

Analisis Fenotip dan Genomik terhadap Resistensi Antibiotik dari Bakteri Probiotik Indigenus di Indonesia

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INTISARI

Empat strain *Lactobacillus* (*L. plantarum* Dad-13, Mut-7, T-3, dan *L. paracasei* SNP-2) yang diisolasi dari makanan fermentasi Indonesia dan materi fekal bayi sehat telah *discreening* kemampuannya sebagai agen probiotik. Tujuan penelitian ini adalah untuk mengetahui resistensi antibiotik dan potensi transfer gen resisten antibiotik pada *L. plantarum* Dad-13, Mut-7, T-3, dan *L. paracasei* SNP-2 terhadap delapan antibiotik (amoksisilin, tetrasiklin, eritromisin, klindamisin, kloramfenikol, streptomisin, kanamisin, dan siprofloksasin). Keempat stok isolat ditumbuhkan dalam media cair de Man, Rogosa, Sharpe pada suhu 37 °C selama 24 jam. Resistensi antibiotik secara fenotip dievaluasi menggunakan metode difusi disk. Sekuensing genom dilakukan menggunakan sistem platform NovaSeq 6000. Genom dianotasi menggunakan *Rapid Annotation using Subsystem Technology* (RAST) V 2.0. Setiap kelompok dari prediksi gen resisten selanjutnya diselaraskan menggunakan *Multiple Sequence Comparison by Log-Expectation* (MUSCLE) dan dikonfirmasi fungsinya menggunakan *Comprehensive Antibiotic Resistance Database* (CARD) 2020. Semua strain menunjukkan resistensi terhadap streptomisin, kanamisin, dan siprofloksasin tetapi rentan terhadap amoksisilin, klindamisin, dan eritromisin. Resistensi terhadap kloramfenikol dan tetrasiklin bervariasi antar strain. Dua strain rentan dan yang lainnya menunjukkan resistensi menengah terhadap kloramfenikol. Hanya satu strain yang resisten terhadap tetrasiklin, sedangkan tiga strain lainnya menunjukkan resistensi menengah. Sekuensing genom dari empat strain dikonfirmasi memiliki gen resistensi terhadap tetrasiklin, amoksisilin, siprofloksasin, dan pompa efluks *multidrug*. Hasil gen resisten berkorelasi dengan hasil fenotipnya, kecuali pada antibiotik amoksisilin dan aminoglikosida (streptomisin dan kanamisin). Kerentanan amoksisilin masih ambigu dan perlu penelitian genomik lebih lanjut, sedangkan resistensi aminoglikosida terjadi karena sifatnya dan dianggap sebagai resistensi intrinsik. Anotasi RAST menunjukkan bahwa semua strain *Lactobacillus* tidak memiliki elemen transposabel, agen transfer gen, dan fungsi terkait plasmid, sehingga transfer horizontal gen resistensi antibiotik mungkin tidak terjadi.

Kata Kunci : Resistensi Antibiotik, Genomik, *Lactobacillus*, Probiotik

Phenotype and Genomic Analysis of Antibiotic Resistance in Indonesian Indigenous Probiotic Bacteria

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ABSTRACT

Four *Lactobacillus* strains (*L. plantarum* Dad-13, Mut-7, T-3, and *L. paracasei* SNP-2) isolated from fermented Indonesian food and the fecal of healthy infants were screened for their ability as probiotic agents. The purpose of this study was to determine the antibiotic resistance and the potential for antibiotic resistance gene transfer to *L. plantarum* Dad-13, Mut-7, T-3, and *L. paracasei* SNP-2 against eight antibiotics (amoxicillin, tetracycline, erythromycin, clindamycin, chloramphenicol, streptomycin, kanamycin, and ciprofloxacin). The four isolate stocks were cultured in de Man, Rogosa, Sharpe broth at 37 °C for 24 hours. Antibiotic susceptibility was evaluated using the disk diffusion method. Genome sequencing was performed using the NovaSeq 6000 sequencing system platform. The genome was annotated using Rapid Annotation using Subsystem Technology (RAST) V 2.0. Each group of the predicted product of resistant genes was further aligned using Multiple Sequence Comparison by Log-Expectation (MUSCLE) and confirmed their function using Comprehensive Antibiotic Resistance Database (CARD) 2020. All strains showed resistance to streptomycin, kanamycin, and ciprofloxacin but susceptible to amoxicillin, clindamycin, and erythromycin. Resistance to chloramphenicol and tetracyclines varied between strains. Two strains were susceptible and the other showed intermediate resistance to chloramphenicol. Only one strain was resistant to tetracyclines, while the other three isolates showed intermediate resistance. Genome sequencing of four strains was confirmed the presence of resistance genes to tetracycline, amoxicillin, ciprofloxacin, and multidrug efflux pump. The occurrence of resistance gene was correlated with the phenotypic results, except for amoxicillin and aminoglycoside antibiotics (streptomycin and kanamycin). Amoxicillin susceptibility is still ambiguous and needs further genomic research, whereas aminoglycoside resistance occurs due to its nature and is considered as intrinsic resistance. The RAST annotation indicates that all *Lactobacillus* strains do not have transposable elements, gene transfer agents, and plasmid-related functions, thus horizontal transfer of antibiotic resistance genes unlikely will occur.

Keyword: antibiotic resistance, genomics, *Lactobacillus*, probiotics