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Sharing Session Probiotik

Endang Sutriswati Rahayu
Mifta Gatya
PUIPT – Probiotik, Pusat Studi Pangan dan Gizi,
Universitas Gadjah Mada
21 Juli 2025

Garis Besar Pelaksanaan Uji Klinis

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- Memperoleh dokumen etik
- Rekruitment subjek sesuai dengan kriteria inklusi

Persiapan

Skrining dan Visit

- Sosialisasi kegiatan penelitian
- Penandatanganan informed consent bagi yang bersedia berpartisipasi sebagai subjek
- Pengisian kuisioner skrining
- Pengukuran parameter klinis (sesuai dengan parameter pada *study design*)
- Subjek diminta untuk tidak mengonsumsi produk probiotik lain serta antibiotik selama penelitian berlangsung
- Menjalankan tahap pembiasaan (baseline)

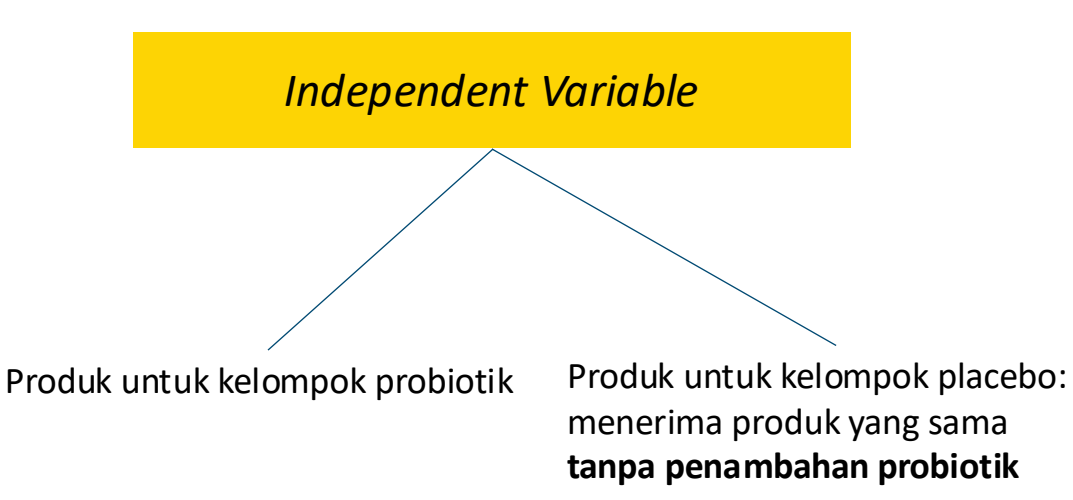
Uji Klinis

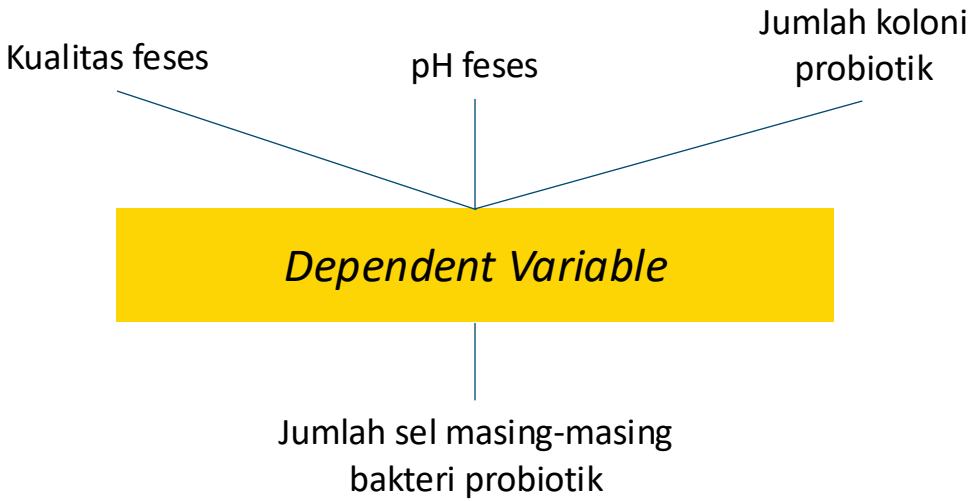
- Pada hari pertama setelah tahap pembiasaan, subjek diminta untuk mengumpulkan feses dan pemeriksaan parameter klinis

a. Blinding dan Randomization (double blind study)
b. Melakukan visit penelitian secara berkala untuk melakukan:

- Pembagian produk
- Pengukuran parameter klinis subjek
- Pembagian kuisioner catatan harian berisi konsumsi produk, konsumsi produk probiotik dan prebiotik, aktivitas fisik, catatan Kesehatan, konsumsi obat, dan catatan pola makan, frekuensi BAB dan kualitasnya

Variabel Penelitian





Parameter penelitian lainnya

- Frekuensi BAB
- Pola makan subjek
- Kondisi kesehatan dan konsumsi obat

Kit Koleksi Sampel Feses

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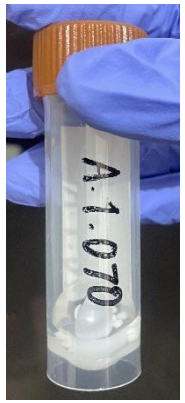
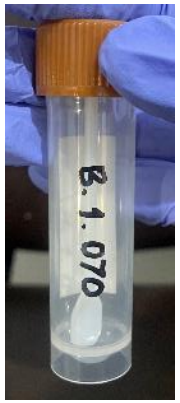
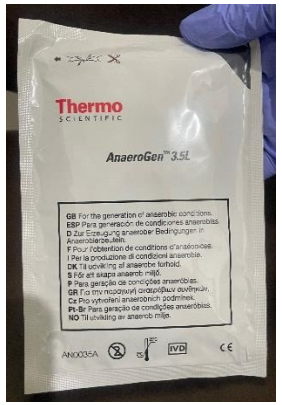
Thermo Bag	
	Digunakan untuk membawa sampel feses – stabil terhadap suhu

