

# Isolation and Characterisation of Potential Probiotic Microorganisms from Kefir

Surachai Khorkamphon\* and Thararat Chitov

Microbiology, Department of Biology, Faculty of Science, Chiang ai University, Chiang Mai, 50200, Thailand

\*Email: surachai\_kh@cmu.ac.th



## ABSTRACT

Kefir is a fermented dairy product produced using kefir grains, which contain a variety of microorganisms, some of which have probiotic potential. However, kefir microorganisms are rarely identified, despite their potential advantages. This study investigated and characterised microbial species present in kefir grains and evaluates their probiotic potential of kefir. Lactic acid bacteria, acetic acid bacteria, and yeasts were isolated from kefir grains cultured in cow's milk using de Man-Rogosa Sharpe-cysteine-bromophenol blue agar, acetic acid bacteria selective agar, and dichloran rose Bengal chloramphenicol agar, respectively. The bacterial and yeast isolates were identified through their 16S *rRNA* and *its* sequences, respectively. The probiotic potential was evaluated by examining tolerance to acidic pH (pH 2.0) and bile salts (0.4%). The results showed that microorganisms isolated from kefir grains include *Lentilactobacillus kefir*, *Lactocaseibacillus paracasei*, *Leuconostoc mesenteroides*, *Pichia fermentans*, *Saccharomyces cerevisiae*, and *Kluyveromyces marxianus*. The isolates exhibited acid tolerance with survival rates ranging from 65.4% to 100% and demonstrated bile tolerance with survival rates between 50.3% to 100%. The hydrophobicity rates ranging from 53.67% to 90.15%. In conclusion, kefir represents a significant source of beneficial lactic acid bacteria and yeasts with probiotic potential, as well as serving as an antioxidant source.

## INTRODUCTION

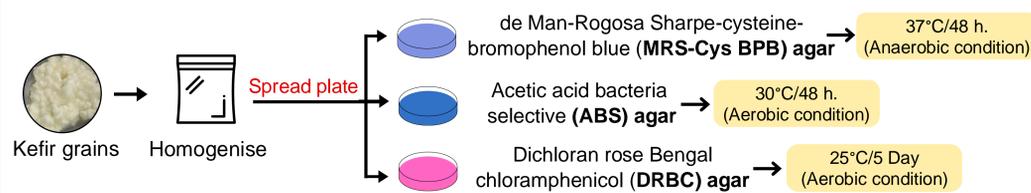
Kefir is a fermented milk product of the Caucasian origin made by fermenting milk with a variety of beneficial microorganisms in kefir grains, with include lactic acid bacteria, acetic acid bacteria, and yeasts. Kefir microorganisms are known to have probiotic potential. In recent years, kefir has gained popularity worldwide, especially among health-conscious individuals who seek nutritious food options. However, microorganism in kefir are rarely identified and their health-related properties are not known. Therefore, this study aimed to investigate the microbial groups present in kefir and their potential as probiotics.

## OBJECTIVES

- To isolate and identify microorganisms in kefir grains
- To examine characteristics of kefir microorganisms related to probiotic properties

## MATERIALS AND METHODS

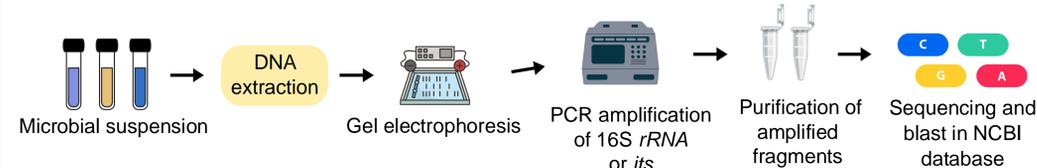
### 1. Isolation of microorganisms from kefir



