Isolasi, Identifikasi dan Aplikasi Bakteri Asam Laktat asal Susu Kambing Peranakan Etawah pada Proses Fermentasi Susu

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KAMBING PERAH

SAANEN DAN TOGENBURG

PERBANDINGAN KANDUNGAN NUTRISI (Park *et al.*, 2007; Wong *et al.*, 1988)

Komposisi (g/100g)	Sapi	Kambing	Kerbau
Lemak	3.9	7.2	7.4
Protein	3.3	4.6	3.8
• Casein	2.6	3.9	2.6
• Whey	0.7	0.7	0.6
Laktosa	4.6	4.8	4.8
Abu	0.7	0.9	0.8
Total Solid	12.5	17.5	16.83

Kambing Peranakan Etawah (PE)

- Hasil perkawinan antara kambing Kacang dengan kambing Etawah (Jamnapari)

- Mampu menghasilkan susu segar 0,5 – 1,5 liter/ekor/hari

Keunggulan Susu Kambing PE

- Komposisi lemak lebih kecil dan homogen dan lebih mudah dicerna tubuh (Novita *et al*., 2005)
- Kandungan CLA *(conjugated linoleic acid)* sebagai senyawa anti kanker tinggi (Jennes, 1980)
- Kandungan Vitamin B1 tinggi (Sarwono, 1993)
- Protein lebih lembut dan mudah dicerna
- Kandungan antiseptik (*fluorin*) tinggi sehingga menekan bakteri penyebab penyakit TBC dan penyakit paru lainnya

Susu Kambing PE Sebagai Bahan Dasar Produk Keju

SUMBER SUSU KAMBING DI YUMMY

SAANEN

ETTAWA dan Peranakan Lokal

GOAT MILK POWDER

MAIN INGREDIENTS

Goat Milk

Cream Milk

Cow Milk

Satrter Culture

Powder Milk

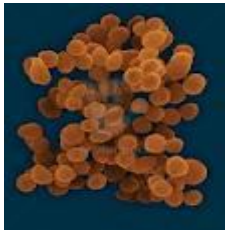
Tujuan Penelitian

- Isolasi dan Identifikasi Bakteri Asam Laktat dari susu Kambing Peranakan Ettawa (PE)
- Uji Potensi sebagai starter dalam fermentasi susu kambing PE

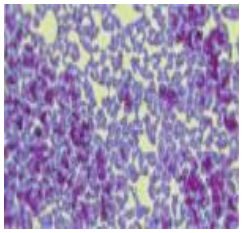


BAKTERI ASAM LAKTAT (Salminen *et al.*, 2004)

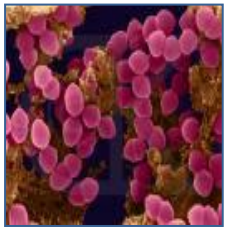
Aerococcus



Carnobacterium



Enterococcus



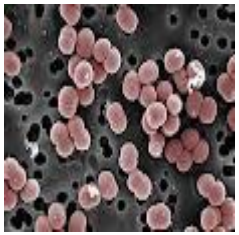
Lactobacillus

Lactococcus

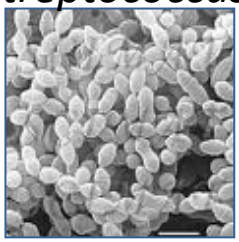
Leuconostoc

Oenococcus

Pediococcus



Streptococcus



Tetragenococcus

Vagococcus

Weissella

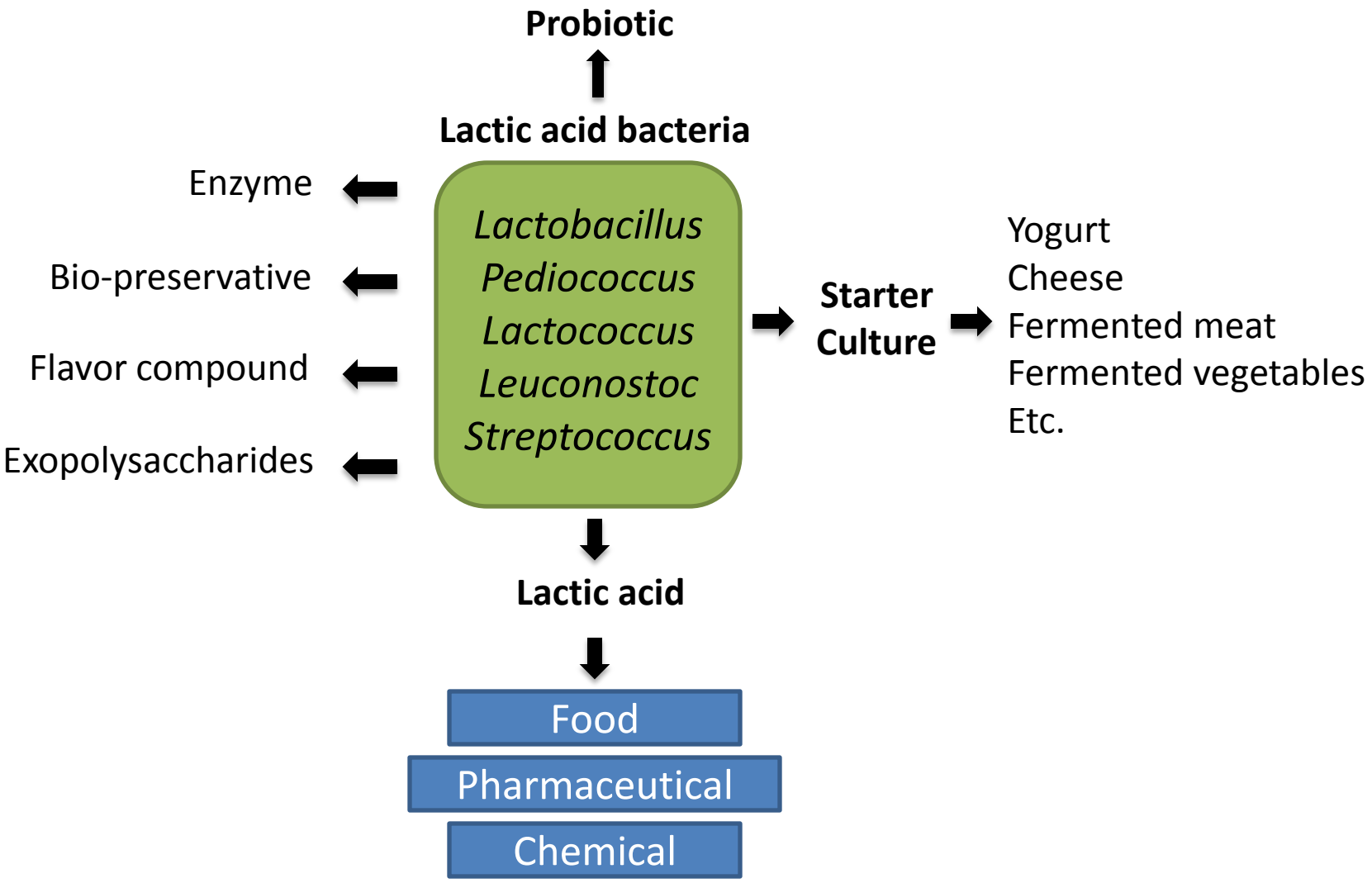
http://www.google.co.id/search?q=Lactic acidbacteria.

