INTISARI

Dadih adalah makanan fermentasi tradisional Indonesia yang berasal dari Sumatra Barat, dibuat dari susu kerbau dengan cara fermentasi secara spontan. Fermentasi dadih melibatkan bakteri asam laktat (BAL). Dalam penelitian ini, BAL yang diharapkan dapat memproduksi asam folat diisolasi dari dadih. Asam folat berfungsi dalam metabolisme asam amino dan nukleotida di dalam sel, seperti biosintesis DNA dan RNA; yaitu biosintesis purin, guanin, adenin, pirimidin dan timin; biosintesis gugus metil; vitamin; dan beberapa asam amino. Kekurangan asam folat dapat menyebabkan resiko cacat lahir pada bayi, misalnya cacat pembuluh syaraf (*neural tube defect*/NTD), mencegah menurunnya kemampuan mendengar, resiko terkena osteoporosis, penyakit jantung, *dementia* dan Alzheimer's, anemia serta meningkatnya resiko kanker. Tujuan penelitian ini adalah untuk mempelajari potensi BAL yang diisolasi dari dadih sebagai agensia penghasil asam folat dan peranannya dalam meningkatkan kandungan asam folat dalam susu fermentasi yang berasa enak sebagaimana yogurt, dengan penambahan prekursor biosintesis asam folat: asam glutamat, asam para amino benzoat (pABA), dan ribosa 5-P.

Penelitian dilakukan dengan mengisolasi BAL dari dadih susu kerbau, memurnikan dan mengidentifikasi isolat BAL berdasar morfologi sel, uji katalase, pewarnaan Gram, dan produksi gas dari glukosa. Untuk identifikasi spesiesnya dilakukan pengujian sifat fenotipiknya (menggunakan API 50CHL) dan genotipiknya (sekuen 16S rRNA). Dilanjutkan dengan skrining BAL penghasil asam folat yang dianalisis menggunakan *Vita Fast Folic Acid* (Bioparm). Kemudian dipelajari pola biosintesis asam folat BAL hasil isolasi dengan media susu skim. Dilanjutkan dengan penambahan prekursor biosintesis asam folat yaitu asam glutamat, pABA, dan ribosa 5-P baik secara individual maupun kombinasinya, serta mempelajari pengaruh penyimpanan terhadap stabilitas asam folat susu fermentasi yang dihasilkan.

Hasil penelitian menunjukkan bahwa dari 29 isolat yang diisolasi, 27 isolat merupakan genus *Lactobacillus* dan 2 isolat genus *Enterococcus*. Terdapatnya kelompok Enterococcus menandakan cara pembuatan dadih kurang higienis. Identifikasi penotipik dan genotipik dilakukan terhadap 17 isolat yang menghasilkan susu fermentasi yang strukturnya kokoh dan enak, menunjukkan bahwa 16 isolat merupakan *Lactobacillus plantarum* dan satu isolat yaitu E-5 kemungkinan merupakan spesies baru. BAL yang memproduksi asam folat tinggi yaitu *L. plantarum* Dad-13 menghasilkan asam folat 29,27±3.92 μg/L, *L. plantarum* G-3 menghasilkan 27,84±5,80 μg/L, dan *L. plantarum* H-1 menghasilkan asam folat 26,95±0,27 μg/L. Bakteri asam laktat mensintesa asam folat pada phase pertumbuhan logaritmik. Penambahan prekursor asam glutamat 75 μmol/L dan kombinasinya meningkatkan kadar asam folat susu fermentasi yang diinokulasi *L. plantarum* Dad-13 sebesar 59,86 – 105,83 %, *L. plantarum* G-3 sebesar 81,93 – 127,20 %. Peningkatan kadar asam folat dengan penambahan

pABA dan kombinasinya dengan inokulum *L. plantarum* Dad-13 sebesar 74,85% – 116,67% dan *L. plantarum* G-3 sebesar 81,93 – 142,59 %. Susu fermentasi dengan suplementasi ribosa 5-P 75 µmol dan 100 µmol dengan inokulum *L. plantarum* Dad-13 kadar asam folatnya meningkat sebesar 80,2 – 105,83 %, dengan *L. plantarum* G-3 meningkat sebesar 113,60 – 127,20 %. Penyimpanan susu fermentasi dengan penambahan prekursor kombinasi asam glutamat dan ribosa 5-P pada suhu 4 ^{o}C selama 13 minggu, asam folat pada susu fermentasi yang diinokulasi *L. plantarum* Dad-13 menurun sebesar 11,75% dan *L. plantarum* G-3 menurun sebesar 15,46 %.