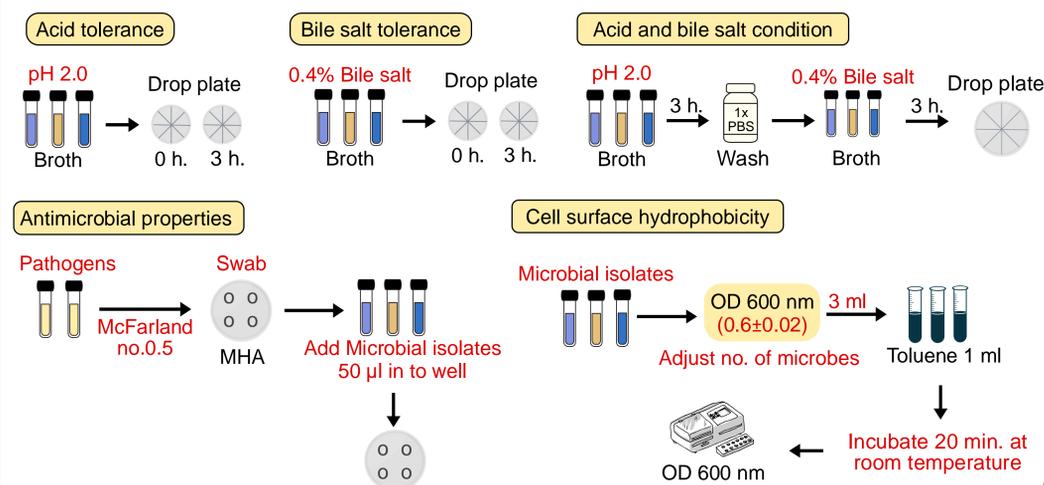
### 2. Study of morphological and biochemical characteristics



### 3. Genetic identification



### 4. Testing probiotic properties

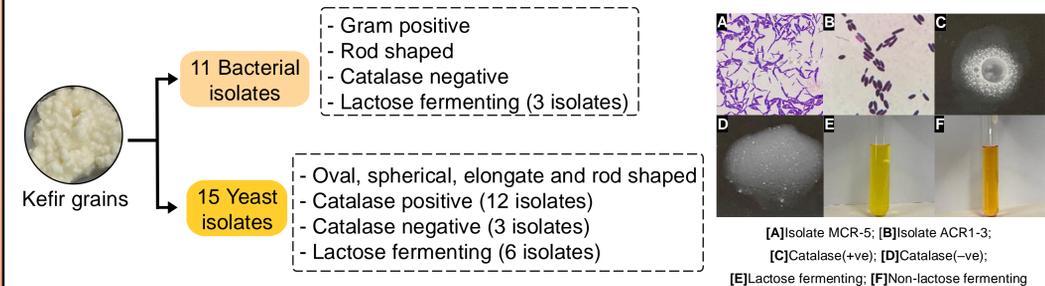


## REFERENCES

- Abraham Majak Gut, Todor Vasiljevic, Thomas Yeager (2021). Kefir characteristics and antibacterial properties – Potential applications in control of enteric bacterial infection. Institute for Sustainable Industries, Victoria University, Australia.
- Conor Slattery, Paul D. Cotter and Paul W. O'Toole. (2019) Analysis of Health Benefits Conferred by *Lactobacillus* Species from Kefir. School of Microbiology and APC Microbiome Institute, University College Cork, T12 Y337 Cork, Ireland.
- Properties of *Lactobacillus* Strains Isolated from Tibetan Kefir Grains. Central Research Laboratory, Second Hospital of Jilin University, Changchun, People's Republic of China.

## RESULTS

### 1. Study of morphological and biochemical characteristics



### 2. Identification of microorganism in kefir grains

#### Bacteria

Isolate	Species	Closest strain	Percent identity
MCR1-1	<i>Leuconostoc mesenteroides</i>	ATCC8293	100%
MCR1-2	<i>Lactocaseibacillus paracasei</i>	R094	100%
MCR1-4	<i>Limosilactobacillus fermentum</i>	CIP102980	99.93%
MCR1-5	<i>Lactocaseibacillus paracasei</i>	NBRC15889	99.93%
MCR1-13	<i>Lentilactobacillus kefir</i>	NBRC15888	99.87%

#### Yeasts

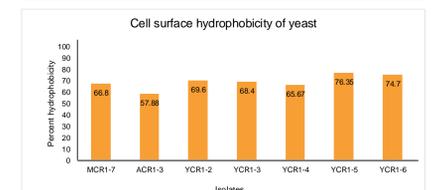
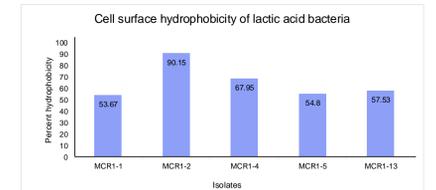
Isolate	Species	Closest strain	Percent identity
MCR1-7	<i>Kluyveromyces marxianus</i>	Dmic154961	99.86%
ACR1-3	<i>Saccharomyces cerevisiae</i>	X68-2	99.75%
YCR1-2	<i>Kluyveromyces marxianus</i>	Kw356/14	100%
YCR1-3	<i>Pichia fermentans</i>	PM19	100%
YCR1-4	<i>Saccharomyces cerevisiae</i>	YN3	100%
YCR1-5	<i>Kluyveromyces marxianus</i>	A2	98.92%
YCR1-6	<i>Saccharomyces cerevisiae</i>	HusL_FF7	100%

