Near Infrared Spectroscopy (NIRS) and Aquaphotomics for Understanding Probiotic Lactic Acid Bacteria (LAB)

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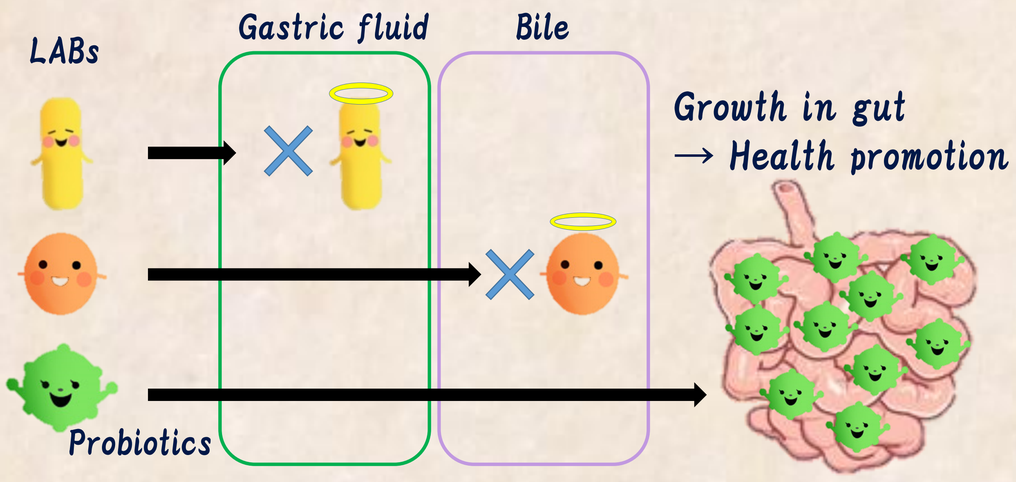
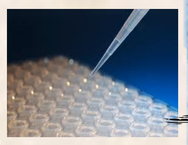
○ Introduction

Probiotics: Live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host [1]



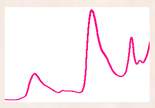
Conventional determination of tolerance to digestive fluids

Genetic method [2]
in vitro method [3]
Simulated gastric fluid and gall
Monitoring of optical density at 665 nm



Expensive, time-consuming, special knowledge and skills (´д`)

NIRS (^▽^)
Cost-effective, rapid, easy to measure, no difficult sample preparation
+
Aquaphotomics [4]



By measureing water, bacterial information can be acquired. Probiotics can be separated from others by its specific water structure.

Objective: To show possibility to classify LAB according to its tolerance to digestive fluids using NIRS and Aquaphotomics

○ Materials & Methods

① in vitro test and classification of tolerance as below

Probiotic (PB): tolerant to digestive fluids
Moderate (M) : which have moderate character
Non-probiotic (NPB): intolerant to digestive fluids

L.: *Lactobacillus*

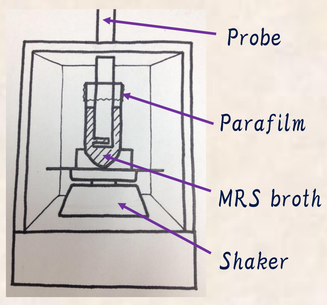
Strains	Recovery after 3h at low pH and Pepsin	Gall
	Recovery (yield of biomass) [-]	Minimal Inhibitory Concentration (MIC) [mg/ml]
L. bulgaricus: S6	0.062	1.25
L. gasseri: S20	0.191	2.50
L. bulgaricus: Y12	0.046	0.625
L. bulgaricus: S2	0	0.156
L. bulgaricus: S3	0.011	0.313
L. bulgaricus: S9	0	0.156

S6, 20, 2, 3, 9 strains were provided from Selur Pharma Ltd. (Bulgaria)
Y12 strain was isolated from a yogurt produced in Japan.

