Isolation, identification, and characterization of lactic acid bacteria from flowers and foods.

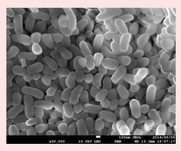
Riko Sakurai and Kanon Sato
Advisor : Tomoaki Kouya
Department of Materials Chemistry and Bioengineering,
National Institute of Technology, Oyama College, Oyama, Tochigi, Japan

Introduction

NIT (KOSEN), Oyama College

Lactic acid bacteria (LAB)

Characteristics
- Gram-positive
- Non motile
- Non spore forming
- Catalase negative
- Cocci- or rod-shaped
- Facultative anaerobes



Products
- Organic acids (Lactic acid, etc.)
- Bacteriocins (antimicrobial peptide)
- Reuterin
- Exopolysaccharides
- Aroma compounds
- etc.

Industrial and Health Applications
Wide variety of fermented foods and health maintenance
e.g. dairy, wine, sake, bread, meats, vegetables, probiotics, etc.

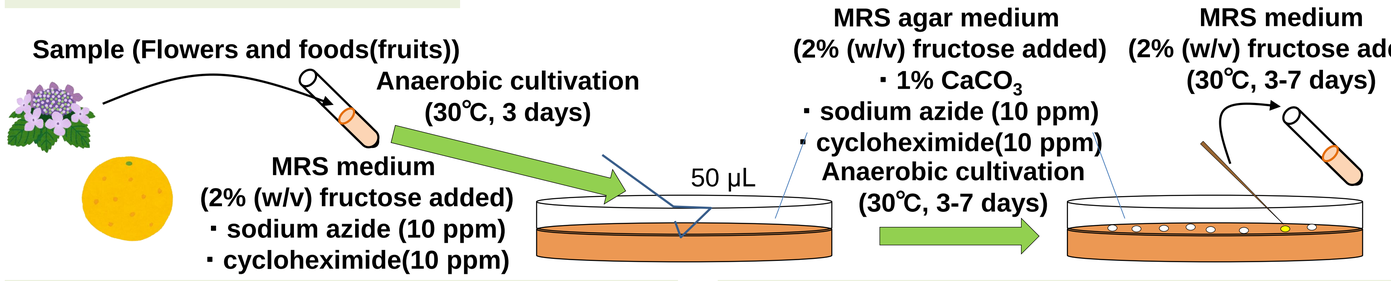
Lactic acid bacteria (LAB) are gram-positive, non-motile, non-spore forming, catalase-negative, and rods or cocci bacteria. LAB is known as probiotics, and they have a beneficial effect on the health of the host [1-3]. Furthermore, many cases of the production of beneficial compounds as biopreservative kind of organic acids, bacteriocins (antimicrobial peptides), and reuterin were reported [4-5] . The aims of this study are to isolate, identify, and characterize LAB from flowers and foods, and to evaluate the antimicrobial activity of LAB against the food spoilage and pathogenic bacterial strains.

Aims of this study

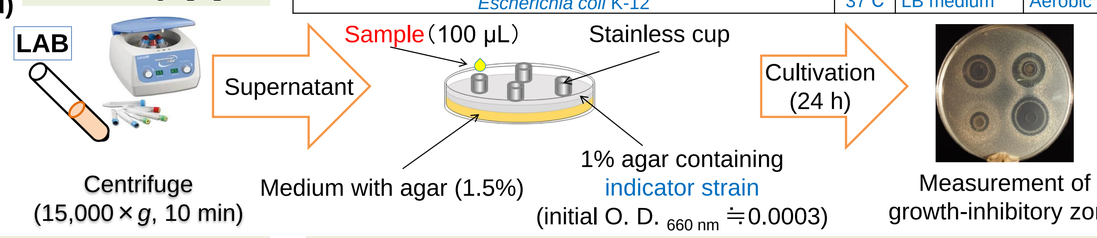
① To isolate, identify, and characterize LAB from flowers and foods (fruits) in Japan.
② To evaluate the antimicrobial activity of LAB against the food spoilage and pathogenic bacterial strains.