Karakteristik BAL

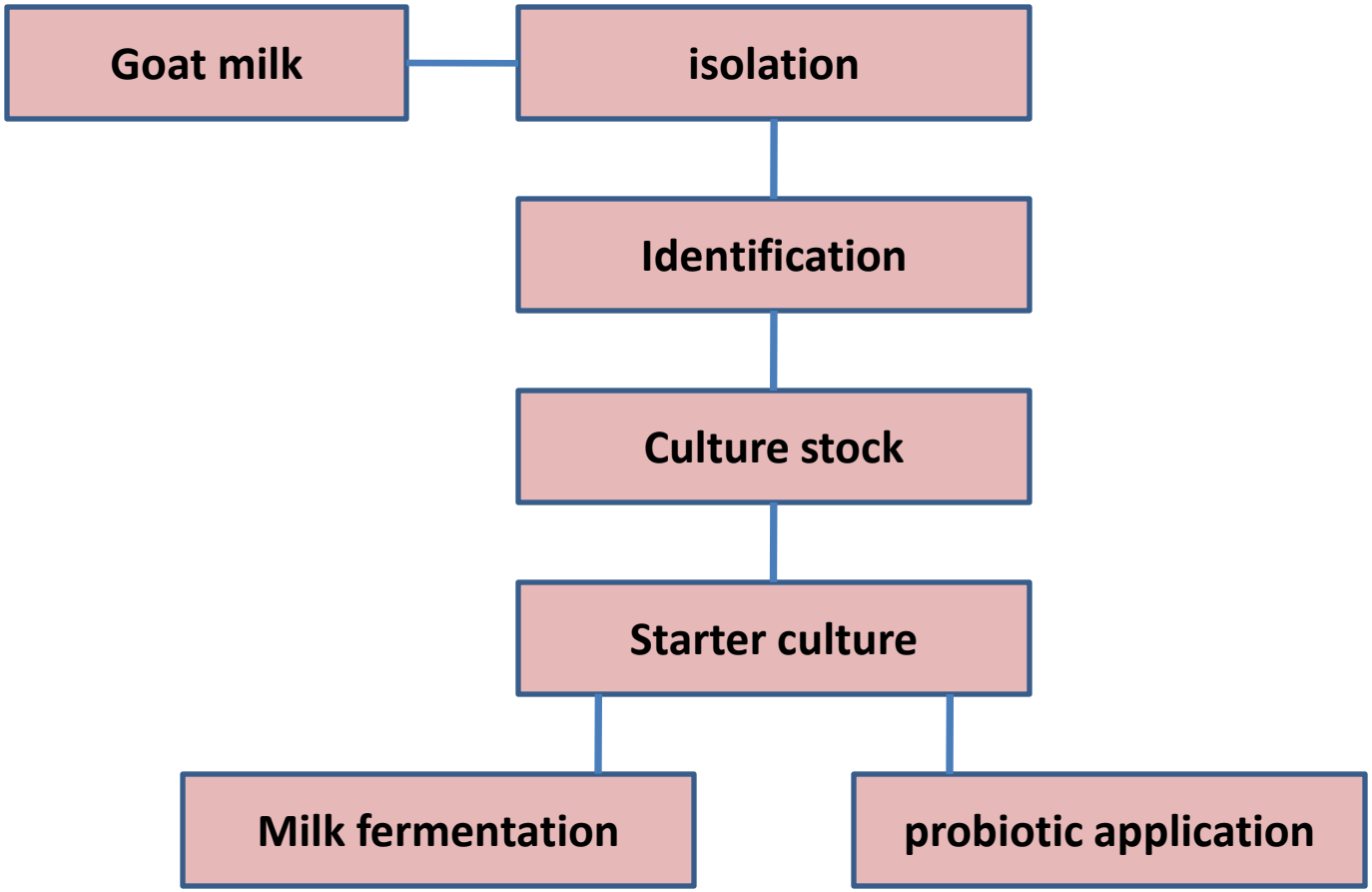
(1) Gram positif dan non spora
(2) Katalase negatif
(3) Fakultatif anaerobik
(4) Fermentasi laktosa menjadi asam laktat
(5) BAL termasuk dalam kategori bakteri yang aman dikonsumsi dan karenanya sering disebut *Generally Recognized as Safe* (GRAS)
(6) Menurunkan pH (agen preservatif)

(Widodo, 2003).