Pack		
	**Masker**	: Untuk mencegah kontaminasi dan bau dari feses
	Sarung tangan	: Untuk menghindari kontaminasi dari tangan subjek
	Kertas steril	: Alas untuk meletakkan feses

Botol feses 1	**Botol feses 2**	**Botol feses 3***	***Anaerobe pouch****	***Zipper pouch****	***Ice gel* (6 pcs)**
					
Terdapat RNA later dan *glass bead*	**Botol kosong**	**Botol kosong**	Untuk kondisi anaerobik	Untuk pouch botol feses 3 dan oxygen absorber	Rendam dalam air, letakkan di freezer – pengganti es batu sementara
Untuk analisa qPCR	Untuk analisa *culture methode*	Untuk analisa *culture methode*			

NB: tanda (*) menunjukkan bahwa perlengkapan tersebut hanya digunakan apabila karakter bakteri adalah anaerob obligat

Tata Cara Sampling Feses

1. Rendam ice gel ke dalam baskom berisi air. Pastikan ice gel sudah menggembung, kemudian masukkan ice gel ke dalam freezer.
2. Apabila sudah akan buang air besar, gunakan masker, sarung tangan dan pasang kertas steril pada ujung-ujung toilet. Pastikan bahwa yang tertampung pada kertas steril hanya feses saja tanpa ada air seni ataupun air toilet
3. Ambil feses yang tertampung pada kertas steril di 3 titik menggunakan sendok yang ada pada botol feses 1. Pastikan RNA later dan glass bead pada botol feses tidak tumpah.
4. Pastikan sampel feses tercampur seluruhnya pada RNA later dan tutup dengan rapat.
5. Ambil botol feses 2 dan masukkan feses hingga 2/3 penuh menggunakan sendok yang ada pada botol, kemudian tutup rapat.
6. **Ambil botol feses 3, masukkan feses hingga 2/3 penuh menggunakan sendok yang ada pada botol.**
7. **Buka zipper pouch, kemudian masukkan botol feses 3 dalam kondisi sedikit terbuka ke dalam zipper pouch.**
8. **Segera buka anaerob pouch, ambil kantong yang ada di dalam pouch dan masukkan ke dalam zipper pouch.**
9. **Sebelum zipper ditutup, tekan dari bagian bawah hingga ke atas untuk mengeluarkan udara yang ada di dalam zipper pouch, kemudian tutup zipper dengan rapat**
10. Masukkan botol feses 1,2, dan 3, lalu masukkan ice gel yang sudah jadi ke dalam *thermo bag*.

NB: Tahap 6-9 hanya dilakukan jika terdapat bakteri anaerob obligat

Pembagian *Sampling Kit*

- *Sampling kit* dibagikan dengan melakukan penjelasan langsung cara sampling kepada subjek **satu per satu** hingga cara treatment untuk sampel hingga sampel sampai ke tangan peneliti
- Sediakan video cara sampling feses

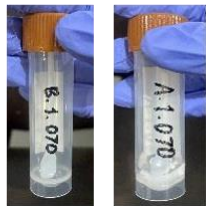
Penanganan dan Pengiriman Sampel

- **Sampel feses sudah siap dikirim**
- **Subjek menghubungi peneliti**

Sampel feses dikirim menggunakan aplikasi "GOJEK" langsung setelah feses sudah siap kirim

Diterima dalam konsisi baik oleh tim peneliti dalam waktu 30 menit

Feses untuk *culture methode* segera di platting setelah sampai



Sample untuk qPCR ditimbang, dihomogenisasi dan disimpan dalam *deep freeze* (-80 °C) sebelum di analisa

