted on the logistic model to obtain the coefficient of carrying capacity in comparing the performance of the strains. The results of the carrying capacity coefficients of isolates KFA 3, KFA 4, KFA 7, KFA 9, and KFB 1 were 0.382; 0.259; 0.389; 0.478; and 0.415 h⁻¹. These results narrowed the prospective strains into isolates KFA 3, KFA 7, KFA 9, and KFB 1 which were all similar in growth rates as indicated by their carrying capacity coefficients. From the results of kinetic modeling, it was revealed that KFB 1 and KFA 3 strains were used as a single starter and compared with kefir grains with a 5% (v/v) inoculation concentration each into goat's milk. All treatments were incubated at 30°C for 18 hours. The results of inoculation using a single starter KFA 3 and KFB 1 as well as kefir grains did not significantly affect the total yeast, moisture content, total solids, viscosity, and syneresis but did affect pH and alcohol content. The conclusion obtained is that starters KFA 3 and KFB 1 as a single starter can ferment goat's milk.

Keywords: *Kluyveromyces marxianus*, kefir starter culture, fermentation kinetics, fermented milk quality