Secara umum dapat disimpulkan bahwa BAL penghasil asam folat, yaitu *L. plantarum* sp. dapat diisolasi dari dadih, dan dapat dikembangkan potensinya sebagai starter produksi susu fermentasi yang kaya asam folat. Penambahan prekursor biosintesis asam folat bisa digunakan untuk meningkatkan kadar asam folat susu fermentasi, dengan hasil terbaik asam para amino benzoat dan kombinasi asam glutamat serta ribosa 5-P 75 µmol/L.

Kata-kata kunci: Bakteri asam laktat, fermentasi, asam folat, prekursor biosintesis asam folat

ABSTRACT

Dadih is Indonesian traditional fermented food coming from West Sumatra as it is derived from buffalo milk fermented spontaneously. During spontaneous fermentation, Lactic Acid Bacteria (LAB) are involved. On this present study, LAB tested was expected to produce folate that was isolated from dadih. Folate plays a role in amino acid and nucleotide metabolism, such as DNA and RNA biosynthesis including purine, guanine, adenine, pyrimidine, and thymine biosynthesis as well as biosynthesis of methyl group, vitamin, and some amino acids. An insufficient amount of folate is responsible for the risk of certain birth defect such as neural tube defect (NTD), auditory ability, heart disease, dementia, Alzheimer's, osteoporosis, anemia, and, cancer. Thus, the objectives of study were to investigate the potency of LAB as folate producing agents and their role in increasing folate content in fermented milk which had a good flavor as yogurt by the addition of folate biosynthesis precursor: glutamic acid, para amino benzoic acid (pABA), and ribose 5-P precursors.

The study was conducted by isolating LAB from dadih derived from buffalo milk, purifying and identifying LAB isolates based on cell morphology, catalase test, Gram stained, and gas production from glucose. Species identification done by phenotypic and genotypic attributes using API 50CHL software and 16S rRNA sequence respectively. Screening of folate producing LAB was analyzed by using *Vita Fast Folic Acid* (Bioparm). Afterwards, the folate biosynthesis model was examined in skim milk medium followed by the addition of precursors of folate biosynthesis as individual or combination, namely glutamic acid, pABA, and ribose 5-P. Last, the effect of storage on the folate stability in the resulting fermented milk was also investigated.

The result of study explained that from 29 LAB isolates obtained, 27 isolates were from Lactobacillus, whilst 2 isolates were from Enterococcus. The presence of Enterococcus indicated an unhygienic process of dadih production. The result of phenotypic and genotypic identification showed that from 17 isolates producing fermented milk that had firm structure and good flavor, 16 isolates were classified into a group of *Lactobacillus plantarum* and one isolate, E-5, was completely unknown and presumed to be a novel species. The folate produced by *Lactobacillus plantarum* Dad-13, *L. plantarum* G-3, and *L. plantarum* H-1 was 29.27±3.92 µg/L, 27.84±5.80 µg/L, and 26.95±0.27 µg/L, respectively. During the logarithmic phase of bacterial growth cycle, folic acid was synthesized by LAB. When the glutamic acid precursor was added at a concentration of 75 µmol/L combined with other precursors, the increase of 59.86-105.83% was occurred in fermented milk inoculated by *L. plantarum* Dad-13 and the increase of 81.93—127.20%. A percentage increase in folic acid content added with pABA precursors and other precursors by *L. plantarum* Dad-13 inoculum were 74.85%–116.67%, whereas the fermented milk inoculated by *L. plantarum* G-3 experienced an increase of 81.93–142.59%. Fermented milk with ribose 5-P

supplementation of 75 μmol and 100 μmol inoculated by *L. plantarum* Dad-13 yielded an increase of 80.20–105.83% in folate content, whilst the higher result of folate content was yielded by the inoculation of *L. plantarum* G-3 (113.60—127.20%). The storage of fermented milk added with glutamic acid and ribose 5-P precursor for 13 weeks at 4^{o}C caused a decrease of 11.75% and 15.46% in folic acid content that was occurred in fermented milk inoculated by *L. plantarum* Dad-13 and *L. plantarum* G-3, respectively.

In conclusion, folate producing LAB, *L. plantarum* sp. was generally able to be isolated from dadih and its potency as a starter in the production of fermented milk enriched by folic acid can be developed. The addition of precursor in folate biosynthesis could be used to improve folic acid content of fermented milk, and the best results were pABA and a combination of glutamic acid and ribose 5-P 75 μmol/L.

Key words: Lactic acid bacteria, fermentation, folic acid, precursor of folate biosynthesis