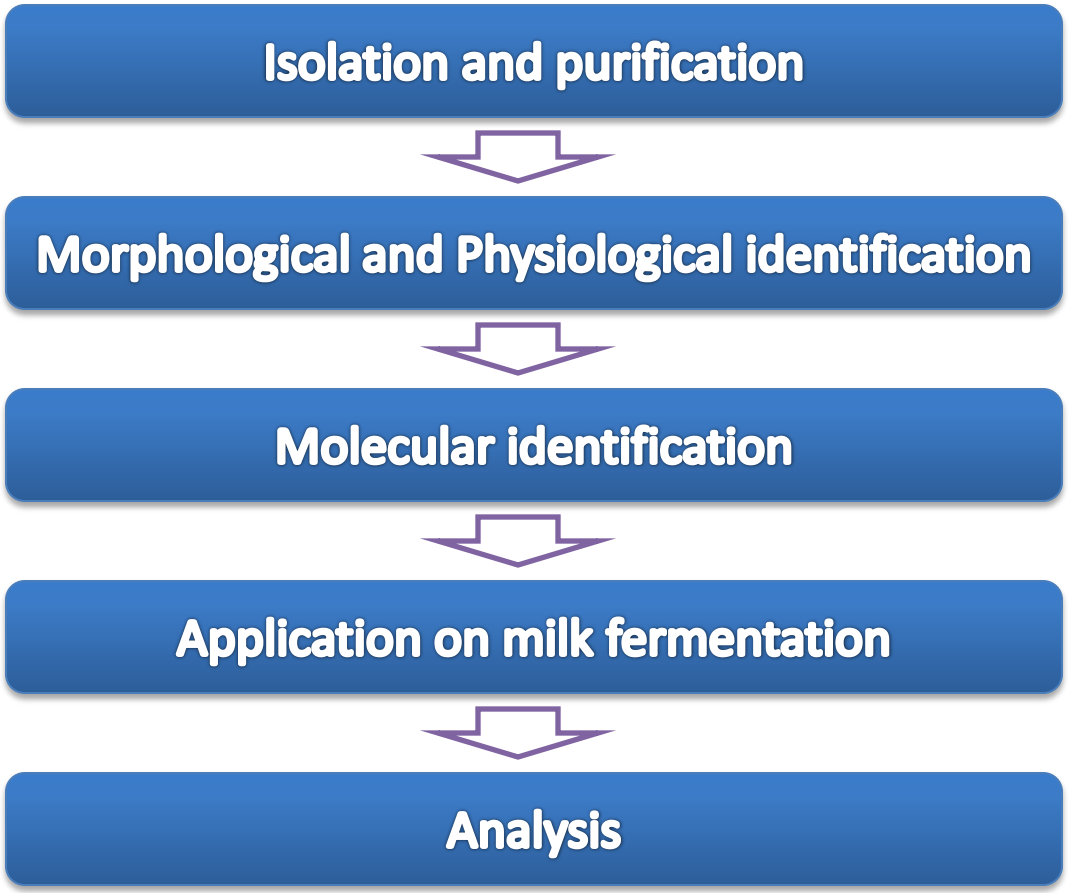
Peranan Bakteri Asam Laktat



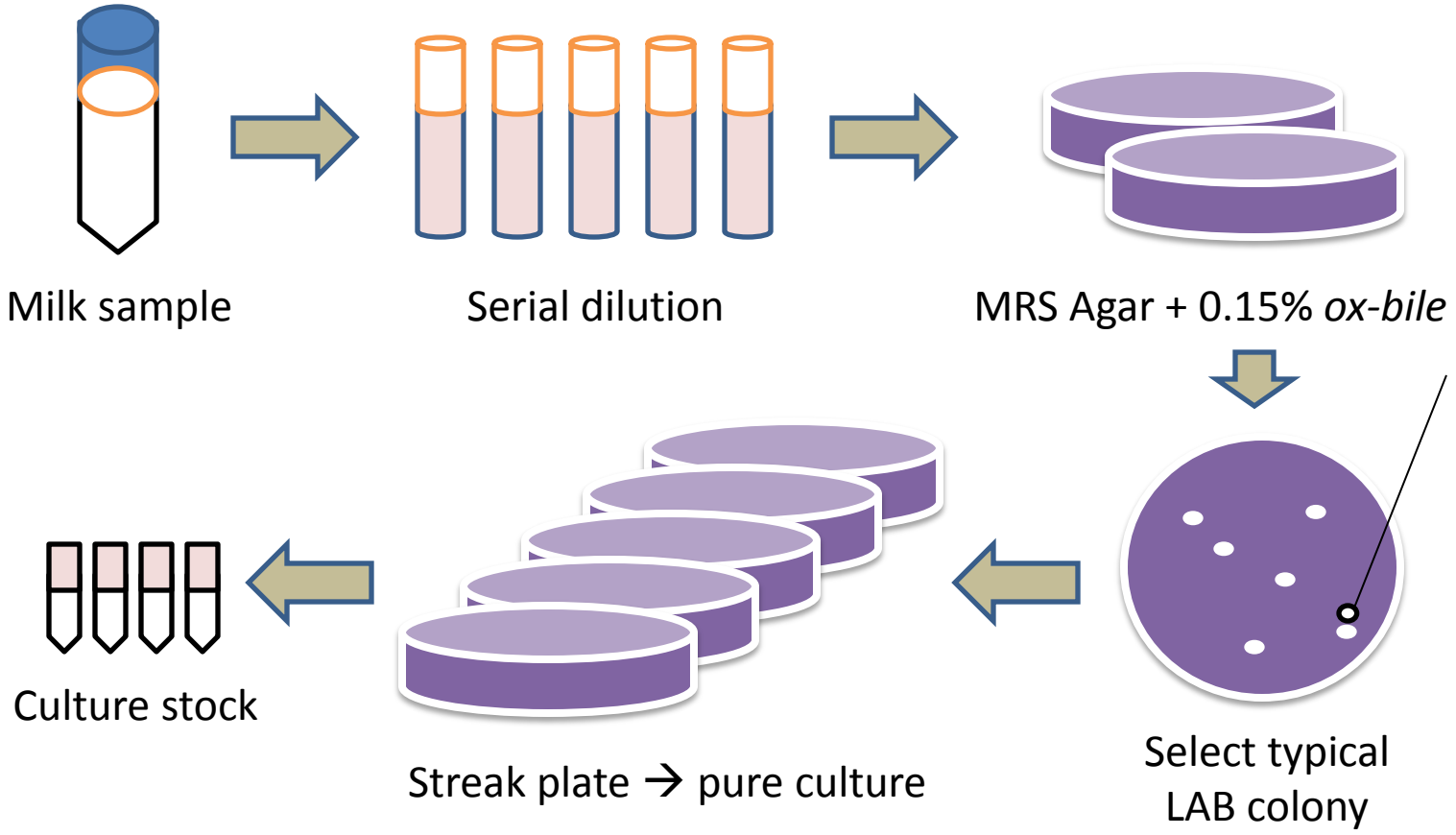
Research outline



Tahapan Penelitian



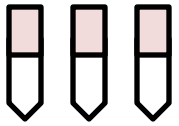
Isolation and purification



Morphological and Physiological identification

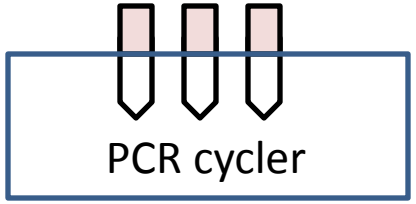
- Gram staining
- Motility
- Catalase test
- CO_2 from glucose
- Growth
  - Different pH
  - Different temperature
  - Different carbon source

Molecular identification



Overnight culture

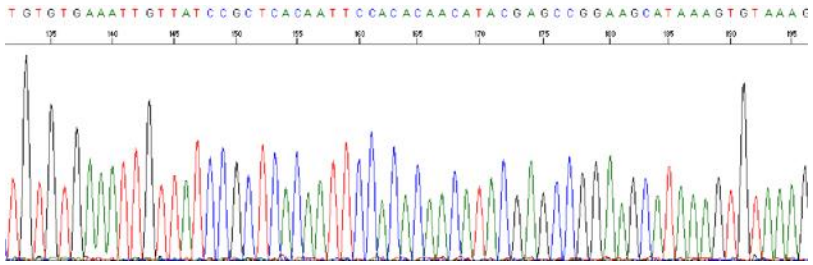
DNA isolation



Denaturation 95°C; 30 s
Annealing 56°C; 30 s
Extension 72°C; 45 s
Final Extension 72°C

