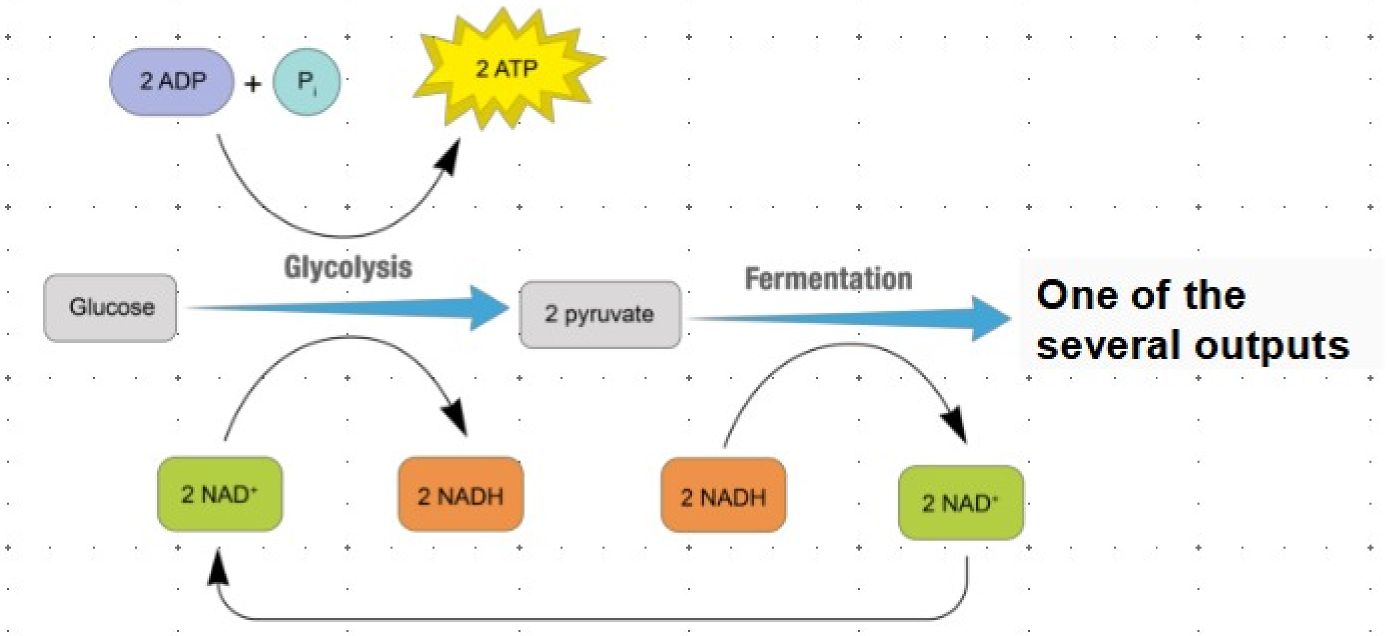
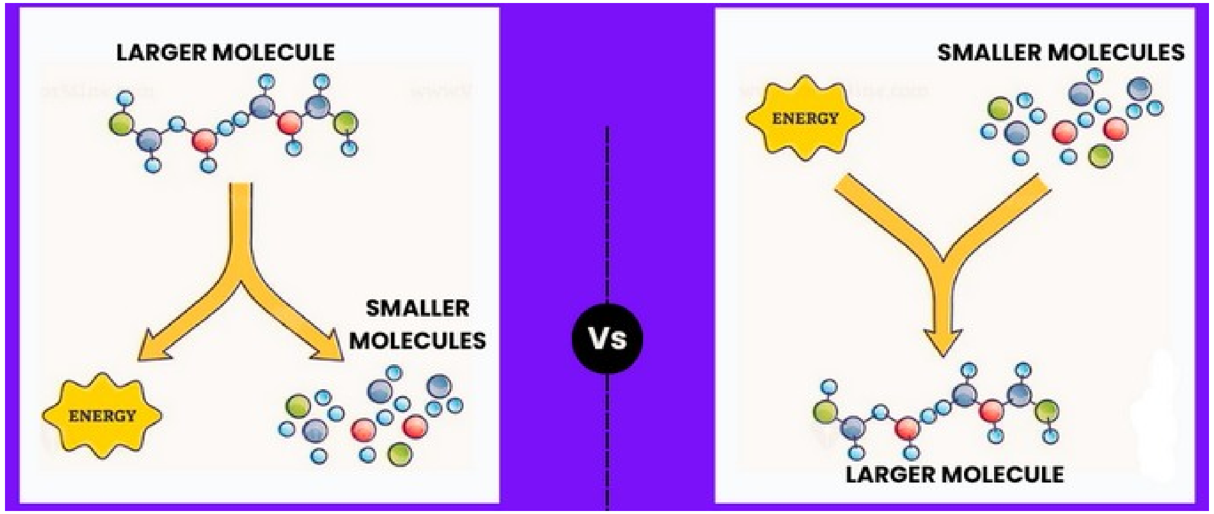
Selected plant-based (q)Fermentations from SEA and beyond

Peter HANTZ
Eötvös University, Dept. of Organic Chemistry
Guest researcher, Singapore Institute of Technology
Fibervar Ltd.

“Fermentation”



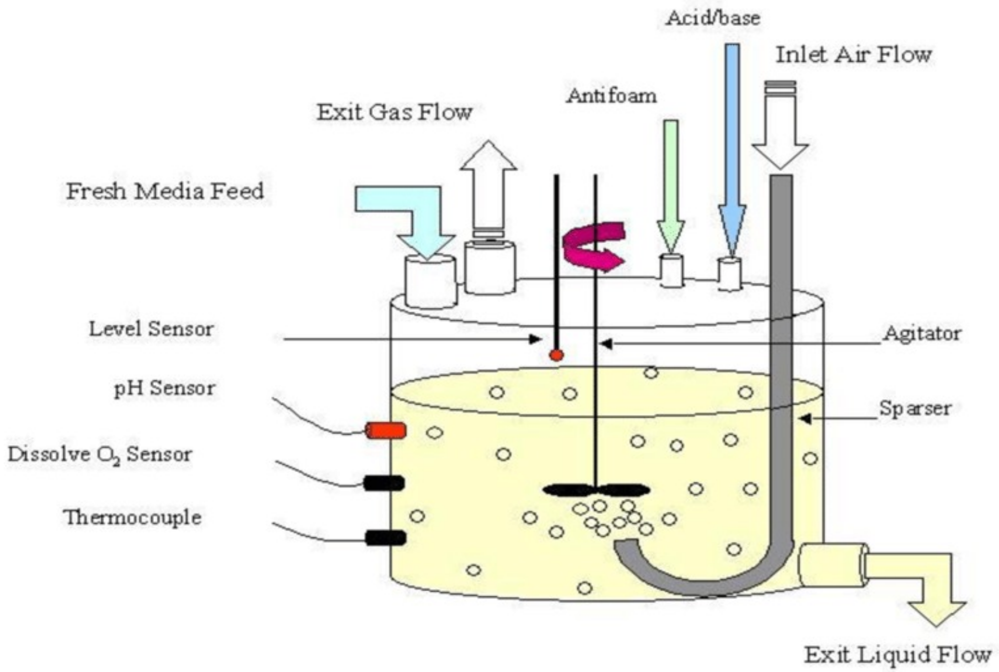
Real fermentation
Anaerobic process
The aerobic @ pyruvate:
→ SztGy-K cycle
→ NAD+ regenerates
→ more effective



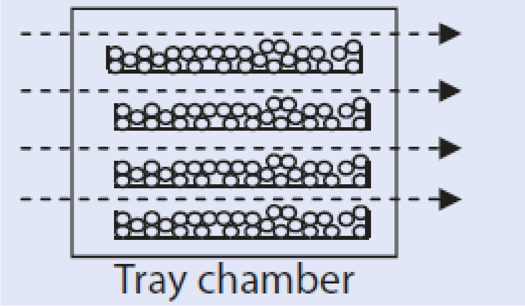
Aerobic fungal catabolism
Strictly-speaking NOT fermentation
→ followed by anabolism

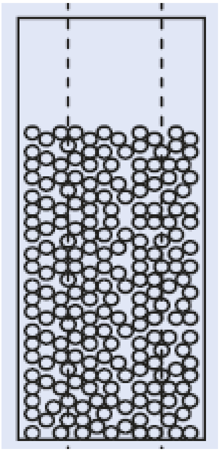
“animal stomach out”

Submerged



Solid-state (SSF)





300 BCE in China, to Japan 700 CE

1876: Hermann Ahlburg isolated & described

National mold of Japan, 2006

Culinary applications:

Amazake (“sweetjuice”)

Sake (“rice-beerwine”)

Mirin (rice liquor)

Miso (+salt+water+soy)

Soy sauce (+salt+soy+wheat)