Materials and methods

Isolation of LAB



Antimicrobial assay [7]

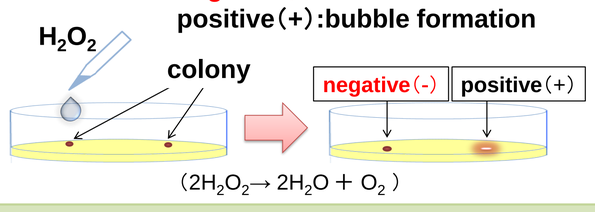


Leuconostoc mesenteroides subsp. *dextranicum* NBRC 100495^T	30℃	MRS medium	Anaerobic
Streptococcus mutans JCM 5705^T	37℃	BHI medium	Aerobic
Escherichia coli JCM 20377	37℃	LB medium	Aerobic
Escherichia coli K-12	37℃	LB medium	Aerobic

Catalase test : $2H_2O_2 \rightarrow 2H_2O + O_2$

Lactic acid bacteria : negative

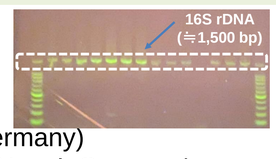
negative(-) : no bubble formation
positive(+) :bubble formation



Identification of lactic acid bacteria (LAB)

16S rDNA Sequence analysis（Database : NCBI ）

①DNA extraction and purification
・NucleoSpin® Tissue (Macherey-Nagel, Germany)



②PCR and clean-up of 16S rDNA
・Bacterial 16S rDNA PCR Kit (Macherey-Nagel, Germany)
・ NucleoSpin® Gel and PCR Clean-up (Macherey-Nagel, Germany)

③16S rDNA sequencing and identification of strains
・BLAST (NCBI : https://blast.ncbi.nlm.nih.gov/Blast.cgi)

Organic acids production : HPLC [6]

Lactic acid, Acetic acid

Hemolysis test

α-hemolysis : partial hemolysis
β-hemolysis : complete hemolysis
γ-hemolysis : no hemolysis

Results and Discussion

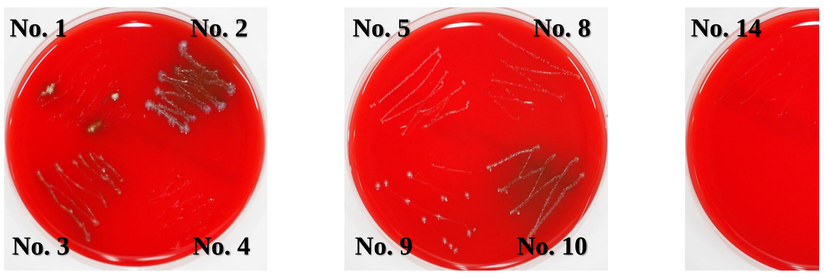


Figure 1. Hemolysis test for isolated LAB.

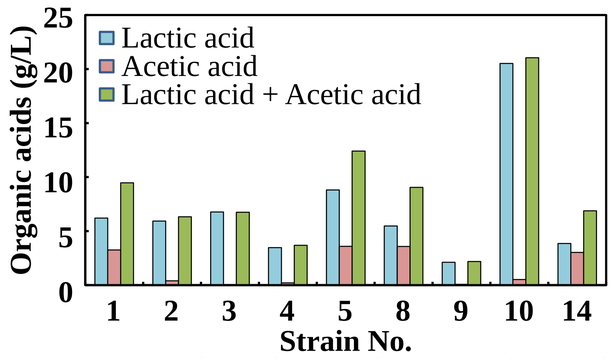


Figure 2. Production of organic acids by isolated strains.

Table 1. Identification of isolated LAB.

Strain No.	Source	No. of nucleotides ((%) Identity) [a]	Closest phylogenetic relative [b] (NCBI accession no.)
1	Uwa gold (Japanese grapefruit)	1081/1089 (99.27%)	*Weissella paramesenteroides* (MF429230.1)
2	African lily	1190/1198 (99.33%)	*Enterococcus faecalis* (MT573065.1)
3	Hydrangea	1177/1180 (99.75%)	*Lactococcus lactis* (MF348239.1)
4	Asiatic dayflower	1178/1184 (99.49%)	*Holzapfelia floricola* (LC519858.1)
5	Cockscomb	1067/1072 (99.53%)	*Levilactobacillus brevis* (MN416307.1)
8	White clover	1191/1198 (99.42%)	*Apilactobacillus kunkeei* (OX335183.1)
9	Peach	1288/1296 (99.38%)	*Streptococcus salivarius* (MT573576.1)
10	Marigold	1300/1309 (99.31%)	*Lactiplantibacillus plantarum* (MH773184.1)
14	Horse nettle	1256/1261 (99.60%)	*Apilactobacillus micheneri* (KX656660.1)

[a] The number of 16S rDNA nucleotides used for the alignment. [b] The % identity with the closest phylogenetic relative.