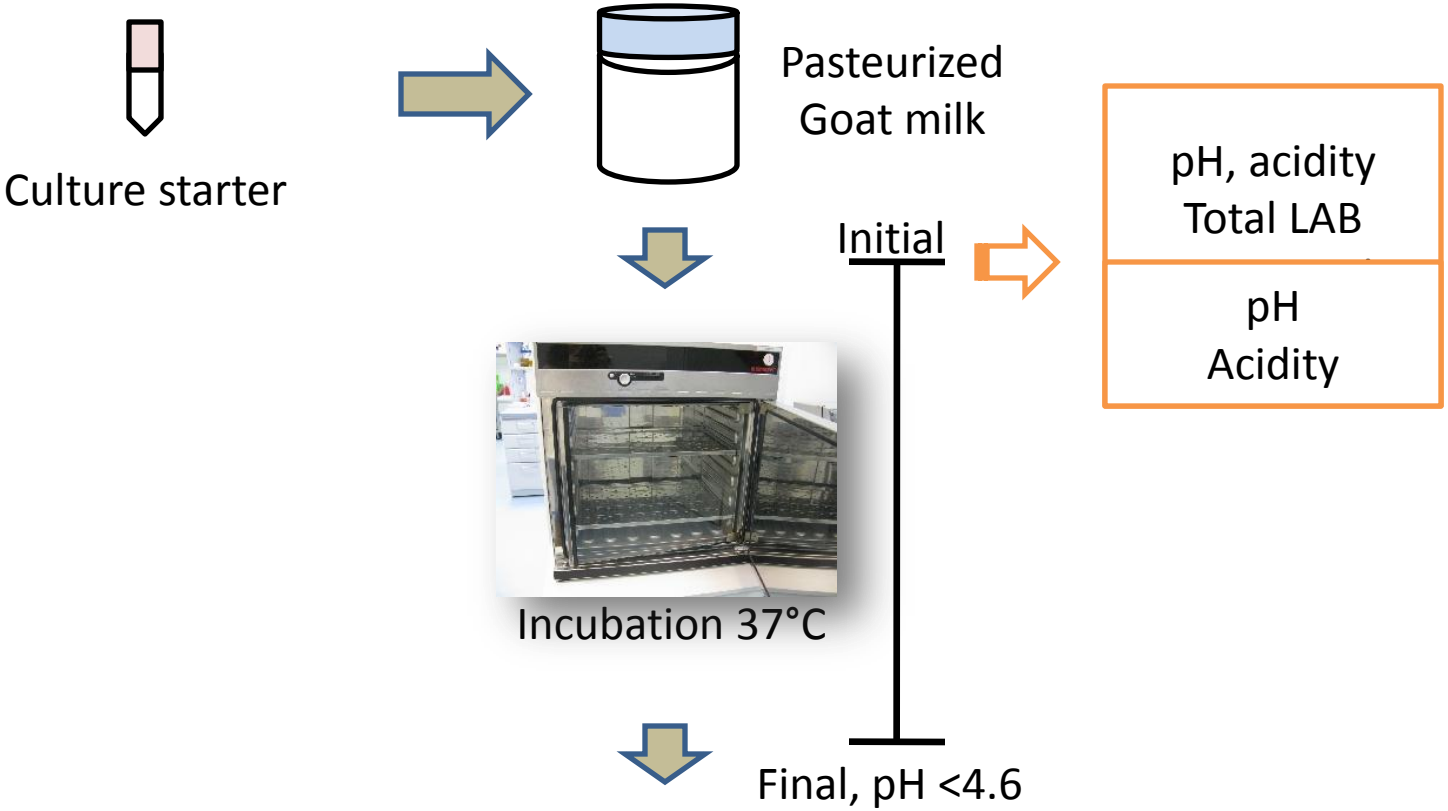
32x

Electrophoresis



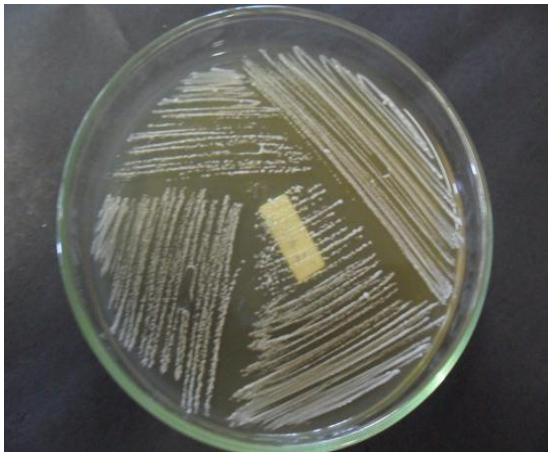
Sequencing

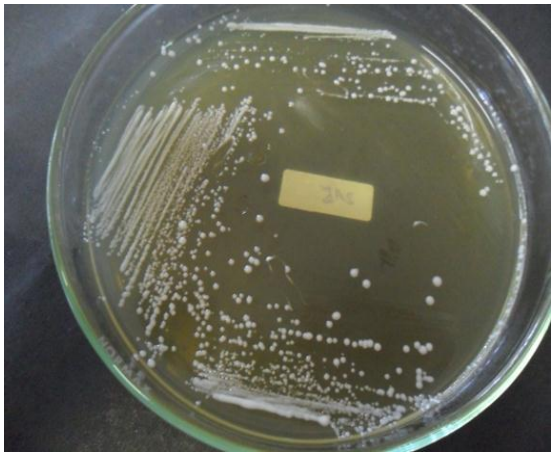
Application on milk fermentation

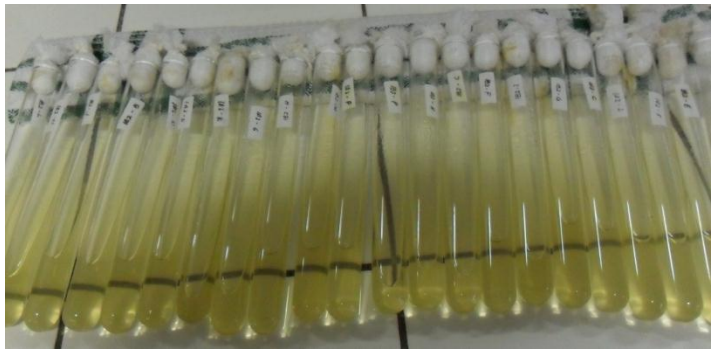


Data analysis

Isolasi Bakteri Asam Laktat







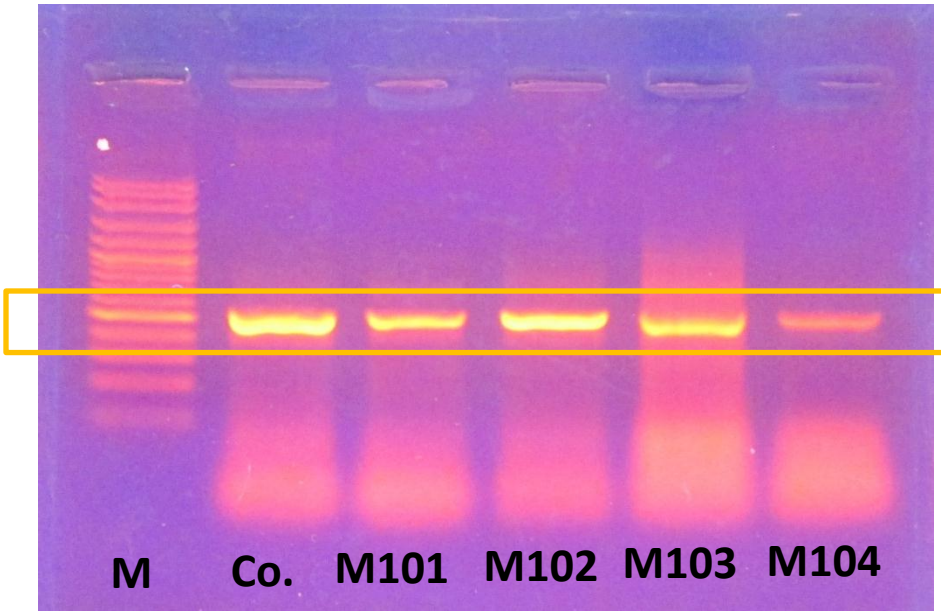
Bacterial Identification

- Media for isolation was De Man-Rogosa-Sharpe (MRS, Oxoid) supplemented with ox bile 0.15%
- Plates were incubated at 37°C for 48h

Morphology and physiological identification

Characteristic	M101	M102	M103	M104
Cell morphology	Cocci	Cocci	Cocci	Bacilli
Gram staining	+	+	+	+
Spore formation	-	-	-	-
Catalase activity	-	-	-	-
Fermentation type	Homo	Homo	Homo	Homo
Glucose fermentation	+	+	+	+
Casein hydrolysis	+	+	+	+
Growth at pH 4.5	-	+	+	+
Growth at 10°C	-	-	-	-
Growth at 45°C	+	-	+	+
Growth at NaCl 6.5%	-	-	-	-