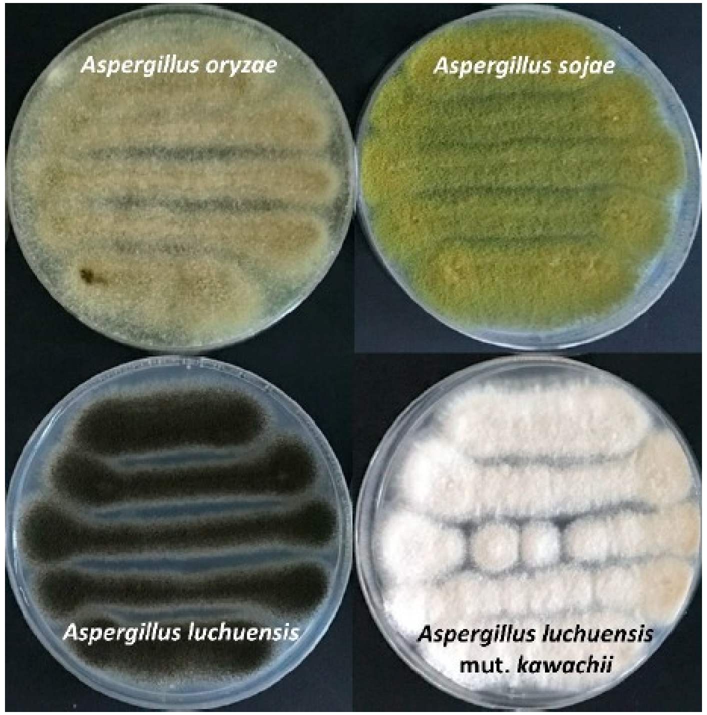
Biology of koji

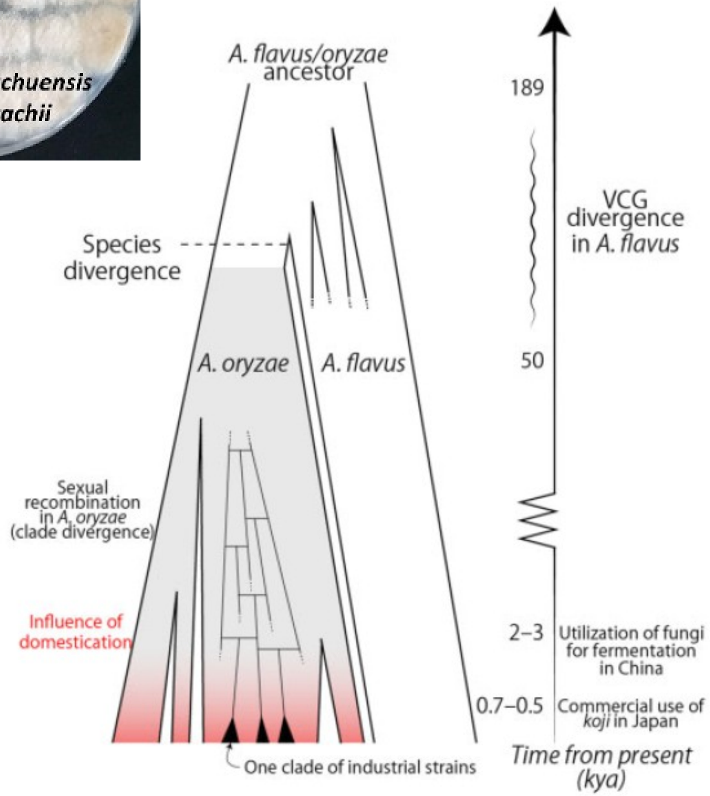
Aspergillus oryzae "yellow"
A. sojae
A. luchuensis "black"
mutation: "white"
NO AFLATOXIN

A. flavus
WITH ALFATOXIN

Genome size (~37 Mb),
8 chromosomes
12.000 genes

Loss of toxins: cca 80k
Domestication: cca 3k

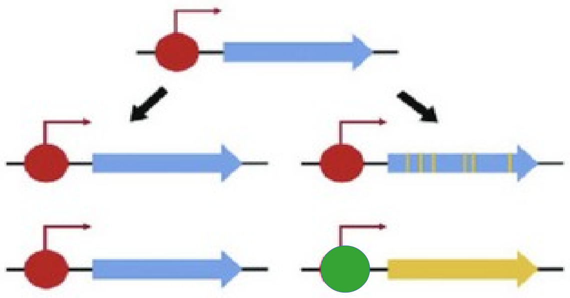




Enzyme redundancy

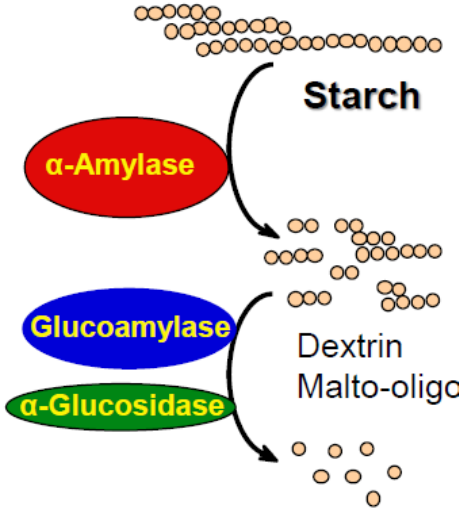
***SLIGHT DIFFERENCES**
@HUGE NEED – MORE

Dublication + divergence
subfunc.
neofunc.

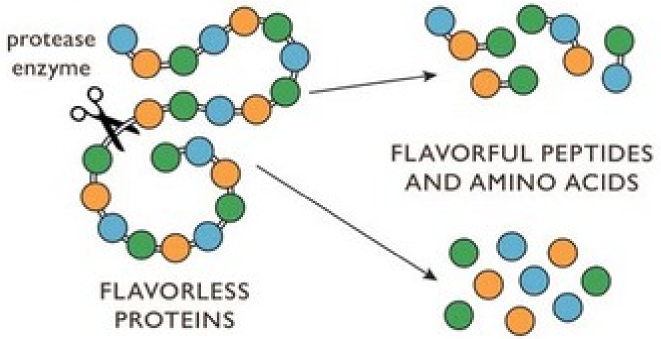


```
CAZymes (~500)   Carbohydrate-active enzymes HUGE#
|
├── Glycoside Hydrolases (GH) (~250)
|    |
|    ├── STARCH (~150)
|    |    |
|    |    ├── α-Amylase        → endo α-1,4
|    |    ├── α-Glucosidase    → exo glucose
|    |    └── Glucoamylase     → exo α-1,4 / α-1,6
|    |
|    ├── Cellulase + Xylanase (~20)
|    |
|    └── Other GHs (~80)
|
└── Auxiliary / Building enzymes (~250)
```

Proteases
~ 120
acidic, neutral, alkaline
serin proteases - rough
metalloproteases, etc. - fine



CAZymes:
DO NOT USE Cu pots
Possibly not Fe, Al pots
AVOID Ca-chelators



Carbon Catabolite Repression

Carbon Catabolite Repression in Filamentous Fungi

Muhammad Adnan[1,2], Wenhui Zheng[3,4], Waqar Islam[5], Muhammad Arif[6], Yakubu Saddeeq Abubakar[7,8], Zonghua Wang[9,10], Guodong Lu[11,12]

"sensors"
Nutrients, T, pH, Mech
- GPCR
- Ca2+ sensors

"Ground level" production
- solid +low water
  - → FlbC → growths
- maltose (glucose-glucose disach.)
  - → AmyR @ promoter → amylase → glucose
- glucose
  - → CreA @ promoter **X** block amylase

```
1. SPATIAL GRADIENTS
   Glucose produced AT TIP (far from sensor)
   ↓
   Sensor in SUBAPICAL ZONE (20-50 μm behind tip)
   Low glucose at sensor despite high at tip

2. FAST UPTAKE
   Glucose consumed faster than produced
   ↓
   [Glucose]local stays LOW
```

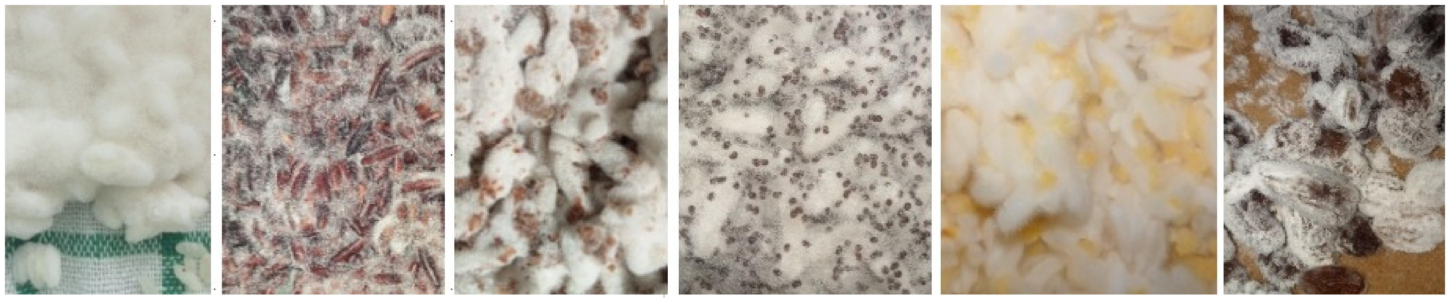
```
KOJI NEEDS ALL THREE:

1. Solid substrate  →  FlbC ON   →  "I'm on solid, enzyme-ready"
2. Glucose LOW      →  CreA OFF  →  "Brake released"
3. Maltose HIGH     →  AmyR ON   →  "Accelerator ON, make enzymes!"
```

(+no physical dilution)

(no Carb. Catab. Repr.)

Recipe & protocol

steamed / boiled herder rice (mix w other grains, dehulled soy, ...)
steam: soak before 6h
boil: 1 : 1.25 – 1 : 2 rice:water
influences enzymes, microbiome



Key parameters:
Moisture content
~70-80% RH, expt. 95%
→ consistency, taste

Oxygen diffusion
~2-3 cm
Slight textile covering

Temperature ~30-37 oC
enzymes, microbiome
→ taste

Time: 44-48h

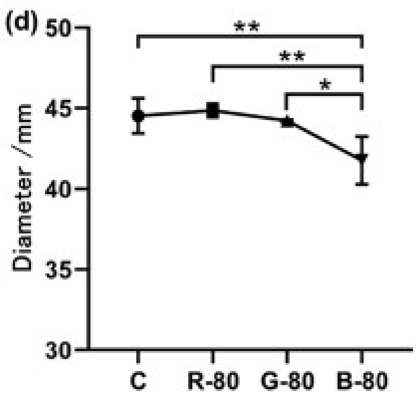
Japan: koji room (koji-muro)

Light? - less relevant

mycelium growth inhibited
@ green and blue light (520 and 475 nm)

secondary metabolites may also be influenced

Likely: flavin chromophore + White-Collar protein conf. ch.