Uji koloni pada Culture Methods

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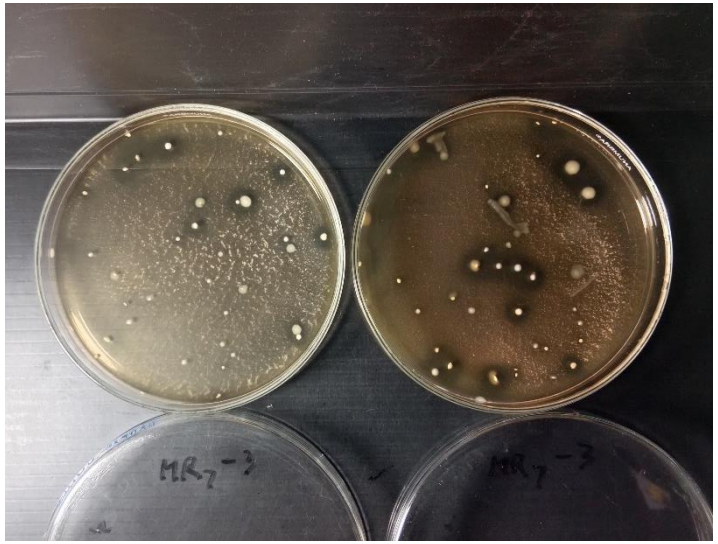
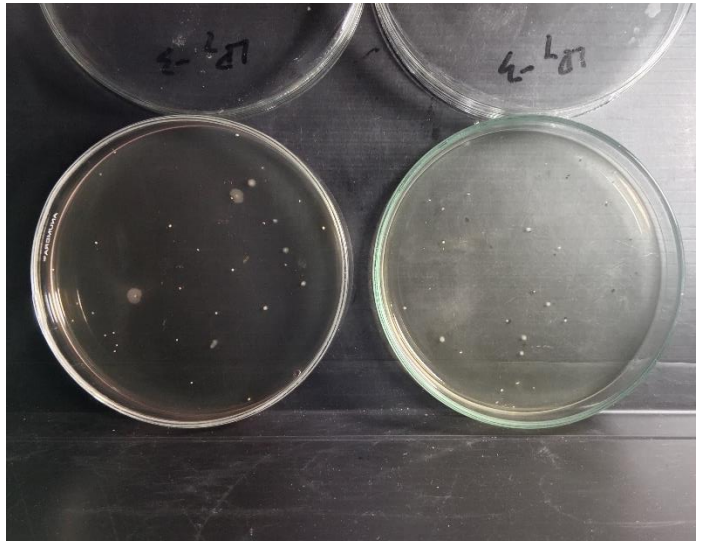
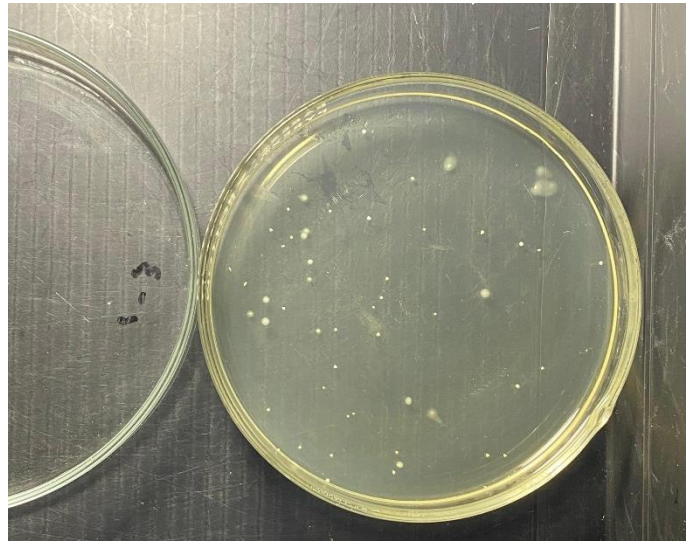
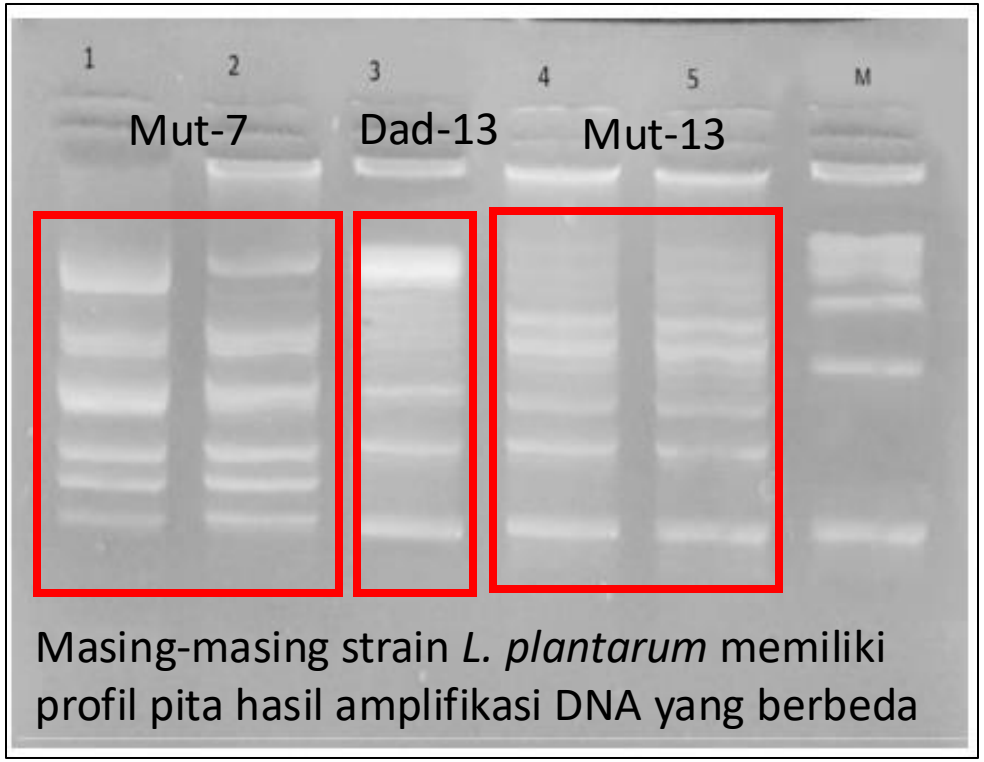
		
Koloni BAL pada media MRS	Koloni *L. plantarum* pada media LPSM	Koloni *Bifidobacterium* Pada media BSM

Uji koloni pada Culture Methods

- Tujuan uji ini adalah untuk menentukan kemampuan masing-masing strain hidup di feses – dengan menguji beberapa koloni yang ada di culture methods (diambil secara acak) – dilakukan analisis PCR menggunakan primer BOX-A1R bersama dengan strain probiotik
- Primer BOX-A1R - 5'-CTACGGCAAGGCGACGCTGACG-3’.
- Masing-masing strain akan memiliki pola pita yang spesifik
- Apabila koloni dari culture method memiliki pola spesifik yang sama dengan strain murni probiotik – berarti strain probiotik tsb dapat berkoloni (hidup) dalam usus



Masing-masing strain *L. plantarum* memiliki profil pita hasil amplifikasi DNA yang berbeda

Figure 1. Result of REP-PCR using BOX A1R primers. 1- *L. plantarum* Mut-7; 2- *L. plantarum* Mut-7 20x dilution; 3- *L. plantarum* Dad13; 4- *L. plantarum* Mut-13; 5- *L. plantarum* Mut-13 20x dilution; M: Molecular weight markers (100-3000 bp). (sumber author/ESR)
BOX A1R Primer:
(5 'CTACGGCAAGGCGACGCTGACGCTGACG-3')

ELSEVIER

FEMS Microbiology Letters 205 (2001) 31–36

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Applicability of rep-PCR fingerprinting for identification of *Lactobacillus* species

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[b] *BCCM/TM/LMG Bacteria Collection, Department of Biochemistry, Physiology and Microbiology, Ghent University, K.L. Ledeganckstraat 35, 9000 Gent, Belgium*