Simulated gastric fluid: HCl (0.2 M), NaCl (0.08 M), Pepsin (9000 U/mL)
Simulated gall: repetition of double fold dilutions from 5 mg/mL solution

② NIRS measurement in the process of bacteria pure culture

XDS (FOSS)
Wavelength: 400-2500 nm
Step: 0.5 nm
Module: Optiprobe Analyzer
Mode: Transflectance
Pathlength: 1.0 mm

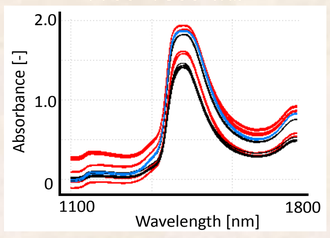


Measurement was done at 37℃ every 4 minutes.

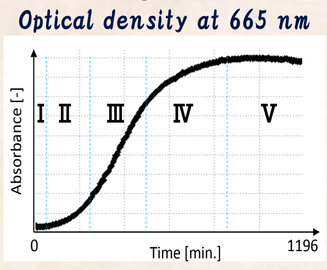
③ Investigation of correlation between in vitro test and NIRS data using Chemometrics and Aquaphotomics approach

○ Analysis

〈Raw spectra〉 1100-1800 nm



〈Bacteria growth curve〉 Optical density at 665 nm



Phase definition
Ⅰ: Lag phase
Ⅱ: Transitional phase
Ⅲ: Log phase
Ⅳ: Transitional phase
Ⅴ: Stationary phase

1100-1800 nm is strongly absorbed by water molecules and enables Aquaphotomics to be more supportive of results.

(i) PCA on each growth phase
Preprocessing: mean centering
Transformation: MSC
To check the separation of probiotic LAB from others and find wavelengths effective for the separation

(ii) Aquagram based on tolerances

$$A = \frac{a - \mu}{\sigma}$$

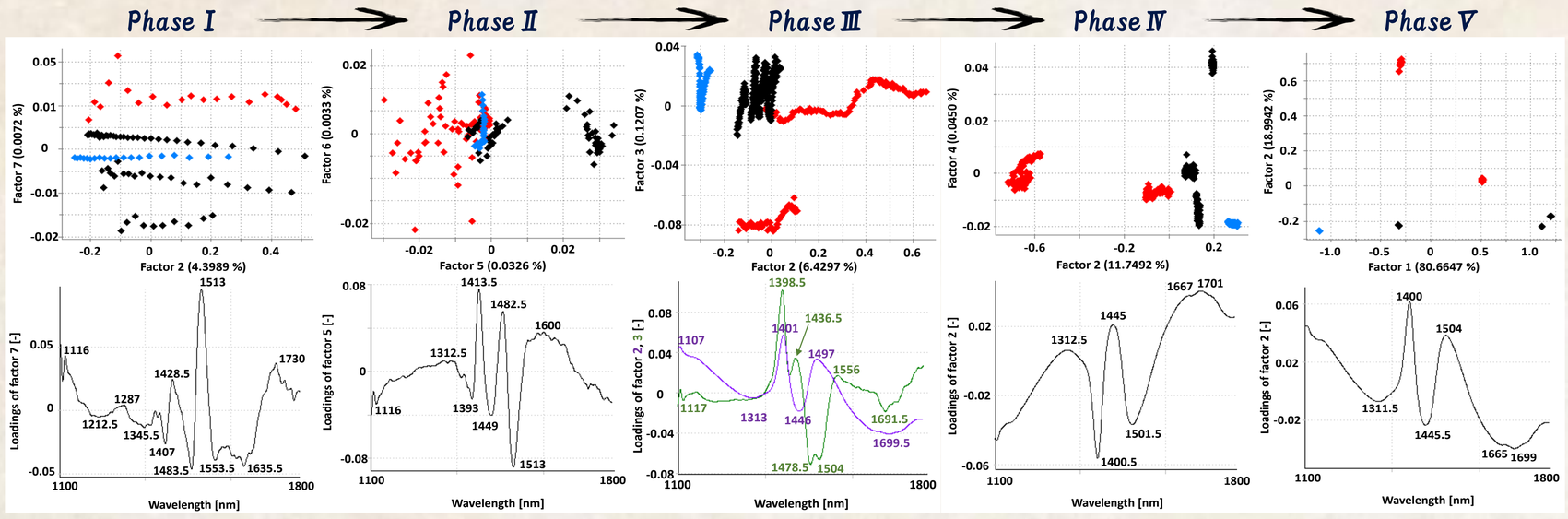
A = Aquagram value
a = absorbance after MSC of all group spectra
μ = average of all spectra in a group
σ = SD of all spectra in a group