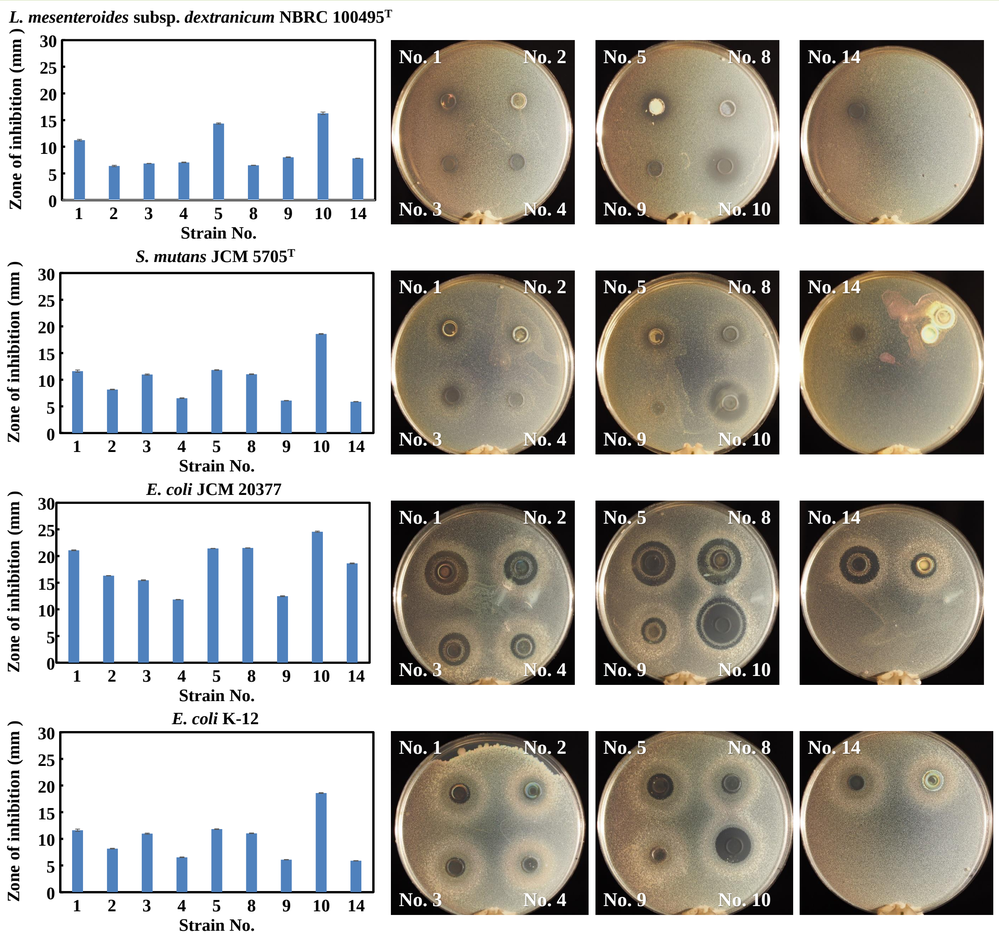


Figure 3. Antimicrobial activity of isolated strains against harmful bacteria.

Conclusions

- **Nine LAB were successfully isolated from flowers (african lily, hydrangea, asiatic dayflower, cockscomb, white clover, marigold, and horse nettle) and foods (Uwa gold and peach).**
- **The 16S rDNA sequence analysis revealed these strains were related to *Levilactobacillus*, *Apilactobaillus*, *Lactiplantibacillus*, *Holzapfelia*, *Lactococcus*, *Weissella*, *Streptococcus*, and *Enterococcus*.**
- **Some LAB had antimicrobial activity against the food spoilage and pathogenic bacterial strains, especially strain No. 10 (*L. plantarum*) also suppressed the growth of *Escherichia coli* (JCM 20377, K-12) and dental caries-associated bacteria (*S. mutans* JCM 5705^T).**

References

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[7] Ishiyama Y. *et al.* (2008). Food Sci. Technol. Res., 14 (3), 239-248.

[Corresponding] Tomoaki Kouya (tkouya@oyama-ct.ac.jp)