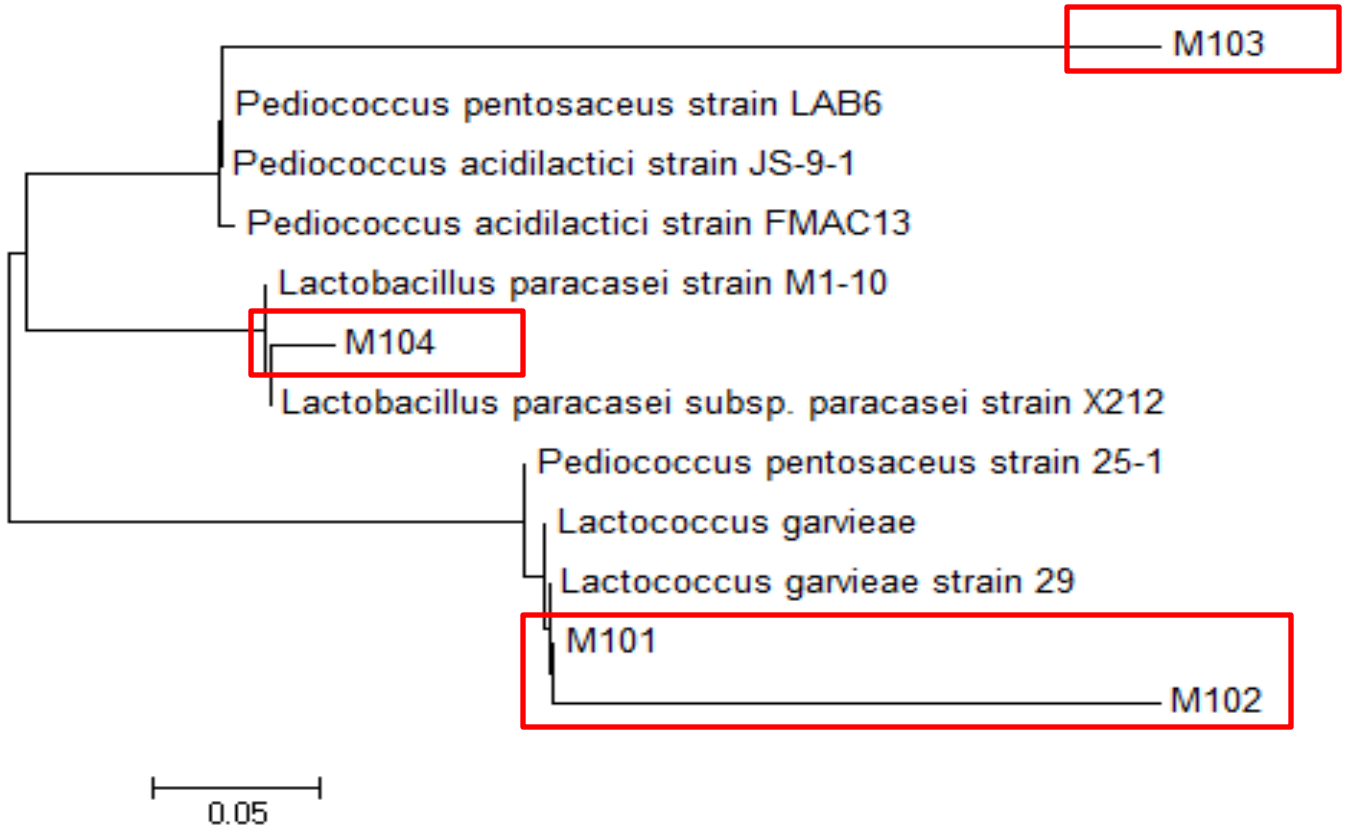
16S rRNA gene amplification



Approx. 518 bp

Amplified bands of PCR using plb16 and mlb16 primer

Phylogenetic tree



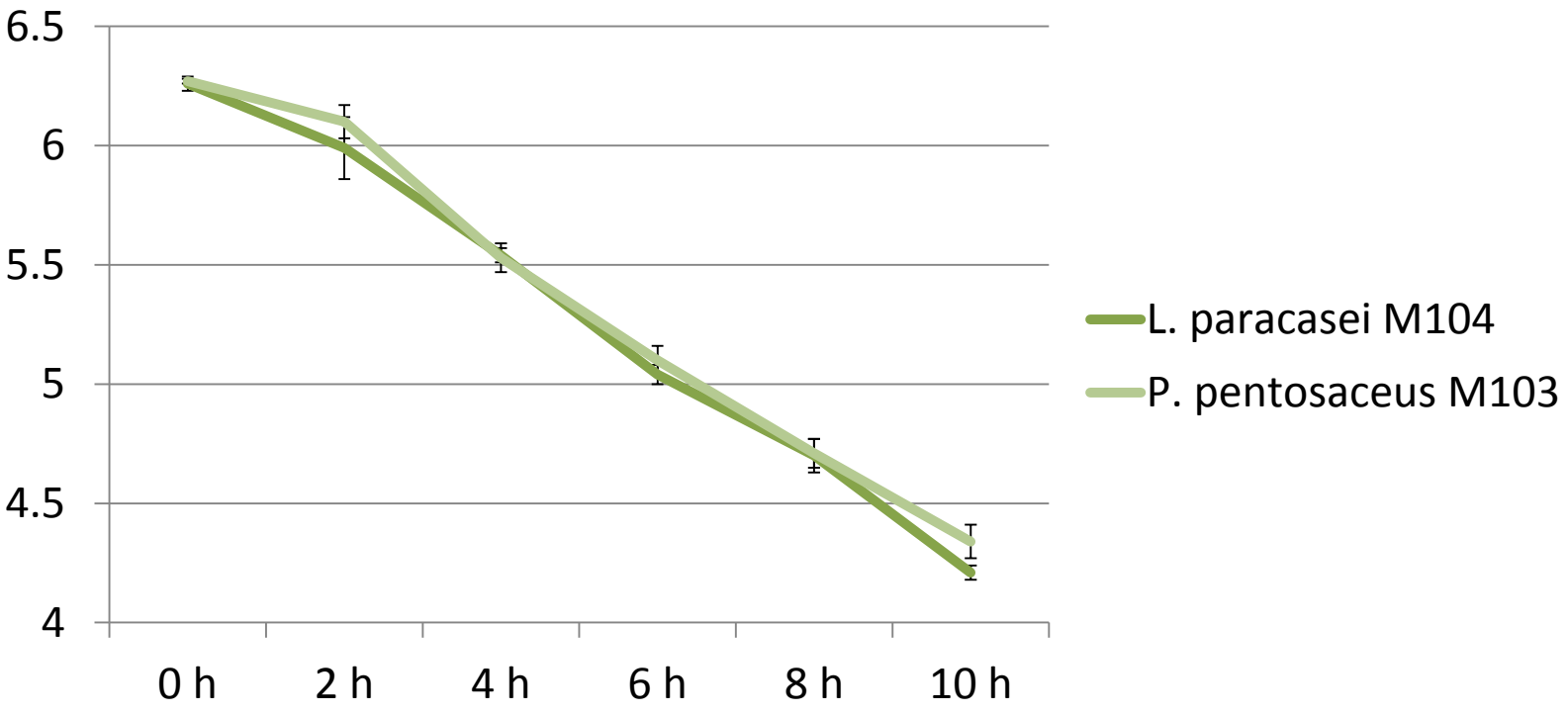
Phylogenetic tree of 4 isolates, analyzed wit MEGA 6.05 Software

Seleksi awal sebagai kultur starter