Visualization of differences between tolerances

○ Results & Discussions

PCA scores and loadings separating probiotic LABs from others (Color in plots: PB, M, NPB)

Phase Ⅰ → Phase Ⅱ → Phase Ⅲ → Phase Ⅳ → Phase Ⅴ



Characteristic common water peaks

Phase	Factor (Variance)	Wavelength [nm]					
		(a)	(b)	(c)	(d)	(e)	(f)
Ⅰ	7 (0.0072 %)	1116, +					
Ⅱ	5 (0.0326 %)	1116, +	1312.5, −				
Ⅲ	2 (5.4297 %)		1313	1401	1446		1699.5
	3 (0.1207 %)	1117				1504	
Ⅳ	2 (11.7492 %)		1312.5, −	1400.5, +	1445, −	1501.5, +	1701, −
Ⅴ	2 (18.9942 %)		1311.5, −	1400, +	1445.5, −	1504, +	1699, −

(a) $H_{13}O_6^+$, H-bonded OH str., K. Mizuse
(b) Unknown
(c) $H_5O_2^+$, asymmetric str., R. Tsenkova
(d) H_2O, symmetric str. + asymmetric str., R. Tsenkova
(e) H_2O, symmetric str., Y. Ozaki
(f) $O_2^- \cdot (H_2O)_4$, J. M. Weber

一. Separation of probiotics becomes easier with phase advancement.
二. Protonated water can have positive correlation to probiotic character and superoxide tetrahydrate can have negative correlation.

Aquagrams showing different patterns in terms of the tolerance to digestive fluids

一. Three different groups of LAB show different specific water spectral pattern.
二. Interaction of probiotic LAB with water molecules is different from phases.
三. Moderate LABs interact especially with S_0 and S_4.
四. Non-probiotic LABs interact especially with S_1, S_2, S_3.

S_i: a water molecule which has i hydrogen bonds

Band Assignment in WAMACS [4]
1344 nm: ν3, 1364 nm: water shell
1372 nm: ν1+ν3
1382 nm: water shell, superoxide
1410 nm: S_0, 1438 nm: S_1, 1464 nm: S_2
1474 nm: S_3, 1492 nm: S_4, 1518 nm: ν1, ν2

* Wavelengths were selected based on WAMACS [4] and specific wavelengths written above.

○ Conclusions

It is possible to classify LAB according to its tolerance to digestive fluids using NIRS and Aquaphotomics.
Our result shows possibility of shortening the process of selection of probiotics.

○ References

[1] FAO/WHO (2002). Joint Working Group Report on Drafting Guidelines for the Evaluation of Probiotic in Food, London, Ontario, Canada, April 30 and May 1.
[2] T. R. Klaenhammer et al., Selection and Design of Probiotics, *International Journal of Food Microbiology*, 50, 45-57 (1999)
[3] I. Pitino et al., Survival of Lactobacillus rhamnosus Strains in the Upper Gastrointestinal Tract, *Food Microbiology*, 27, 1121-1127 (2010)
[4] R., Tsenkova, Introduction Aquaphotomics: Dynamic Spectroscopy of Aqueous and Biological Systems Describes Peculiarities of Water, *J. Near Infrared Spectrosc.*, 17, 303-313 (2009)