- 520 nm, 20 mmol → ~4.6 W/m^2
- 520 nm, 60 mmol → ~13.8 W/m^2
- 475 nm, 80 mmol → ~20 W/m^2

AMERICAN SOCIETY FOR MICROBIOLOGY Microbiology Spectrum

RESEARCH ARTICLE

Check for updates

Inhibition of *Aspergillus oryzae* Mycelium Growth and Conidium Production by Irradiation with Light at Different Wavelengths and Intensities

Shangfei Lin,[a,b] Haokuan Qin,[a] Xiaolin Zhang,[c] Wenqi Li,[a] Muqing Liu[a,b,c]

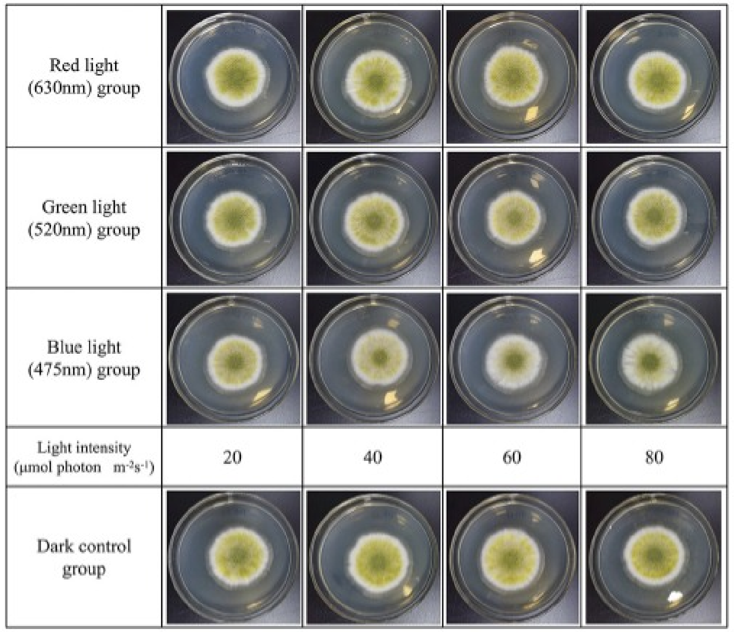


FIG 2 Influence of different types of light irradiation on *A. oryzae* GDMCC 3.31. Strains were incubated on PDA plates for 72 h at 30°C. Until incubation, each plate was examined and photos were obtained.

Associated microbes

Less well-grounded research than expected
Some role @ bacteria
Several substartes

Lactic acid bacteria (LAB) – dominant functional group

Weissella	- heterof. (LA+AcA, CO_2)	→ soft acidity,	→ B9, ↓infl. cytokines
Lactobacillus	- homo/heterof. (LA /AcA), enz.	→ tangy acidity, umami	→ B-vitamins, ↓infl. cytokines
Pediococcus	- homof. (LA only)	→ clean acidity,	-
Leuconostoc	- heterof. LA+AcA, CO_2, diacetyl	→ buttery	→ SCFA, ↓infl. cytokines
Lactococcus	- homof. LA + some diacetyl	→ mild acidity	-

Also

Staphylococcus	-umami; proteolysis → amino acids, ammonia, volatile sulfur compounds
Bacillus	-intense umami; extracell. proteases → glutamic acid, peptides, ammonia

Soy and rice differences

Bacs source: Sporulating ones – survive boiling
not just contaminants — they shape the flavor baseline

Bacterial Communities and Prediction of Microbial Metabolic Pathway in Rice Wine Koji From Different Regions in China

Xinxin Zhao[1,2†], Fanshu Xiang[1,2†], Fengxian Tang[1,2], Wenchao Cai[1,2], Zhuang Guo[3], Qiangchuan Hou[3], Xinquan Yang[1], Wen Song[1,2] and Chunhui Shan[1,2]*

“Active compounds”

Less well-grounded research than expected
NOT A MEDICINE – A GOOD FOOD!

vitamin B1: found a gene @ synthesis overexpressed

Construction of a thiamine pyrophosphate high-producing strain of *Aspergillus oryzae* by overexpression of three genes involved in thiamine biosynthesis

Misato Tokui,[1,*] Takafumi Kubodera,[1] Katsuya Gomi,[2] Nobuo Yamashita,[1] and Akira Nishimura[1]

Traditional Koji application

Improvement of skin conditions by ingestion of *Aspergillus kawachii* (Koji) extract containing 14-dehydroergosterol in a randomized, double-blind, controlled trial

Food Sci. Technol. Res., 17 (3), 209–218, 2011

Brown rice: more antioxidants - not a surprise
Bioavailability may be enhances

Radical Scavenging Activity of the Japanese Traditional Food, *Amazake*

Solid-state cultivation of multiple industrial strains of *koji* mold on different Thai unpolished rice cultivars: biotransformation of phenolic compounds and their effects on antioxidant activity

Some secondary metabolites

REVIEW

The ancient koji mold (*Aspergillus oryzae*) as a modern biotechnological tool

Ghoson M. Daba[*], Faten A. Mostafa[*] and Waill A. Elkhateeb

Alternative substrates

Fruits / Roots:

Bael fruit

Sporadic attempts on BSG → miso

Papers: some quality red flags...

Upcycling apple peels:

-Glycosylated flavonoids → aglycones

- more digestible fibers

Tailoring the composition, antioxidant activity, and prebiotic potential of apple peel by *Aspergillus oryzae* fermentation

Nitrogen enrichment:

25g wet rice + 0.6g Urea + Amm-sulphate

→2.3x more proteins w lysin!

hint: Amm-citrate

https://doi.org/10.1038/s41538-025-00463-2

Fast and easy edible protein production by nitrogen-supplemented koji fermentation

Iron enrichment:

1-5 mMol FeSO4

→ even 7% dry m/m% iron!

Iron Absorption from Iron-Enriched *Aspergillus oryzae* Is Similar to Ferrous Sulfate in Healthy Female Subjects

Amazake & drinks

Classical amazake:

Steamed rice + koji + water

Heat 50-60 oC o/n

Simplified amazake:

Just koji + water

Variants:

+ fruits, chesnut, ginger, plant extracts

Kuro Mirin (& Florium)

>100 aroma compunds:
alcohols, acids, ethyl esters,
Strecker aldehydes, phenols
D-Amino acids!

Microbiome
→ **some enzymes @ EtOH?**
EtOH
→ acetaldehyde / condensation
polyphenol condensation
→ **brown polymers**
phenolic acids:
→ glycosides during the aging
Sugar dehydration
→ HMF 5-Hydroxymethylfurfural
HMF + acetaldehyde
→ dark polymers
Acetaldehyde + amino acids
→ **Strecker aldehydes**
Acids+ethanol:
→ estherification?
Reducing Sugars + AA (N):
→ **extremely slow Maillard?**

Bioscience, Biotechnology, and Biochemistry, 2015
Vol. 79, No. 3, 484–487

Note

Aroma compounds in Japanese sweet rice wine (Mirin) screened by aroma extract dilution analysis (AEDA)

Shu Kaneko* and Kenji Kumazawa

Ogawa & Co. Ltd., Urayasu, Japan

Metabolomic investigation of differences in components and taste between hon-mirin and mirin-like-seasoning

Kana Yamamoto,[1] Moyu Taniguchi,[1] Takeharu Nakahara,[2] Yusuke Ito,[2] Ryoichi Sasano,[3] and Eiichiro Fukusaki[1,*]

Department of Biotechnology, Graduate School of Engineering, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan,[1] Research and Development Division, Kikkoman Corporation, 338 Noda, Noda, Chiba 278-0037, Japan,[2] and AiSTI SCIENCE CO., Ltd., 120-6 Kuroda, Wakayama, Wakayama 640-8341, Japan[3]

Starters & strains

RIB = Research Institute of Brewing (Japan)
RIB strains
    curated, well-characterized strains
    A, B, C: types of inactve Aflatoxin genes

Some EU vendors: wrong labeling

TABLE 3. Classification of *A. oryzae* RIB strains based on PCR amplification patterns of aflatoxin biosynthesis gene homologs

Classification	Aflatoxin biosynthesis genes amplified by PCR[a]	No. of strains[b]	Ratio (%)
Group 1	*aflT, nor-1, aflR, norA, avnA, verB, vbs*	122	58.1
Group 2	*avnA, verB, vbs*	77	36.7
Group 3	*verB, vbs* or *vbs*	9	4.3
Others	No shared patterns	2	0.9

www.higuchi-m.co.jp/en/choice/

How to Select the Koji Starter

versus

Tempeh

traditional Indonesian fermented food
produced by SSF
of legumes, cereals
with filamentous fungi of the gen. Rhizopus
physically binding hyphae
commonly Rhizopus oligosporus.