- Isolat ditumbuhkan pada media susu skim 18% (w/v) dan diinkubasi pada suhu 37°C selama 18 jam
- pH akhir fermentasi kultur starter:

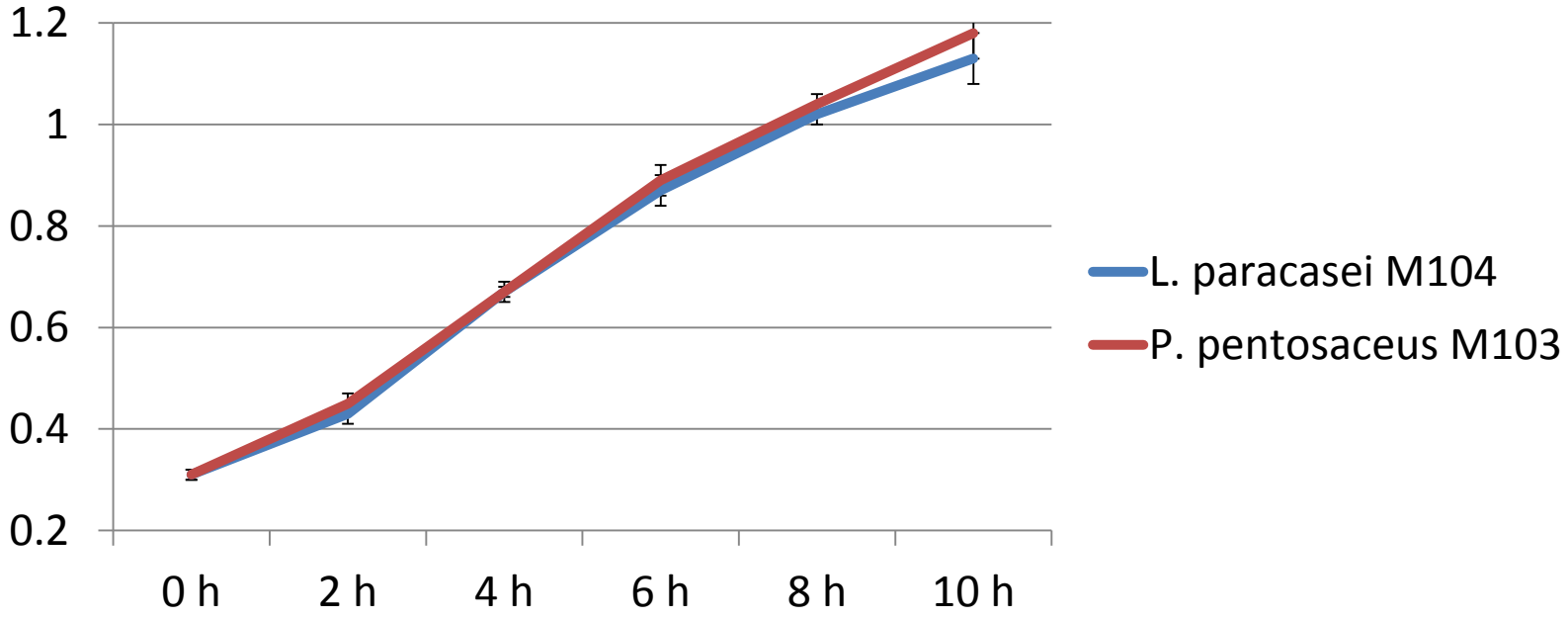
Isolat	pH akhir
L. garvieae M101	5,18 ± 0,12
L. garvieae M102	5,24 ± 0,17
P. Pentosaceus M103	4,52 ± 0,08
L. Paracasei M104	4,50 ± 0,05

