Naturally Fermented Milk from Indonesia: A Study of Microbial Diversity and Probiotic Potency for the Potential Treatment of Intestinal Mucositis

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Abbreviations

ACE	Angiotensin I-converting Enzyme
ATCC	American Type Culture Collection
BLAST	Basic Local Alignment Search Tool
bsh	Bile salts hydrolase
Caco-2	Adenocarcinoma of the colon
CD	Crypt depth
CFS	Cell-free supernatant
cfu	Colony Forming Units
dATP	2-deoxy-adenosine-5-triphosphate
DC	Dendritic cells
dCTP	2-deoxy-cytidine-5-triphosphate
dGTP	2-deoxy-guanosine-5-triphosphate
DM	Dry matter
DNA	Deoxyribose nucleic acid
dNTPs	Deoxynucleotide triphosphates
DMEM	Dulbecco's modified Eagle Medium
dTTP	2-deoxy-thymin-5-triphosphate
EDTA	Ethylenediamine tetra acetic acid
FCS	Feta calf serum
5-FU	5-fluorouracil
GABA	Gamma-aminobutyric acid
H&E	Haematoxylin and eosin
HEPES	4-(2-hydroxyethyl)-1-piperazine ethanesulfonic
IDZ	Inhibition diameter zones
IgA	Immunoglobulin A
IL	Interleukin
ITS	Intergenic transcribed spacer
LAB	Lactic acid bacteria
Lacl	*Lactococcus lactis* subsp. *lactis* SL3.34
Lp	*Lactobacillus plantarum* S1.30
LSD	Least significance difference

LSM	Lactic acid bacteria Susceptibility test Medium
MAPK	Mitogen-activated protein kinase
MilliQ H_2O	Milli-Q filtered deionized water
MPO	Myeloperoxidase
MRS	de Man, Rogosa and Sharpe
msa	Mannose-specific adhesion
MTX	Methotrexate
NCFS	Neutralized cell-free supernatant
NF-$_K$B	Nuclear factor-kappa B
NFM	Naturally fermented milk
NGS	Next generation sequencing
NSLAB	Non-lactic acid bacteria
OTU	Operational Taxonomy Unit
PBS	Phosphate-buffered saline
PD	Phylogenetic distance
*pln*A	*Plantaricin* A
RFLP	Restriction Fragment Length Polymorphism
rRNA	Ribosomal RNA
ROS	Reactive oxygen species
SD	Standard deviation
SEM	Standard error of the mean
TAE	Tris-acetic EDTA
Taq	*Thermus aquaticus* DNA polymerase
TNF-α	Tumour Necrosis Factor-Alpha
Tris	Tris (hydroxymethyl) aminomethane
TSA	Tryptone soya agar
TSA	Tryptone soya agar
TSB	Tryptone soya broth
VH	Villus height
YEPD	Yeast extract peptone d-glucose
YMA	Yeast mannitol agar
YPD	Yeast peptone dextrose

Abstract

Naturally fermented milk (NFM) is prepared from fresh milk which is fermented spontaneously, without any inoculation of starter cultures. With increasing interest in novel dairy products, naturally fermented milks have become of interest to food microbiologists as a result of their potential as technologically important microorganisms. Dadih is a well-known naturally fermented milk product developed by local people in West Sumatra, Indonesia. This product is manufactured using unpasteurized buffalo milk which is then fermented spontaneously at ambient temperature. Dangke is prepared from heat-treated buffalo milk, and then processed enzymatically utilizing papain from papaya latex. Identification and characterization of the indigenous microbiota is essential for understanding how the microbial ecology impacts on the organoleptic, safety and potential health benefits of dadih and dangke. In addition, the presence of probiotic microorganisms in these products was also evaluated by investigating their potential to reduce the severity of chemotherapy-induced intestinal mucositis in rats. Probiotics have been defined as 'live microorganisms that, when administered in adequate amounts, confer a health benefit on the host' (Hill et al. 2014).

Lactic acid bacterial (LAB) groups detected using culture-dependent techniques in dadih were *Lactobacillus plantarum, Lactococcus lactis* subsp. *lactis*, and *Enterococcus faecium* (**Chapter 2**). Only one species of acetic acid bacteria was found, namely *Acetobacter orientalis,* while yeasts isolates were identified as *Saccharomyces cerevisiae*, *Candida metapsilosis* and *Kluyveromyces marxianus,* with *C. metapsilosis* as the principal yeast (**Chapter 3**). Other bacteria detected included *Klebsiella oxytoca*, *Klebsiella* sp. and *Bacillus pumilus.* Among these bacteria, *L. plantarum* was the most frequently isolated LAB from dadih, followed by *L. lactis* subsp. *lactis.*

Indigenous microbiota detected in dangke were relatively similar to dadih (**Chapter 2**). However, *E. faecium* and *B. pumilus* were not found in dangke. *Lactococcus lactis* subsp. *lactis* was the most predominant LAB, while *S. cerevisiae* was the most frequently isolated yeast (**Chapter 3**). Moreover, based on a culture-independent method (pyrosequencing), genus *Lactococcus* had the greatest relative abundance in dadih. Based on pyrosequencing results, a more diverse population of mesophilic LAB was found in dangke sourced from cow's milk; while family Enterobacteriaceae dominated dangke samples from buffalo milk.

Lactobacillus plantarum S1.30 isolated from dadih demonstrated probiotic properties which included tolerance to low pH and bile salts, antimicrobial activity and the presence of a bacteriocin regulating gene (plantaricin A) and *msa* and *bsh* genes, susceptibility to antibiotics and ability to adhere to Caco-2 cells (**Chapter 4**). From these probiotic features, only antimicrobial activity and the presence of *msa* and *bsh* genes were not demonstrated by *L. lactis* subsp. *lactis* SL3.34. However, from the pyrosequencing results, this strain was selected as the representative of the dominant genus/species in dadih.

The efficacy of probiotics evaluated in the present study was variable at treating 5-fluorouracil (5-FU)-induced intestinal damage *in vivo* (**Chapter 5**). The results suggested that *L. plantarum* S1.30 and *L. lactis* subsp. *lactis* SL3.34 could have beneficial effects through partially improving metabolic parameters such as water intake, urine output, food intake, and fecal output in 5-FU challenged rats. The severity of damage in the jejunum and ileum was also reduced following probiotic culture treatment.

In conclusion, this insight into the microbial composition of dadih and dangke will assist in the development of sustainable and technologically feasible starter cultures with probiotic properties. This information has the potential to enhance human health, food safety and food security from locally produced traditional fermented milk products.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Signed: Date: June 2017

Yoga Dwi Jatmiko

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Conference Presentations Arising from this Thesis

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Jatmiko, Y.D., Howarth, G.S. & Barton, M., 2015. Preliminary assessment of bacterial diversity of Indonesian naturally fermented milk products by pyrosequencing. The Australian Society for Microbiology Annual General Meeting July 2015, Canberra, Australia (Poster).

Jatmiko, Y.D., Howarth, G.S. & Barton, M., 2014. A preliminary study of bacterial community during fermentation of Dadih using pyrosequencing and PCR ITS-RFLP. The Australian Society for Microbiology Annual General Meeting July 2014, Melbourne, Australia (Oral Presenter).