1815 – earliest reference to tempe
@ Javanese Serat Centhini manuscript

1936 – attempt @ India but
“they did not have any interest in
this unknown fermentation product”

1946 – Netherland
the first tempeh-making company in Europe

1964 – use of perforated plastic bags and tubes
proposed by Martinelli and Hesseltine

2010 – a “soy scare” on the Internet
→ from other types of grain legumes

Rhizopus

```
               Rhizopus MRCA
      (ancient WGD ~300 Mya)
                        |
            --------------------
            |                  |
Rhizopus microsporus    --------------
(incl. var.                  |             |
oligosporus)      Rhizopus stolonifer  Rhizopus arrhizus /
(tempeh)                (bread mold)   Rhizopus delemar
                                       (pathogen / industry)
```

Mucoromycota, Mucor…. Gen. Rhyzopus

Main mold:
Rhizopus microsporus var. oligosporus

genome sizes (24-26 Mb)
10.000-12.000 genes
6 or 7 chromosomes (!), haploid=one set

Whole-genome duplication (~300 Mya; Devon–Carbon)
@common ancestor of Rhizopus

15-25% transposable elements
(post-WGD restructuring)

No toxins, BUT
Associated bacteria may produce toxins

Enzymes comparison

Enzyme / Activity	Rhizopus oligosporus	Aspergillus oryzae / sojae	Functional Winner	Relevance
Amylases	Moderate	Very high	Aspergillus	Decompose starch
Protease profile	Moderate, alkaline-biased	High, acid/neutral-biased	Context-dependent	Peptides, AA-s
Phytase	Very high	Moderate	Rhizopus	Improvement of mineral bioavailability
β-Glucosidase	Very high	Moderate	Rhizopus	Isoflavone → aglycone
Lipase	Moderate–high	Moderate	Rhizopus (slight)	Fat mobilization, flavor development
Laccase / Phenol oxidase	Significant	Minimal / absent	Rhizopus	Polyphenol modification
Cellulase	Low-moderate	Low	-	Surface loosening

Tempeh requires more protein - builds edible mycelial biomass.
Koji primarily relies on starch-rich substrates.

Nitrogen Catabolite Repression

NCR:
a system that prioritizes
“easy” nitrogen sources (e.g. urea, ammonium, AA) over proteins,
transcription factors such as AreA,
repressing the transcription of genes @ proteolysis
avoiding the unnecessary expression

J Gen Microbiol. 1992 Dec;138(12):2539-44. doi: 10.1099/00221287-138-12-2539.

Regulation of the secretion of Rhizopus oligosporus extracellular carboxyl proteinase

P C Farley [1], L Ikasari

Microbiology (Reading). 1998 Aug:144 (Pt 8):2355-2366. doi: 10.1099/00221287-144-8-2355.

The Rhizopus oryzae secreted aspartic proteinase gene family: an analysis of gene expression

Recipe & protocol

(Soy)beans, soaking 12h
Remove hulls, cooking-steaming 45-60 mins
Slightly dry
May spray acid

Starter added cca 0.4 m/m %, mix
Fermented for 1-2d 30-32 oC

MicroAerobic conditions are essential: HOLES
- thin, btw beans, holder holes, 2.5 cm thick
- importance of a_w

Refrigerator for 1w
Eventually cooked, steamed, dried, baked

Anaerobic:
bacteria/yeasts
→ turn to EtOH fermentation

Too much air:
→ surface sporulation → bad taste

Over-ripe:
AA → ketoacid → ammonia
+ extra bacteria

Variants

species :: evolutionary lineage
genetically & phylogenetically distinct
mating
Subspecies:: a stable subgroup
Strain:: a single genetic isolate with minor differences

Development of Salt-Tolerant *Rhizopus oligosporus* for Tempeh Production

Tatsuo Kai, Etsuko Nishizaki, Yasuyo Sakamoto, Yuko Ishimoto

Starting strain: max 0.6% NaCl
10 generations
Slows only at 1.2% NaCl

Phenotypic, Metabolic, and Genetic Diversity of the Indonesian Isolates of *Rhizopus oligosporus*

CAHYA PRIHATNA[1] AND ANTONIUS SUWANTO[2*]

32 oC grew all 15
45 oC grew only FB-06
50 oC none

Nutrient use:
FB-07: 46/47 (most)
FB-06: 44/47
FB-01: 19/47 (least)

Associated microbiome

Tempeh:: not fermented by Rhizopus alone
Bacterial community
→ nutrition, taste, and metabolites
→ optimal acidic pH

Core microbiome
Lactic acid bacteria (Firmicutes G+)
Limosilactobacillus →vitB12, ↓cytokines (@EPS, peptides)
Leuconostoc → SCFA, ↓cytokines (@EPS, SCFA)
Enterococcus (!)
Enterobacteriaceae (Proteobacteria G-)
Enterobacter → acids, aroma 2,3butanediol
Klebsiella→vitB 1,2,6,7,9
Citrobacter →vitB 1,2,6,7,9

Cooking kills most bacteria - not the spores
Starter, natural contamination
Fermentation selects and rebuilds
LABs grow fast

Microbiome & oligos

Raffinose & stachyose reduction in tempe fermentation
“flatulence oligosaccharides”

Tempe mold alone practically insufficient
(Rehms & Barz, 1995; Graffham et al., 1995)

Mixed cultures (mold + bacteria) are required
(Egountley 2003, but no bacs identified)

Likely:
Lactic acid bacteria (LAB) with α-galactosidase
Lactobacillus plantarum (strain-dependent)

Recent approaches

community:
- metabarcoding
- metatranscriptomics
- analytics
- time series

co-cultures

isolate:
- substrate spectum
- growth parameters
- analytics
- transcriptomics

Recent results @ SIT

Innovation: Brewer Spent Grain + protocol for supplementation

Koji @
BSG with starch / starch + protein

Tempe @
BSG with starch

“Active compounds”

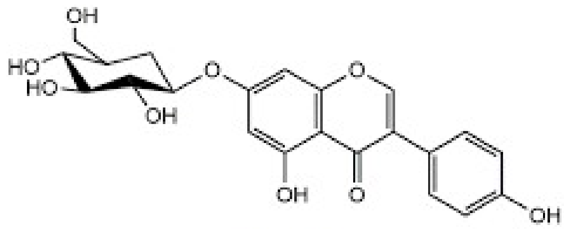
Assciated bacteria have a very omportant role.
More important than in Tempeh

(Soy) Isoflavones

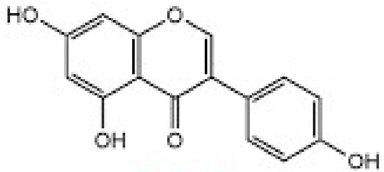
R. oligosporus: β-glucosidase :: glucosides→ aglycons

MOST IMPORTANT: BIOAVAILABILITY

genistin → genistein

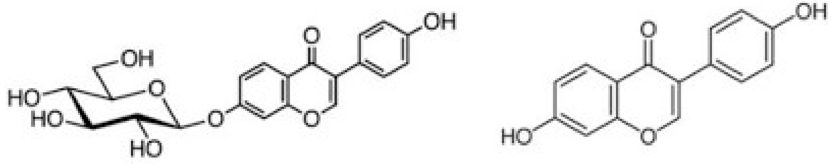


Genistin



Genistein

A Daidzin B Daidzein



B-vitamines, esp. B12

by bacteria

Anti-inflammatory EPS, peptides

by bacteria

Probiotic effect

anaerobic bacteria

Alternative substrates

Tempeh requires more protein. Rhizopus builds edible mycelial biomass.
Koji fermentation primarily relies on starch-rich substrates.

Various protein-rich beans:

bean, green peas, barley, oat, … 20-40 protein dry mass m/m%

fruits: apple dice, wild apple
nice but...
no nitrogen - weak biomass


okara (5% protein dry mass m/m%)
coffee (10+%)
algae (10+%)



Tapai

fermenting starchy ingredients (rice or cassava)
with a mixed starter culture
containing amylolytic yeasts/molds and bacteria
that saccharifies starch

Spread all over South Eastern Asia
Indigenous, likely >500 years old
Calmette, 1892: Banh men “Levure chinoise” *A. rouxii*

Calmette, A. (1892). La levure chinoise, ferment de l’amidon. *Annales de l’Institut Pasteur*, *6*, 604–620.

Why so complex?
Ecosystem
molds = aerobic
LABs = anaerobic
am. yeasts = mix

amylolytic enzymes = several types

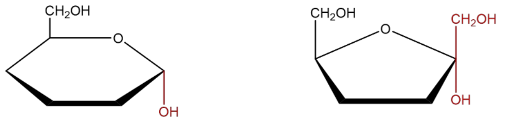
Amylolysis

amylolytic enzymes::
α-glycosidic bonds in starch

β-Glucosidase NOT in amylolysis
exo-acts on β-1,4-glycosidic in cellulose

Form of amylolysis	Enzyme(s)	Bond cleaved	Main product(s)	Mode of action
Endo-amylolysis	α-Amylase	α-1,4 (internal)	dextrins, oligosaccharides	HITTING
Exo-amylolysis	β-Amylase	α-1,4 (chain ends)	maltose	SLIDING
Exo-amylolysis	Glucoamylase (amyloglucosidase)	α-1,4 and α-1,6 (chain ends)	glucose	SLIDING
Debranching	Pullulanase, Isoamylase	α-1,6	linear chains	HITTING

α anomers

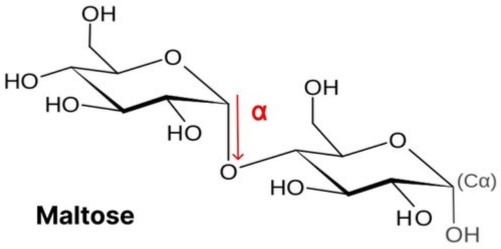


D/L and ref.: C5–CH_2OH

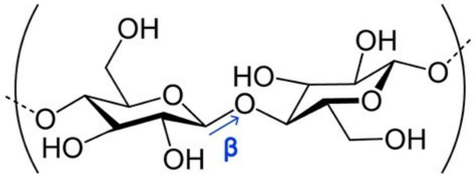
β anomers

Glucose(6) and Fructose(6)