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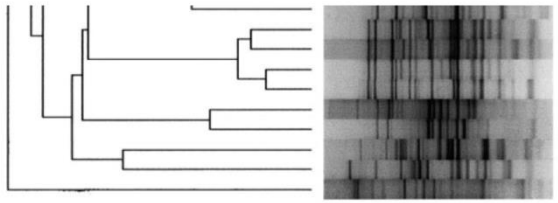
- PCR amplification of repetitive bacterial DNA elements (rep-PCR) has characteristics: (i) a high discriminatory power, (ii) low cost, (iii) suitable for a high-throughput of strains, and (iv) considered to be a reliable tool for classifying and typing a wide range bacteria
- Three Primers used in this study
- REP1R-I (5'-IIIICGICGICATCIGGC-3') and REP2-I (5'-IIICGNCGNCATCNGGC-3'),
- BOXA1R (5'- CTACGGCAAGGCGACGCTGACG-3'), and
- $(GTG)_5$ (5'-GTGGTGGTGGTGGTG-3')
- Result – as shown in Fig 1. GTG primer could discriminate strain of *Lactobacillus plantarum* (part of the Fig.1)



LMG 13087[T]	B	*Lactobacillus paracasei* subsp. *paracasei*
LMG 1284	B	*Lactobacillus plantarum*
LMG 9206	B	*Lactobacillus plantarum*
LMG 6907[T]	B	*Lactobacillus plantarum*
LMG 18021	B	*Lactobacillus plantarum*
LMG 10755[T]	B	*Lactobacillus pentosus*
LMG 18401	B	*Lactobacillus pentosus*
LMG 16673[T]	B	*Lactobacillus paraplantarum*
LMG 19456	LAB	*Enterococcus faecalis*
LMG 9469[T]	LAB	*Weisella halotolerans*

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Fig. 1. Dendrogram generated after cluster analysis of the digitized $(GTG)_5$-PCR fingerprints of the reference strains. The dendrogram was constructed using the unweighted pair-group method using arithmetic averages with correlation levels expressed as percentage values of the Pearson correlation coefficient. [a]LMG: BCCM/TM/LMG bacteria collection (Laboratory of Microbiology, Ghent University, Belgium, http://www.belspo.be/bccm); T: type strain; [b]Group A: obligately homofermentative lactobacilli, B: facultatively heterofermentative lactobacilli, and C: obligately heterofermentative lactobacilli.

Metoda molekuler, berbasis PCR menggunakan spesifik primer dapat digunakan untuk membedakan strain pada spesies yang sama. Contohnya adalah *Lactobacillus plantarum* berbagai strain memiliki profil pita yang berbeda dari hasil amplifikasi menggunakan primer spesifik

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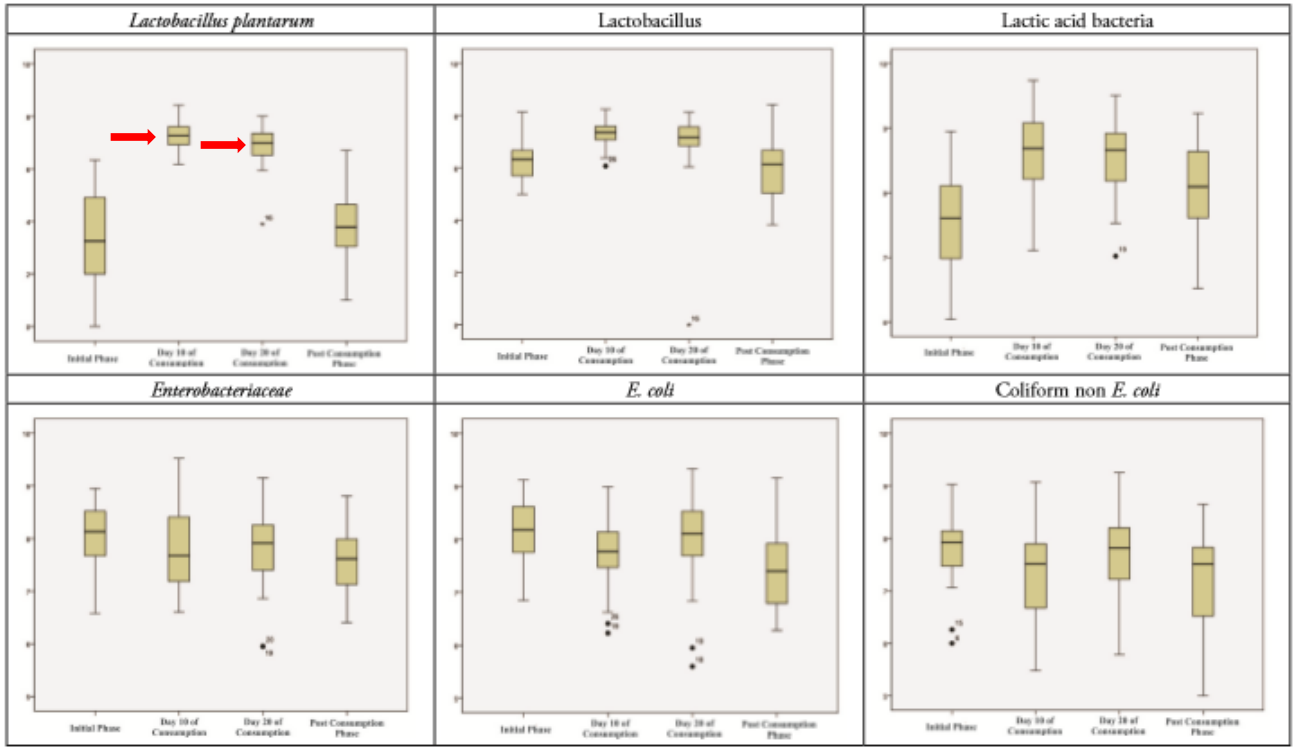
EFFECTS OF CONSUMPTION OF FERMENTED MILK CONTAINING INDIGENOUS PROBIOTIC *LACTOBACILLUS PLANTARUM* DAD-13 ON THE FECAL MICROBIOTA OF HEALTHY INDONESIAN VOLUNTEERS