Fermentation analysis - pH



Sterter culture (2%, w/v)	Fermentation time (h)					
	0	2	4	6	8	10
L. paracasei M104	6.26±0.01^{a}	5.99±0.07^{b}	5.54±0.06^{c}	5.04±0.06^{d}	4.70±0.06^{e}	4.21±0.07^{f}
P. pentosaceus M103	6.27±0.03^{a}	6.10±0.13^{b}	5.53±0.03^{c}	5.10±0.04^{d}	4.71±0.07^{e}	4.34±0.03^{f}

Fermentation analysis – Acidity



Sterter culture (2%, w/v)	Fermentation time (h)					
	0	2	4	6	8	10
L. paracasei M104	0.31±0.01[a]	0.43±0.02[b]	0.67±0.02[c]	0.87±0.03[d]	1.02±0.02[e]	1.13±0.05[f]
P. pentosaceus M103	0.31±0.01[a]	0.45±0.02[b]	0.67±0.01[c]	0.89±0.03[d]	1.04±0.02[e]	1.18±0.05[f]

Metabolisme Laktosa dan Prosuksi Asam Laktat

Sample	Lactose concentration (%)	
	0 h	10 h
P. Pentosaceus M103	4.60 ± 0,39[a]	3.24 ± 0.22[b]
L. Paracasei M104	4.60 ± 0,39[a]	3.15 ± 0.35[b]

Sample	Lactic acid concentration (g/L)	
	0 h	10 h
P. Pentosaceus M103	0.1422 ± 0.0210[a]	0.1768 ± 0.0340[b]
L. Paracasei M104	0.1422 ± 0.0210[a]	0.1905 ± 0.0145[b]

Total BAL dan Viscosity

Sample	Total LAB (log cfu/ml)	
	0 h	10 h
P. Pentosaceus M103	7.817±0.053^{a}	9.238±0.330^{b}
L. Paracasei M104	7.719±0.159^{a}	9.637±0.510^{b}

Sample	Apparent viscosity (%)	
	0 h	10 h
P. Pentosaceus M103	1.82±0.12^{a}	1075±25$^{b, A}$
L. Paracasei M104	1.82±0.12^{a}	1475±15$^{b, B}$

KESIMPULAN

- Isolat *Pediococcus pentosaceus* M103 dan *Lactobacillus paracasei* M104 memiliki potensi untuk dikembangkan sebagai kultur starter dalam fermentasi susu.

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ISOLATION AND IDENTIFICATION OF GOAT MILK-DERIVED *Lactobacillus paracasei* M104 AND *Pediococcus pentosaceus* M103 AND THEIR POTENTIAL USE AS STARTER CULTURE FOR FERMENTATION

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ABSTRACT

The aims of this study were to isolate and identify lactic acid bacteria from the fresh milk of crossbred Peranakan Etawah goats in Yogyakarta, Indonesia and assess their potential utility in dairy fermentation. Fresh milk samples were collected from three different farms and plated into de Man Rogosa and Sharpe (MRS) agar supplemented with 0.5% ox bile. Colonies were purified with a streaking method followed by morphological and biochemical analysis using Gram staining, a catalase test, tests of motility and spore formation and growth at different temperatures. Molecular identification was based on nucleotide sequencing of 16S rRNA genes. Four isolates, M101, M102, M103 and M104, were identified. Certain features of isolates M101 and M102 were homologous with *Lactococcus garvieae*, isolates M103 and M104 showed a degree of homology with *Pediococcus pentosaceus* and *Lactobacillus paracasei* subsp. *paracasei* respectively. Selected isolates were used to ferment milk at 37°C for 10 hours. After 10 h, milk fermented with *Lactobacillus paracasei* M104 had a pH of 4.21±0.07 and acidity of 1.13±0.05. Milk fermented with *Pediococcus pentosaceus* M103 had a pH of 4.34±0.03 with acidity of 1.18±0.05. *Lactococcus garvieae* had limited ability to acidify milk, producing only a slight change in pH over