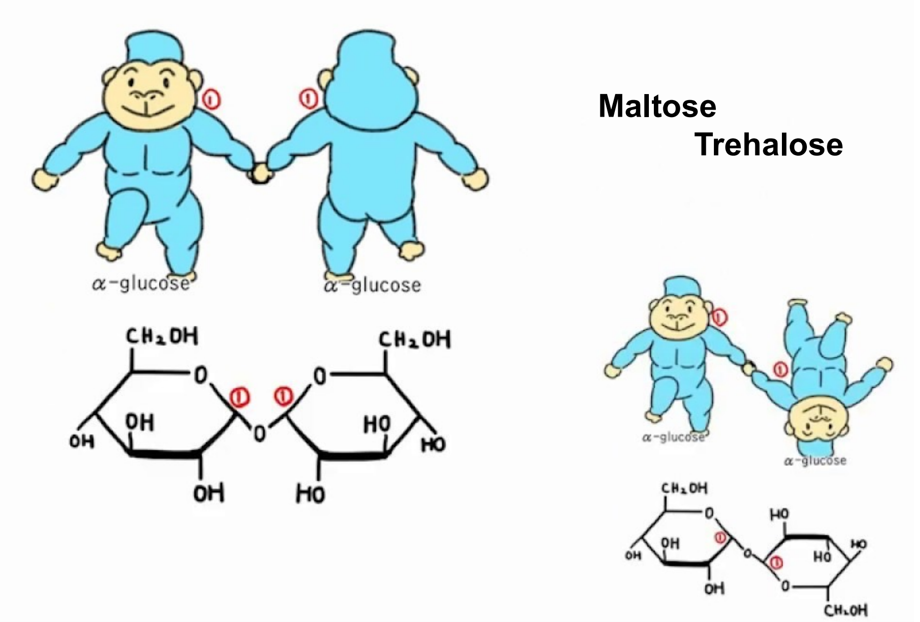
polymers: a- or b- bonds
which is anomeric (OH→ water)
the other gives bond O
sach. not polymer: a-b



Maltose



Cellulose



Inhibition!

Pichia burtonii
alpha-amylase (PBA)glycoprotein @ 51 kDa.
Optimum @ 40 degrees C
inhibited by Cd(2+), Cu(2+), Hg(2+), Al(3+), Zn(2+)

DO NOT USE Cu pots
Possibly not Fe, Al pots

> Biosci Biotechnol Biochem. 2006 Dec;70(12):3019-24. doi: 10.1271/bbb.60430. Epub 2006 Dec 7.

Purification and characterization of an alpha-amylase of Pichia burtonii isolated from the traditional starter "murcha" in Nepal

Akiko Takeuchi [1], Akiko Shimizu-Ibuka, Yoshitaka Nishiyama, Kiyoshi Mura, Sanae Okada, Chiyoko Tokue, Soichi Arai

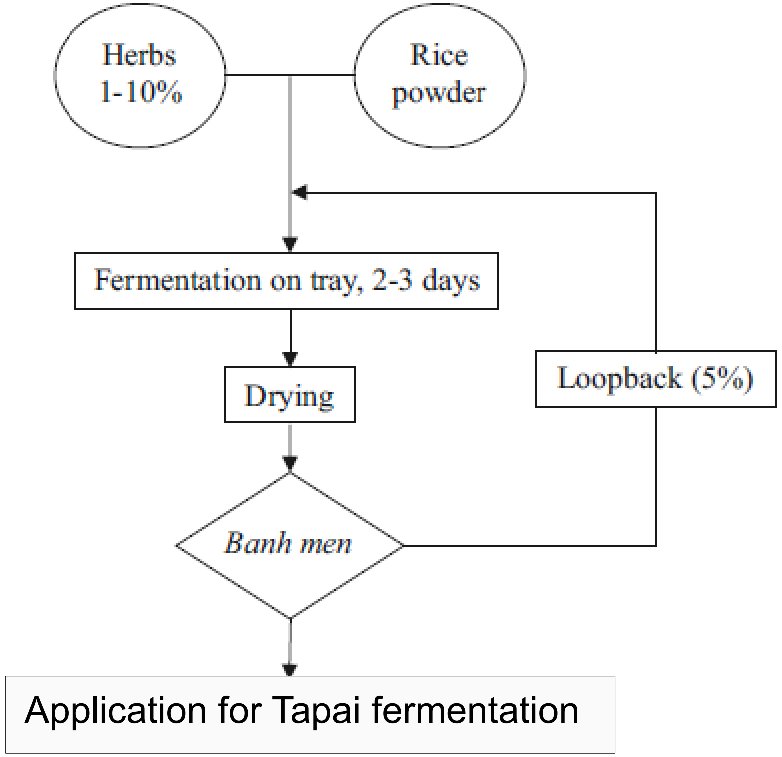
Some of them: Ca^{2+}-dependent
- Ca^{2+} chelators inhibit by removing Ca2+

Non-specific inhibitors: Phenolic compounds
- tannins
- plant polyphenols
- spice-derived phenolics

The starter(s)

Microbi:
- Molds
- Yeasts
- [Bacteria & anti-microbial plants]

Technology:
- starter powder + rice powder + dry/wet plants:
  - spices (e.g. *Piper longum, Piper nigrum, Coriandrum sativum*)
  - sweets (e.g. *Alpinia galanga*)
  - wet phase: e.g. *Allium sativum*



Starter ecosystem:
yeasts, molds, bacteria

Molds: mycelium, aerobic, exoenzymes

Yeasts: budding, respire/ferment

Most important:
Amylolytic Molds

Amylomyces rouxii
Rhizopus microsporus
Mucor indicus

Usually secondary:

Amylolytic Yeats

Saccharomycopsis fibuligera
Candida pelliculosa
Endomycopsis burtonii (syn. Pichia burtonii)

Amylolytic bacteria

Bacillus subtilis

Bacillus amyloliquefaciens
~ Limosilactobacillus fermentum

EtOH:
Yeasts

Saccharomyces cerevisiae (pseudohyphae)

pH, flavors, metabolites:
Bacs - VARY

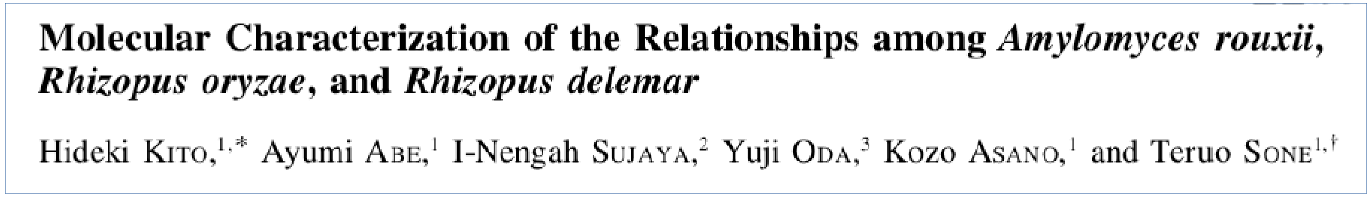
Lactobacillus plantarum (fac. Hetero AcAc + EtOH)
Pediococcus pentosaceus
Weissella confusa

Starter ecosystem

Amylomyces rouxii:: two lineages w strains:

lactic acid – producing
”smooth” taste
related to Rhizopus oryzae

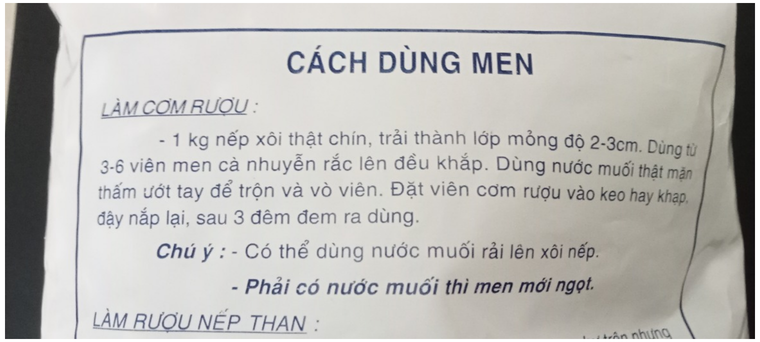
fumaric- and malic-acid – producing
”harsher” taste
related to Rhizopus delemar



Molecular Characterization of the Relationships among *Amylomyces rouxii*, *Rhizopus oryzae*, and *Rhizopus delemar*

Hideki Kito,[1,*] Ayumi Abe,[1] I-Nengah Sujaya,[2] Yuji Oda,[3] Kozo Asano,[1] and Teruo Sone[1,†]

Some Vietnamese starters require the use of salt
→ LABs
medium tolerance ones like: L. plantarum (2-4%)



Banana leaves

Characteristics and bacterial community of black glutinous rice *tapai* with various packaging types during fermentation

[1]*Barus, T., [1]Tanex, R. F. and [2]*Prasasty, V. D.

BGRT-PB. In conclusion, packaging type significantly affected sugar content, organoleptic profiles, and the bacterial community composition of BGRT. Among the three packaging types, BGRT-BL outperformed BGRT-PB and BGRT-RA. BGRT-BL exhibited the highest proportion of the genera *Pediococcus* and *Limosilactobacillus*.