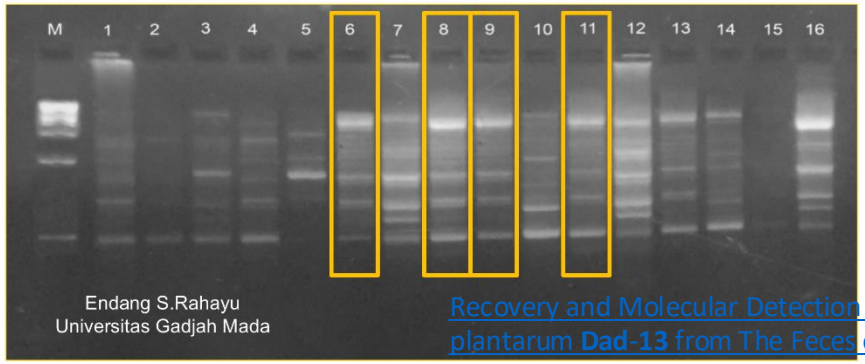
[1]Endang S. Rahayu, [1]Muhammad N. Cahyanto, [1]Mariyatun, [2]Martinus-Agus Sarwoko, [2]Pri Haryono, [1]Linda Windiarti, [2]Joko Sutriyanto, [3]Istiti Kandarina, [4]Sri Nurfiani, [4]Eni Zulaichah, and [1]Tyas Utami

https://drive.google.com/file/d/1L4u6hgZ1p_ip86qWapR-fF3KKyJjU9BM/view

FIGURE 2. Changes of fecal microbiota after consumption of fermented milk containing *L. plantarum* DAD 13 for 20 days



Design study: Pre and post
Total subjects: 30 people; Consumption : 20 days
Enumeration and isolation of *L. plantarum* from fecal samples using LPSM
Result: *Lactobacillus plantarum* Dad-13 **could survive** in the colon - based on the similar profile of PCR using BOX A1R Primer of isolates from subjects fecal after consumption



Recovery and Molecular Detection of Lactobacillus plantarum **Dad-13** from The Feces of Healthy Indonesian Volunteers After Intake of Fermented Milk

Rep-PCR using primer BOX A1R; M : *Molecular weight markers* (100-3000 bp); 1- F1.2; 2- F1.12; 3- F1.14; 4- F1.18; 5- F1.30; **6- *L.plantarum* Dad 13**; 7- F2.1; **8- F2.12; 9- F2.14**; 10- F2.18; **11- F2.30**; 12- F4.2; 13- F4.12; 14- F4.14; 15- F4.18; 16- F4.30

During the 20 days of consumption (F2) of Rep - PCR results showed the same band profile among isolates from stool 3 volunteers (no 12,14,30) with *L. plantarum* Dad13
Conclusion *: L. plantarum* Dad13 can grow in the colon

3 Biotech (2021) 11:270
https://doi.org/10.1007/s13205-021-02765-0

ORIGINAL ARTICLE

Efficacy of BOX-PCR fingerprinting for taxonomic discrimination of bifidobacterial species isolated from diverse sources

Rajashree Jena[1,2] · Prasanta Kumar Choudhury[1,2] · Anil Kumar Puniya[1] · Sudhir Kumar Tomar[1]

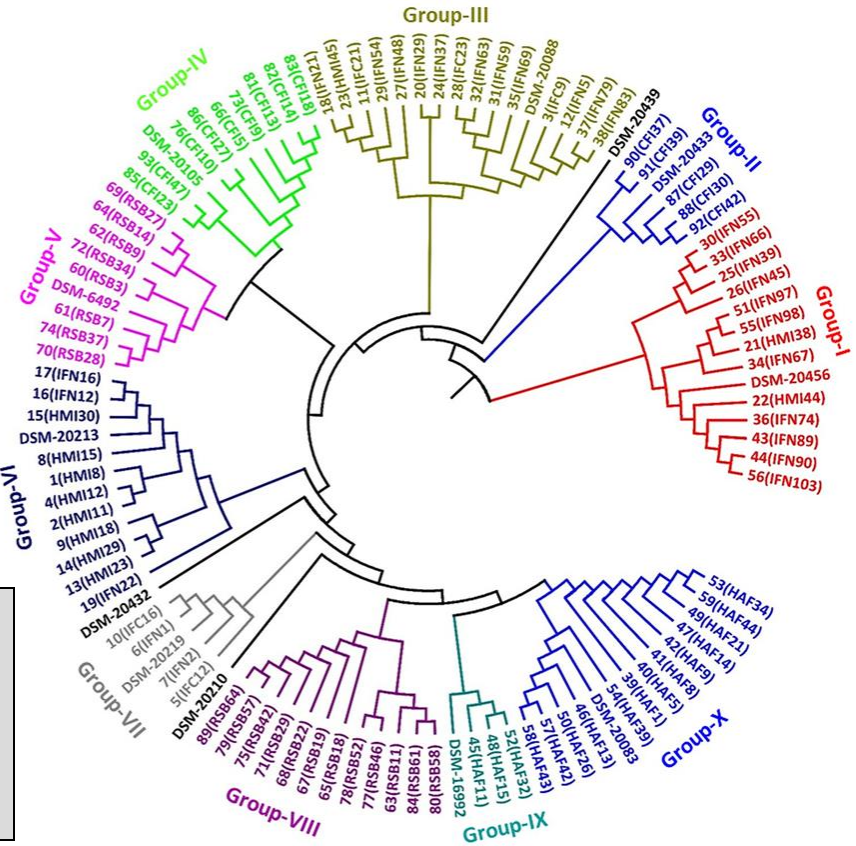


Fig. 2 Dendrogram constructed from the amplified BOX PCR fingerprints with reference strains