### 3. Probiotic properties

#### 3.1 Acid and Bile salt tolerance

Isolate	Species	Percent survival		
		Acid (pH 2.0)	Bile (0.4%)	Acid + Bile
MCR1-1	<i>Leuconostoc mesenteroides</i>	65.40	50.30	4.74
MCR1-2	<i>Lactocaseibacillus paracasei</i>	93.06	47.07	77.00
MCR1-4	<i>Limosilactobacillus fermentum</i>	99.69	4.04	3.99
MCR1-5	<i>Lactocaseibacillus paracasei</i>	100.00	54.96	70.52
MCR1-13	<i>Lentilactobacillus kefir</i>	100.00	94.82	4.54
MCR1-7	<i>Kluyveromyces marxianus</i>	82.62	80.29	83.36
ACR1-3	<i>Saccharomyces cerevisiae</i>	104.31	120.34	132.12
YCR1-2	<i>Kluyveromyces marxianus</i>	97.46	68.02	77.41
YCR1-3	<i>Pichia fermentans</i>	73.11	99.82	100.00
YCR1-4	<i>Saccharomyces cerevisiae</i>	90.82	100.00	100.00
YCR1-5	<i>Kluyveromyces marxianus</i>	86.46	74.40	92.77
YCR1-6	<i>Saccharomyces cerevisiae</i>	86.64	4.83	60.27

#### 3.2 Cell surface hydrophobicity test



#### 3.3 Antimicrobial activity

Isolate	Species	Inhibition zone to pathogen (mm)					
		<i>S. aureus</i> DMST 8013	<i>B. cereus</i> B-4ac	<i>B. cereus</i> B-358	<i>E. coli</i> O157:H7	<i>S. enterica</i>	<i>C. albicans</i> DMST 5815
MCR1-1	<i>Leuconostoc mesenteroides</i>	10.83 ± 0.57	9.95 ± 0.08	11.32 ± 0.64	10.93 ± 0.37	11.71 ± 0.33	NI
MCR1-2	<i>Lactocaseibacillus paracasei</i>	NI	9.95 ± 0.08	9.43 ± 0.30	11.00 ± 0.44	10.67 ± 0.40	NI
MCR1-4	<i>Limosilactobacillus fermentum</i>	11.50 ± 0.53	10.93 ± 0.23	10.22 ± 0.10	10.57 ± 0.64	10.55 ± 0.40	NI
MCR1-5	<i>Lactocaseibacillus paracasei</i>	NI	10.63 ± 0.50	10.10 ± 0.35	12.27 ± 0.57	9.30 ± 0.26	NI
MCR1-13	<i>Lentilactobacillus kefir</i>	NI	NI	NI	NI	NI	NI
MCR1-7	<i>Kluyveromyces marxianus</i>	NI	NI	12.50 ± 0.65	NI	NI	NI
ACR1-3	<i>Saccharomyces cerevisiae</i>	NI	NI	NI	NI	NI	NI
YCR1-2	<i>Kluyveromyces marxianus</i>	NI	NI	11.97 ± 0.12	NI	NI	NI
YCR1-3	<i>Pichia fermentans</i>	NI	NI	NI	NI	NI	NI
YCR1-4	<i>Saccharomyces cerevisiae</i>	NI	NI	NI	NI	NI	NI
YCR1-5	<i>Kluyveromyces marxianus</i>	12.10 ± 0.60	NI	12.80 ± 0.26	NI	NI	NI
YCR1-6	<i>Saccharomyces cerevisiae</i>	NI	NI	NI	NI	NI	NI

Antibiotic: Gram positive bacteria (Penicillin), Gram negative bacteria (Polymyxin B), yeast (0.12% Chlorhexidine)  
NI: No inhibition

## CONCLUSIONS

- Various microorganisms were isolated from kefir grains, including five isolates of lactic acid bacteria and seven isolates of yeasts.
- Some of these strains, particularly *Lactocaseibacillus paracasei* isolate MCR1-2 and *Kluyveromyces marxianus* isolate YCR1-5, have a strong probiotic potential due to their high tolerance to acid and bile salts, along with pathogen inhibition properties and high levels cell surface hydrophobicity.

## ACKNOWLEDGEMENTS

The authors would like to thank Asst. Prof. Thararat Chitov, PhD, Mr. Achirawit Ngamsomchat, Mr. Nipitpong Sawongta, members of 2806 Laboratory, and Department of Biology, Faculty of Science, Chiang Mai University.