Black Glutinous Rice Tapai: best with banana leaves > rose apple leaves > plastic

Recipe & protocol

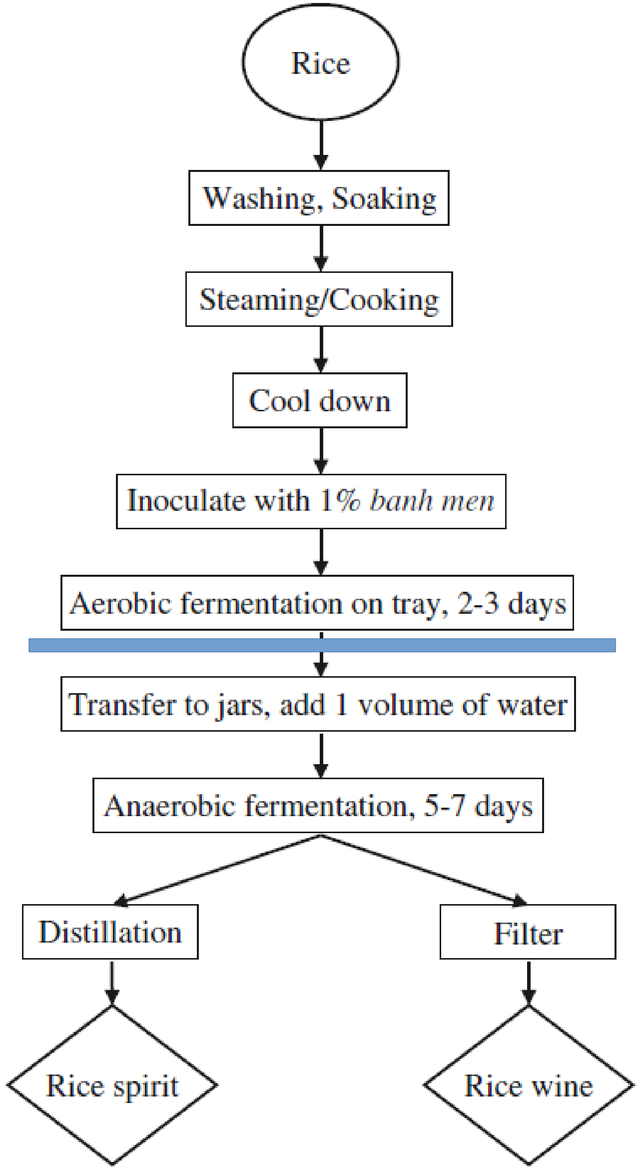
Raw materials:
White/Black rice
Cassava
Corn
...

Humidify, soften, disinfect
Washing
Heat treatment
Boiling – absorption method
added ≈ absorbed
Boiling – excess water
decanting
Steaming

Cool + Dry a bit
Inoculate

Ferment 2-3d
isolate
white rice balls: @ banana leaves
black rice: bulk

EtOH: anaerobic: SSF→
AcAc: aerobic



Kombucha

Sugar: 5-12%
Tea – micronutrients, nitrogen

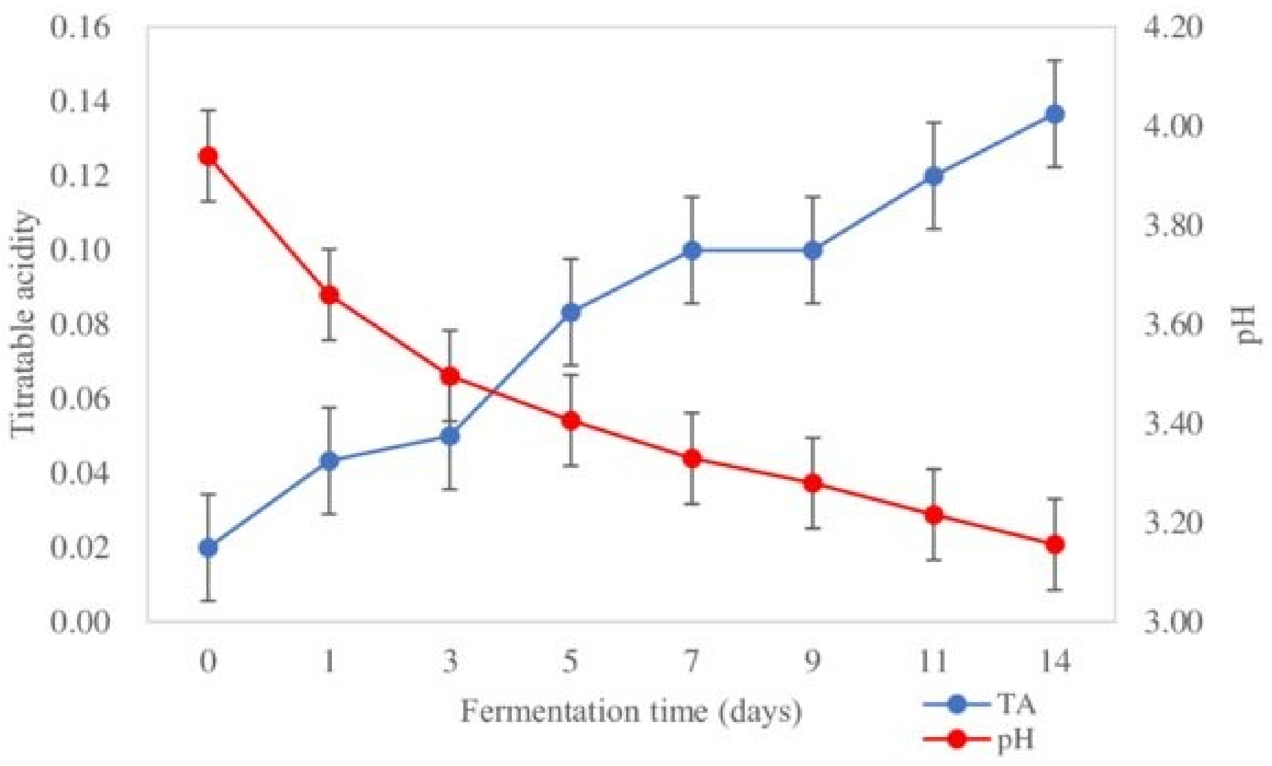
SCOBY - symbiotic culture of bacteria and yeast @ bacterial cellulose

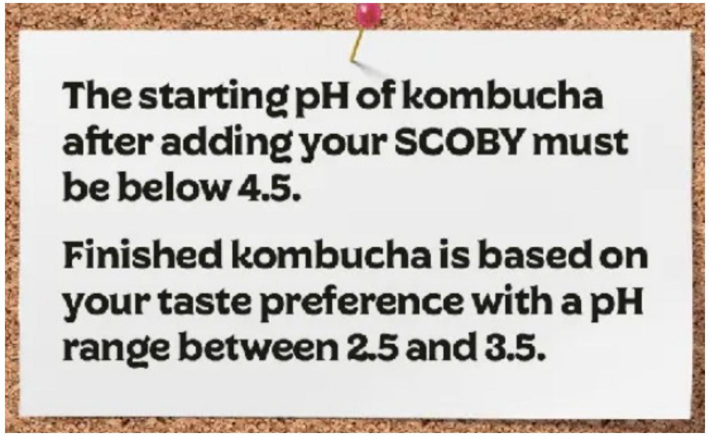
I. Sugars to Ethanol
Yeasts

II. Ethanol to Vinegar
Acetobacter & some Lactobacillus

SCOBY mainly from sugar - @ Komagataeibacter

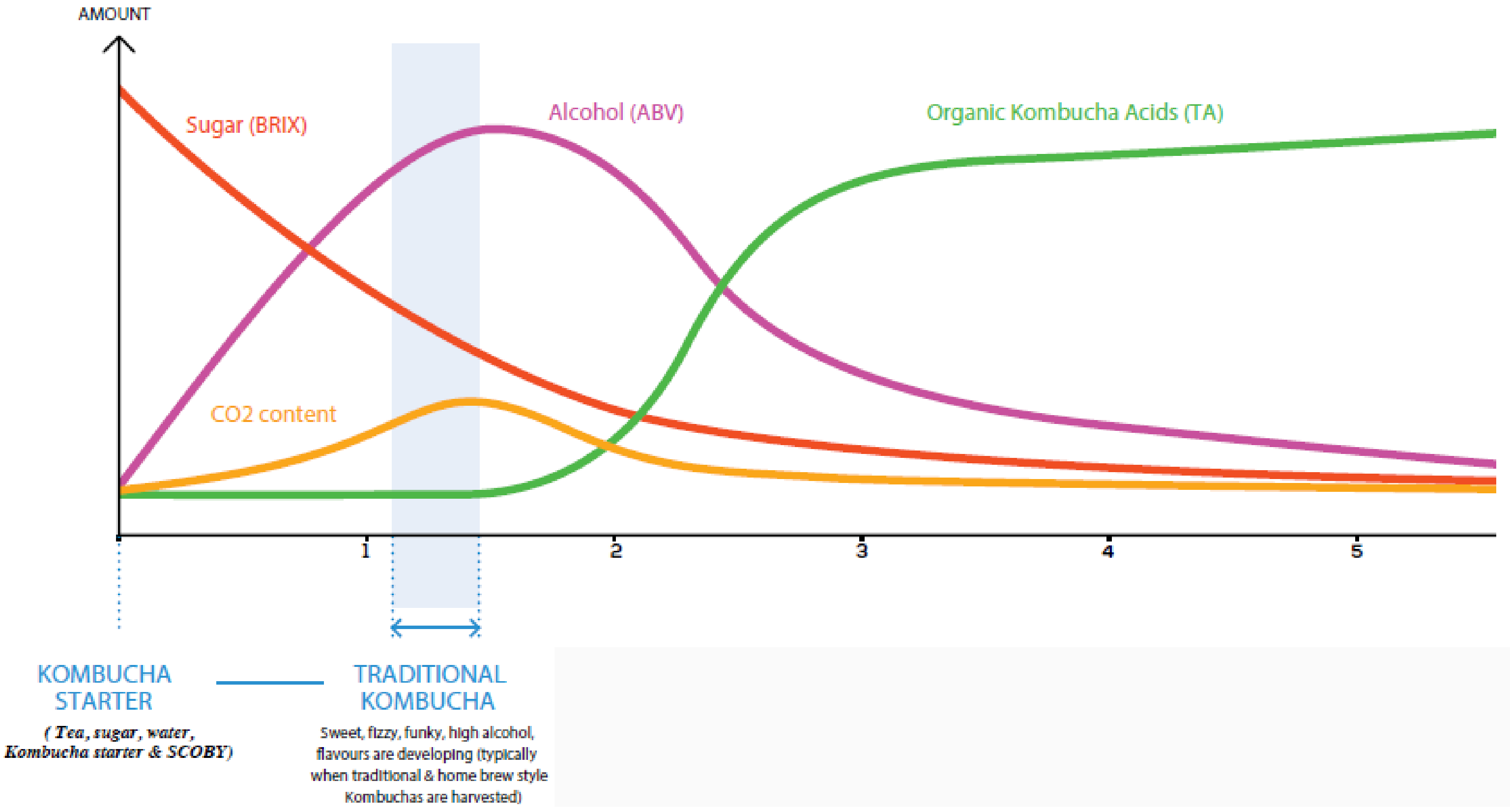
SCOBY layers:
up: Komagatei; AAB (air!)
low: yeasts
LIQ:
yeasts > AAB