Primer BOX-A1R dapat digunakan untuk mengidentifikasi strain yang sama

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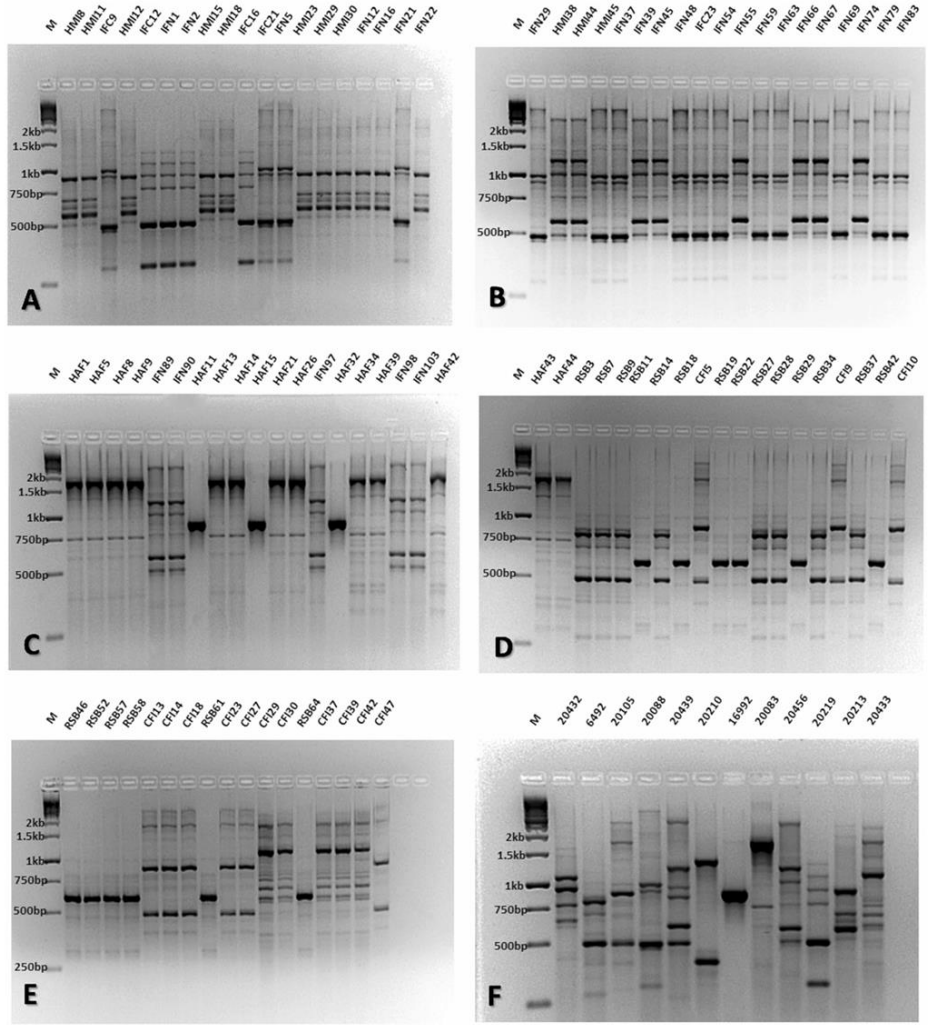

Fig. 1 Agarose gel photographs showing BOX PCR amplification (M-1 kb DNA size ladder) [A] [B] [C] [D] Lane 1–19 amplified product of the respective isolates [E] Lane 1–17 fingerprints of the isolates [F] Lane 1–12 fingerprints of the standard DSMZ isolates

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Culture Method

Bakteri	Selective Medium	Suhu	Durasi28nkubasi	Sumber
Bacillus wiedmannii	BHI, Nutrient Agar, Columbia Blood Agar	30 °C	24 jam	Miller et al., 2016
Bifidobacterium breve	Bifiobacterium selective medium + BSM suplemen	37 °C	24–48 jam	Chen et al., 2020; Chen et al., 2024; Yang et al., 2018
Lactobacillus plantarum	LPSM	30–37 °C	24–48 jam	Bujalance et al., 2006
Lactobacillus rhamnosus	MRS without glucose	37 °C	36-48 jam	Zhang et al., 2019
Lactobacillus casei	MMV (maltose-MRS-Vancomycin)	37 °C	48-72 jam	Lena et al., 2015
Lactobacillus helveticus	MRS	37 °C	24–48 jam	Griffiths and Tellez, 2013
Lactobacillus fermentum	MRS	37 °C	24–48 jam	-
Lactobacillus bulgaricus	Modified Reinforced Clostridial Medium (mRCM)	42 °C	12-24 jam	Nwamaioha et al., 2018
Bifidobacterium longum	Bifiobacterium selective medium + BSM suplemen	37 °C	24–48 jam	Hidalgo-Cantabrana et al., 2014
Pediococcus pentosaceus	Pediococci selective medium (PSM)	37 °C	24–48 jam	Simpson et al., 2006
Rhodopseudomonas palustris	RHODOSPIRILLACEAE MEDIUM (modified) (DSMZ Medium 27)	28 °C	48–72 jam	https://bacdive.dsmz.de/strain/1819

Protokol qPCR

Bakteri	Primer (F / R)	Protokol qPCR	Sumber
Lactobacillus casei	sg-Lcas-F: ACCGCATGGTTCTTGGC sg-Lcas-R: CCGACAACAGTTACTCTGCC	Annealing 55 °C	Matsuda et al., 2009
Lactobacillus plantarum	sg-Lpla-F: CTCTGGTATTGATTGGTGCTTGCAT sg-Lpla-R: GTTCGCCACTCACTCAAATGTAAA	Annealing 60 °C	Matsuda et al., 2009
Lactobacillus fermentum	LFer-1: CCTGATTGATTTTGGTCGCCAAC LFer-2: ACGTATGAACAGTTACTCTCATACGT	Annealing 55 °C	Watanabe, K., 1998
Lactobacillus helveticus	Lh-R: CCAGGTGGTCGTGCTCTTAA Lh-F: TGACCGATCCGATCACTCTT	Annealing 57 °C	Yu et al., 2015
Pediococcus pentosaceus	-	-	-
Bifidobacterium spp.	G-Bifid-F: CTCCTGGAAACGGGTGG G-Bifid-R: ACATCTATAGCCCTTCTTGTGG	Annealing 55 °C	Matsuki et al., 2002
Bifidobacterium breve	BiBRE F: CCGGATGCTCCATCACAC BiBRE R: ACAAAGTGCCTTGCTCCCT	Annealing 55 °C	Matsuki et al., 1999
Bifidobacterium longum	BiLON F: TTCCAGTTGATCGCATGGTC BiLON R: GGGAAGCCGTATCTCTACGA	Annealing 55 °C	Matsuki et al., 1999
Rhodopseudomonas palustris	F: GCGGGAAGATAATGACGGT R: TGGTAGCAACTAAGGACGG	Annealing 65 °C	CN Patent CN101195841A, 2008
Lactobacillus rhamnosus	sg-Lcas-F: ACCGCATGGTTCTTGGC sg-Lcas-R: CCGACAACAGTTACTCTGCC	Annealing 55 °C	Matsuda et al., 2009