Processes will stop: pH ~ 2
Use glass / inox 304/316

Sugar > 1% can remain IF
- AAB activity >> yeast activity
  - @ T low
  - @ O2 high



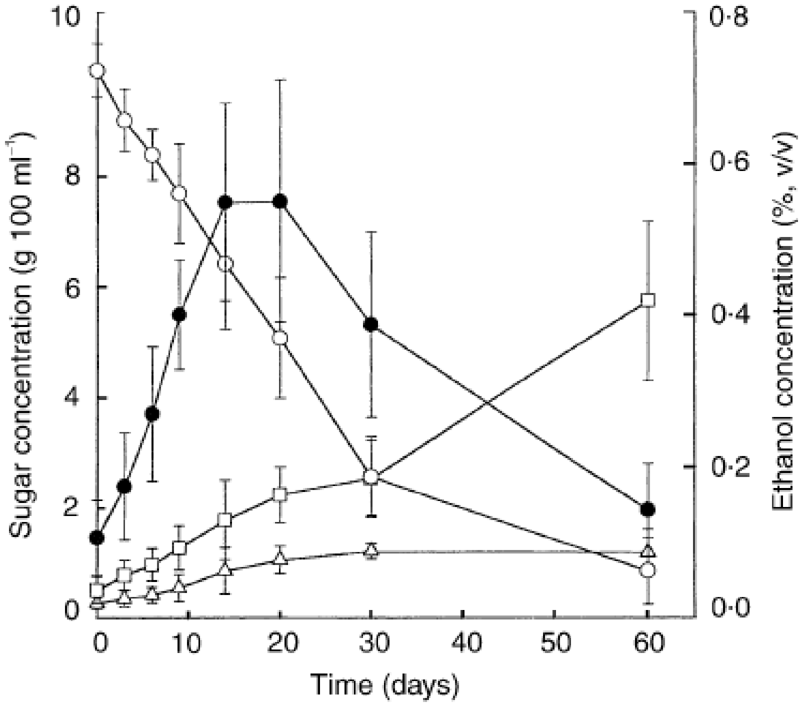


Fig. 1 Changes in residual sucrose, glucose, fructose and ethanol concentrations in tea fungus metabolites during prolonged fermentation. Data presented are the averages of nine samples of tea fungus from different sources, and error bars represent the standard deviations from nine samples. (○), Sucrose; (△), glucose; (□), fructose; (●) ethanol

Yeast, Komagatibacter: invertase
split saccharose
fructose used after glucose...

Recipe and protocol

Brix cca 5-12% (TEA 10g/L + sugar / sterile fruit juice)
Add SCOBY+liquid cca 10% (set pH to cca 4.5)

Aeritaed fermentation - textile
20-24 oC for cca 7-10 days, regular pH (3-3.5) and BRIX check!

"Second fermentation" – filter – may add 10% fruit juice – 2-3d @ 10oC STRONG glass

kombucha growth. The best carbon sources for growth of the nascent kombucha pellicles were sucrose, glucose, and fructose. On maltose, galactose, and lactose, not only did the kombucha pellicles grow poorly but 25% were also contaminated by common airborne molds. Good growth of the kombucha cultures was correlated with low pH of the fermented tea. Antibacterial effects

An unexpected advantage

Aflatoxin B_1 degradation by microorganisms isolated from Kombucha culture

Fadia Ben Taheur [a,*], Chalbia Mansour [a], Khawla Ben Jeddou [b], Yasmine Machreki [a], Bochra Kouidhi [c], Jawaher Abdualbaqi Abdulhakim [c], Kamel Chaieb [a,d]

AFB1 degrading yeasts (in 7d)

- Pichia occidentalis ~59%
- Hanseniaspora opuntiae → ~39%
- Candida sorboxylosa → ~37%

Water-kefir

- Lactic acid bacteria
- Yeast
- Acetic acid bacteria

Fast growth: 3 days << kombucha

Dextrane grains: SCOBY

LAB → exopolysaccharide matrix @ kefir grain

Liquorilactobacillus hordei

LAB → real probiotic

Compare @ Sauerkraut:

liquid, yeast and L. hordei needed, no salt

Compare @ Kefir

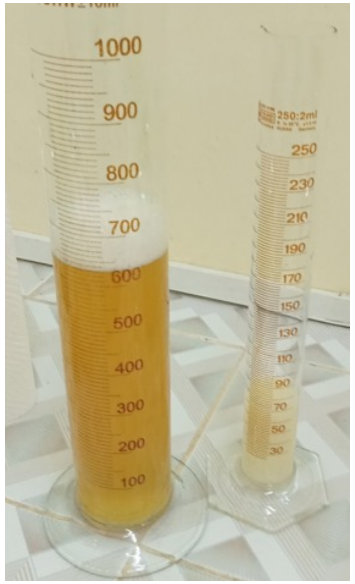
grains: kefiran

Combinations

Ginger, kurkuma, chamomile
anti-inflammatory

Sauerkraut
Water-kefir
LAB
anti-inflammatory

(Ordinary fruits)
hedonic



Cytokines
small signaling proteins
by T-cells & macrophages
start, amplify, or stop inflammation

Pro-inflammatory:
TNF-α
IL-1β
IL-6

Anti-inflammatory agent:
Reduce infl. cytokine production
Block inflammatory gene activation

Bacterial Cellulose

Nata de coco
1949 by Teódula Kalaw África,
National Coconut Corporation

coconut water @ Philippines

Gel from sugar, oxic polymerization
Komagataeibacter nataicola

AcAc bacs need ethanol
Komagataeibacter spp. do NOT need ethanol

Nata de pina (18th century)
Nata de tofu/soya

Biochemical pathways

Komagataeibacter (Gluconacetobacter) species
PQQ (pirroloquinolin-qiinon) coenzyme

$$\text{Glucose} \xrightarrow{\text{PQQ–glucose dehydrogenase}} \text{Gluconic acid} + \text{electrons}$$

Oxidation: Electrons directly @respiratory chain

50-70%

$$\text{Glucose} \rightarrow \text{Glucose-6-P} \rightarrow \text{Glucose-1-P} \rightarrow \text{UDP-glucose} \rightarrow \text{Cellulose}$$

10-30%

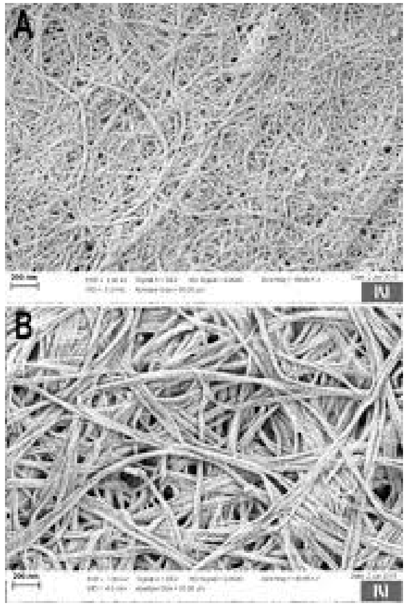
Cellulose synthesis costs energy

Cellulose synthesis rate

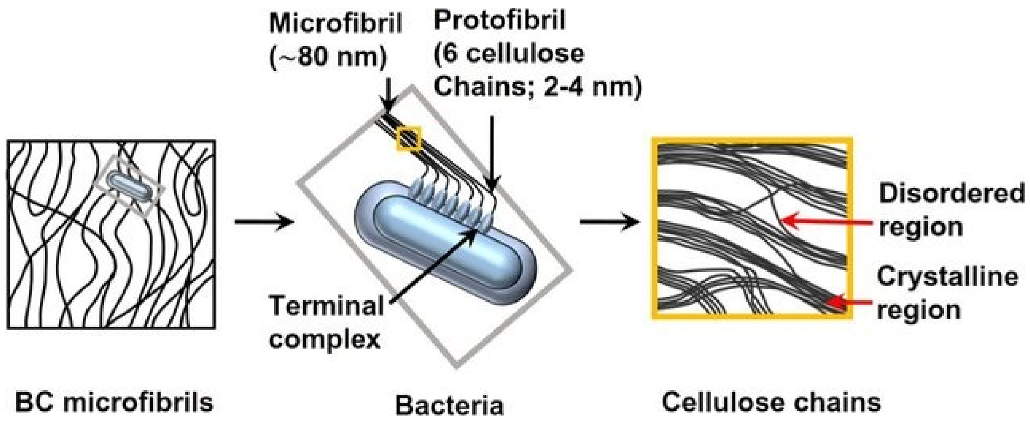
limited by UDP-glucose flux,
not by glucose availability alone

oxygen availability strongly affects

Cellulose biofilm: “mother vinegar” IF there is remanent sugar – SCOBY – NATA - AciKoji