Publikasi terkait strain

UNIVERSITAS GADJAH MADA

Bacillus wiedmaniim

Bakteri	Asal Isolat	Sifat Fisiologis & Genetik	Aktivitas / Potensi Biologis	Pustaka
Bacillus wiedmannii FSL W8-0169T	Susu mentah (USA, 2012)	Genom 5.3 Mb, G+C 35.3%, tidak memfermentasi sukrosa & arginin, MLST baru, rpoB AT 61	- Sitotoksik terhadap sel HeLa - Menghasilkan enterotoksin HBL dan NHE - Psikrotoleran, tumbuh pada 7°C	Miller et al., 2016
Bacillus wiedmannii FSL H7-0353	Produk susu siap konsumsi (2005)	Genom 5.6 Mb, rpoB AT 61	- Sitotoksik (mirip W8-0169T) - Potensial patogen makanan	Miller et al., 2016
Bacillus wiedmannii FSL H8-0032	Produk susu siap konsumsi (2005)	Genom 5.6 Mb, profil MLST baru	Tidak dijelaskan spesifik, diasumsikan serupa dalam kelompok B. wiedmannii	Miller et al., 2016
Bacillus wiedmannii FSL J3-0113	Produk susu (2012)	Genom 5.6 Mb, rpoB AT 417	Tidak dijelaskan rinci, termasuk dalam kelompok sitotoksik dan psikrotoleran	Miller et al., 2016
Bacillus wiedmannii (unspecified)	Tidak disebutkan	Mengandung gen bawA, tidak memiliki sekuens leader (*leaderless bacteriocin*)	- Memproduksi **bacteriocin Bawcin** - Spektrum antibakteri luas terhadap *Listeria monocytogenes*, *B. subtilis*, *Lactococcus lactis*, dll	Budhwani et al., 2023

Publikasi terkait strain

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Bifidobacterium breve CCFM683

No	Model	Treatment	Dose & Duration	Parameters Analyzed	Conclusion	Source
1	Mouse (IMQ-induced psoriasis)	Oral gavage of *B. breve* CCFM683	1×10^{9} CFU/day for 6 days	PASI score, ear thickness, cytokines (IL-17, TNF-α, IL-10), FXR-TLR4/NF-κB signaling, gut microbiota, bile acids	CCFM683 ameliorates psoriasis via microbiota modulation, increased DCA/LCA, and inhibition of inflammatory pathways	Chen et al., 2024
2	Mouse (IMQ-induced psoriasis)	Oral *B. breve* CCFM683 at multiple doses	10^{6} - 10^{10} CFU/day for 6 days	PASI score, histology, cytokines, bile acids, gene expression (K16, K17, involucrin)	Dose-dependent relief; optimal at $\geq10^{8.42}$ CFU/day; improved skin barrier, reduced inflammation via FXR/NF-κB	Chen et al., 2023
3	Mouse (DSS-induced colitis)	Oral gavage *B. breve* CCFM683	1×10^{9} CFU/day for 7 days before and during DSS	DAI, colon length, MPO, cytokines (IL-10, IL-6, TNF-α), PPARγ, gut microbiota, colonic CLA	Ameliorates colitis via CLA production, microbiota modulation, and anti-inflammatory effects	Yang et al., 2018
4	Mouse (ApcMin/+ colorectal tumor model)	Oral *B. breve* CCFM683	10^{9} CFU/day for 8 weeks	Tumor number, gut microbiota, bile acids, gene expression (FXR, TLR4, NF-κB), CLA	Inhibits tumorigenesis via CLA, microbiota regulation, bile acid modulation, suppressed inflammation	Chen et al., 2025
5	In vitro & metabolomics	Linoleic acid induction in two *B. breve* strains (CCFM683 and CCFM1025)	Exposure to LA in culture	Intracellular metabolites, PCA, metabolic fluxes	B. breve CCFM683 shows different metabolic modes and higher CLA production in response to LA	Gao et al., 2021

Publikasi terkait strain

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L. plantarum N13

Bakteri	Asal / Koleksi	Penggunaan	Ciri Fisiologis Utama	Model Uji	Parameter yang Dianalisis	Hasil Utama	Pustaka
Lactobacillus plantarum N13	Susu fermentasi	Tunggal	Produksi CLA tinggi, toleransi asam & empedu, adhesi tinggi ke sel HT-29	DSS-induced colitis pada tikus BALB/c	DAI, panjang kolon, sitokin (IL-6, TNF-α), mikrobiota usus	Menurunkan gejala kolitis, ekspresi IL-6 dan TNF-α turun, mikrobiota kembali seimbang	Liu et al., 2024

***L. rhamnosus* CCFM1064** – belum ada publikasi

***L. casei* CCFM8441** – belum ada publikasi

Publikasi terkait strain

UNIVERSITAS GADJAH MADA

Lactobacillus helveticus strain DLBSA201 dan DLBSA202

Bakteri	Media & Kondisi Kultur	Model Uji	Parameter yang Dianalisis	Hasil (Individu)	Hasil (Kombinasi dengan DLBSA202)	Pustaka
L. helveticus DLBSA201	MRS HiVeg broth, 37 °C, 18–22 jam	In vitro (pH, empedu, Caco-2, E. coli O157:H7, ekspresi gen)	Toleransi asam (pH 3)	Viabilitas 56%	–	Tjandrawinata et al., 2023
			Toleransi empedu (0.5%)	Viabilitas >97%	–	
			Aktivitas BSH	Tidak ada aktivitas	–	
			Adhesi ke sel Caco-2	89% sel bakteri melekat	–	
			Hambatan E. coli O157:H7 (agar difusi)	Zona hambat 12 mm	Zona hambat 15 mm	
			Hambatan pertumbuhan E. coli dalam CFS	Penurunan ±15%	Penurunan ±30%	
			Displacement di sel Caco-2	Tidak signifikan	Signifikan menurunkan kolonisasi E. coli	
			Ekspresi TLR-4, IL-8	Tidak signifikan	Menurun signifikan	
			Ekspresi TNF-α	Tidak signifikan	Menurun signifikan	
			Ekspresi occludin & ZO-1	Tidak signifikan	Meningkat signifikan	
			Ekspresi claudin-2	Tidak signifikan	Tren penurunan ($p \approx 0.09$)	

Publikasi terkait strain

UNIVERSITAS GADJAH MADA

Bifidobacterium longum CECT 7894 dan *Pediococcus pentosaceus* CECT 8330

Bakteri	Model Uji	Dosis & Durasi	Media & Kondisi Kultur	Parameter yang Dianalisis	Hasil Ringkasan	Pustaka
Bifidobacterium longum CECT 7894	DSS-induced colitis, tikus C57BL/6	5×10^8 CFU/hari, 5 hari	MRS anaerobik 37 °C	DAI, panjang kolon, ekspresi ZO-1, occludin, histologi kolon	Menurunkan DAI, memperbaiki kolon, ekspresi tight junction meningkat	Xiao et al., 2022
Pediococcus pentosaceus CECT 8330	DSS-induced colitis, tikus C57BL/6	5×10^8 CFU/hari, 5 hari	MRS mikroaerofilik (5% CO_2), 37 °C	DAI, CMDI, sitokin (TNF-α, IL-1β, IL-6, IL-10), Treg, ZO-1, occludin, SCFA, microbiota	Mengurangi inflamasi, meningkatkan IL-10 & Treg, meningkatkan SCFA, memperbaiki mikrobiota	Dong et al., 2022
B. longum CECT 7894 + *P. pentosaceus* CECT 8330	Uji klinis bayi kolik (Chen et al.)	1×10^9 CFU/hari, 21 hari	Probiotik oral suspensi klinis	Durasi dan frekuensi menangis, konsistensi tinja, frekuensi buang air besar, rasio respon klinis	Durasi menangis menurun signifikan dari hari ke-7, 14, 21; perbaikan tinja & kenyamanan gastrointestinal bayi	Chen et al., 2021
B. longum CECT 7894 + *P. pentosaceus* CECT 8330	Uji klinis pilot bayi kolik (Santas et al.)	1×10^9 CFU/hari, 21 hari	Tidak dilaporkan rinci (oral probiotik)	Durasi menangis, konsistensi tinja	Penurunan durasi menangis (81 → 54 min/hari); tren membaik tapi tidak signifikan (P=0.083)	Santas et al., 2015

Publikasi terkait strain

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Multistrain EMRO

Strain Kombinasi	Model Uji	Metode Uji & Media Kultur	Parameter yang Dianalisis	Hasil Utama	Pustaka
Rhodopseudomonas palustris EMRO201 *Lactobacillus casei* EMRO002 *Lactobacillus casei* EMRO213 *Lactobacillus plantarum* EMRO009 *Lactobacillus fermentum* EMRO211 *Lactobacillus bulgaricus* EMRO212 *Lactobacillus rhamnosus* EMRO014	**In vitro enzyme assay** pada enzim inflamasi MIF (Macrophage Migration Inhibitory Factor)	- Medium fermentasi: madu (1.13%), asam sitrat (20mg/mL), molases (3.07%), aloe vera (1.07%)- Fermentasi selama 45 hari, kondisi anaerob, suhu 26 °C - Hasil: supernatan dengan total populasi ~2×10^6 CFU/mL per strain - Uji kinetik enzim **MIF tautomerase**: metode spektrofotometri dengan atau tanpa preincubation dan dilusi	- IC_{50} (konsentrasi penghambat 50%)- Mode inhibisi (kompetitif / nonkompetitif) - Efek reversibilitas terhadap enzim	- IC_{50} = 7.80 ± 1.96 mg/L- Efek penghambatan **reversibel** terhadap enzim MIF - Dugaan mekanisme **non-kompetitif (allosterik site)**	Nyotohadi & Kok, 2023
Rhodopseudomonas palustris EMRO201 *Lactobacillus casei* EMRO002 *Lactobacillus casei* EMRO213 *Lactobacillus plantarum* EMRO009 *Lactobacillus fermentum* EMRO211 *Lactobacillus bulgaricus* EMRO212 *Lactobacillus rhamnosus* EMRO014	**Uji klinis pada manusia (pilot trial)** – pasien kanker payudara stadium lanjut yang sedang menjalani kemoterapi aktif	- Subjek: pasien kanker payudara stadium lanjut yang sedang menjalani kemoterapi- Intervensi: **PRO EM1 cair**, diminum 3×/hari (7.5–15 mL per kali) - Durasi: 21–30 hari- Total kandungan probiotik: >2×10^6 CFU/mL per strain - Monitoring klinis: fungsi ginjal, hati, kondisi fungsional, dan gejala terkait kemoterapi	- *Karnofsky Performance Score* - Gejala: kelelahan, mual- BUN, kreatinin, ALT, HIT count	- *Karnofsky Score* meningkat signifikan (dari 90 ke 100; $p<0.001$) - BUN menurun signifikan ($p=0.008$)- Gejala mual & kelelahan membaik- ALT, kreatinin, HIT stabil (tidak toksik)	Kirtishanti et al., 2025