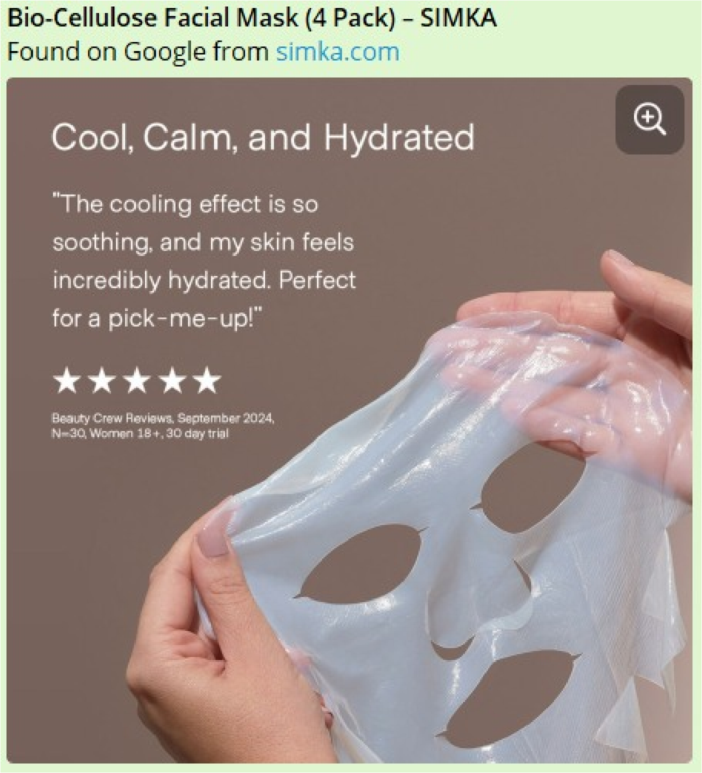


Komagataeibacter spp.



Crystallinity

Ben Tre "Coconut Town"



Culinary

coconut water 1L
sugar 100g
lemon juice cca 20 mL
eventually
squeezed coconut milk
150g meat + 200 mL
water

set pH to 4
boil
cool in flat trays (2-3cm depth)
<u>add the starter > 10%</u>
cover with paper
9-14 days @ 25-30oC

when done, wash (even longer)
flavoring
sweetening: boil in sugar syrup

https://grokipedia.com/page/Nata_de_pi%C3%B1a

https://www.youtube.com/watch?v=xEA-9NEgT7w

Bioplastic

Growth speed

Textile/leather-industry performance parameters

tensile strength (σ=F/A, MPa)
flexibility (bending stiffness)
abrasion resistance
Coating → water resistance

Biocompatible
Biodegrading

Chemical crosslinking

citric acid -COOH and cellulose -OH
esther bound @ 150 oC

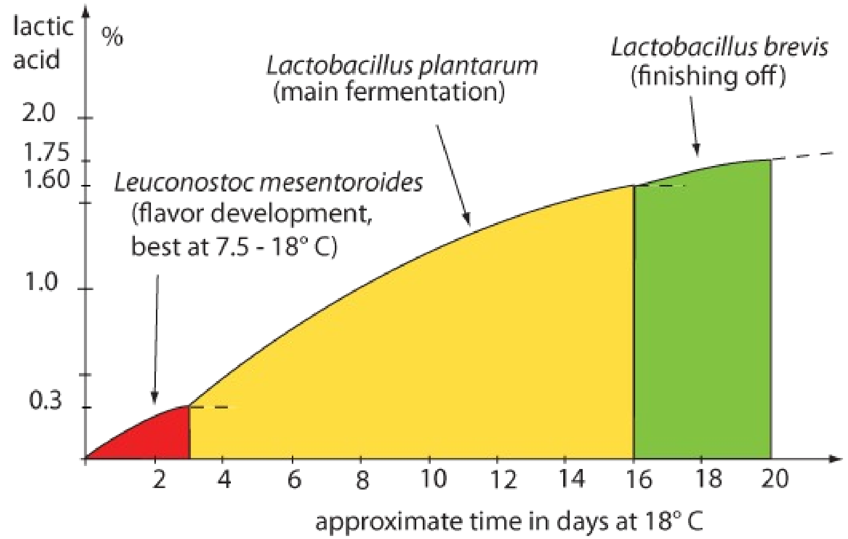
dialdehydes
/toxicity/

Plasticizer

PEG-variants

Sauerkraut

2-3% salt, mix, cover:
osomosis => submerged
ANOXIC



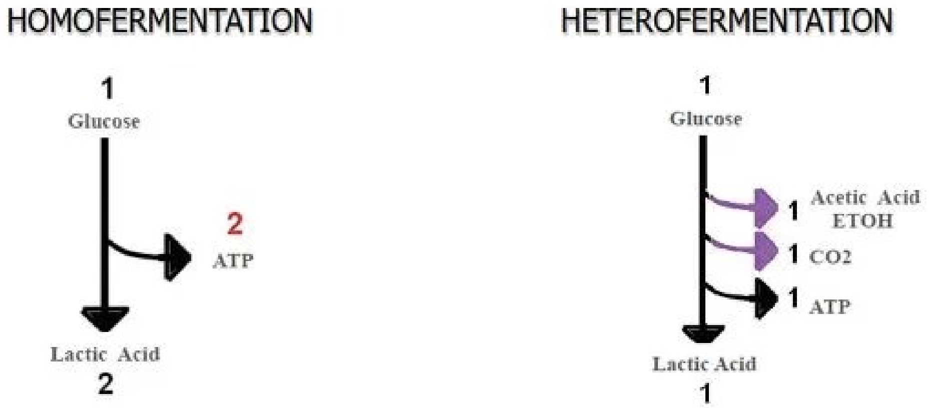
Two-stage fermentation
Day 0: Diverse microbiota (Pseudomonadales, Enterobacteriaceae)

I. Heterofermentative LAB d1-3
Leuconostoc, Weissella => lactic / acetic acid, CO_2, EtOH

II. Homolactic stage d3→
Lactobacillus => mainly lactic acid

Other byproducts: mannitol and sulfur compounds

Nutrients: glucose, fructose
cabbage 2-4%



Kimchi and Sauerkraut

Aspect	Kimchi	Sauerkraut
Salting method	Brine soak (wet salting), then rinse	Dry salt mixed directly with cabbage
Salt purpose	Wilt cabbage, then removed	Draws out moisture, stays in final product
Seasoning	Complex paste: chili, garlic, ginger, fish sauce, etc.	Salt only
Submersion requirement	Not strictly necessary; paste coating excludes oxygen	Must be completely submerged under brine

Metabolic outputs:

Output	Lactobacillus	Leuconostoc	Weissella
Lactic acid	High	Moderate	Moderate
CO_2	Low	High	High
Acetic acid	Low	Moderate	Moderate
Mannitol	Rare	Common	Common
Flavor	Sharp sour	Mild, fruity	Mild, buttery

Stress tolerance:

Stress condition	Lactobacillus	Leuconostoc	Weissella
Low pH (<4.0)	Excellent	Moderate	Lower
High salt	Good	Moderate	Lower
Cold (≤10°C)	Moderate	Excellent	Excellent

	Kimchi	Sauerkraut
Ingredients	Cabbage + salt + garlic, chili, ginger	Cabbage + salt only
Fermentation	2-stage, II. LAB-dominance Some conds. II. Lecunostoc	2-stage, II. LAB-dominance
Flavor evolution	Complex, spicy, umami	Clean, sour, cabbage-forward

Not PRO?

The impact of regular sauerkraut consumption on the human gut microbiota: a crossover intervention trial

Nelly Schropp[1], Alexander Bauer[1], Virginie Stanislas[1], Kun D. Huang[2], Till-Robin Lesker[2], Agata Anna Bielecka[2], Till Strowig[2,3] and Karin B. Michels[1*]

Results Using shotgun metagenomic sequencing, we observed changes in single bacterial species following fresh and pasteurized sauerkraut consumption. More pronounced changes were observed in the pasteurized sauerkraut intervention arm. Only pasteurized sauerkraut consumption increased serum short-chain fatty acids (SCFAs).

Conclusions The gut microbiome of healthy individuals is rather resilient to short-term dietary interventions even

investigated SCFAs produced via effects on gut bacteria

The fermented cabbage metabolome and its protection against cytokine-induced intestinal barrier disruption of Caco-2 monolayers

Lei Wei,[1] Maria L. Marco[1]

Because only partial protection against intestinal barrier disruption was found when individual metabolites (D-PLA, ILA, and lactate) were applied, the findings indicate that the complex mixture of metabolites in a cabbage fermentation offers advantages over single metabolites to benefit intestinal health.

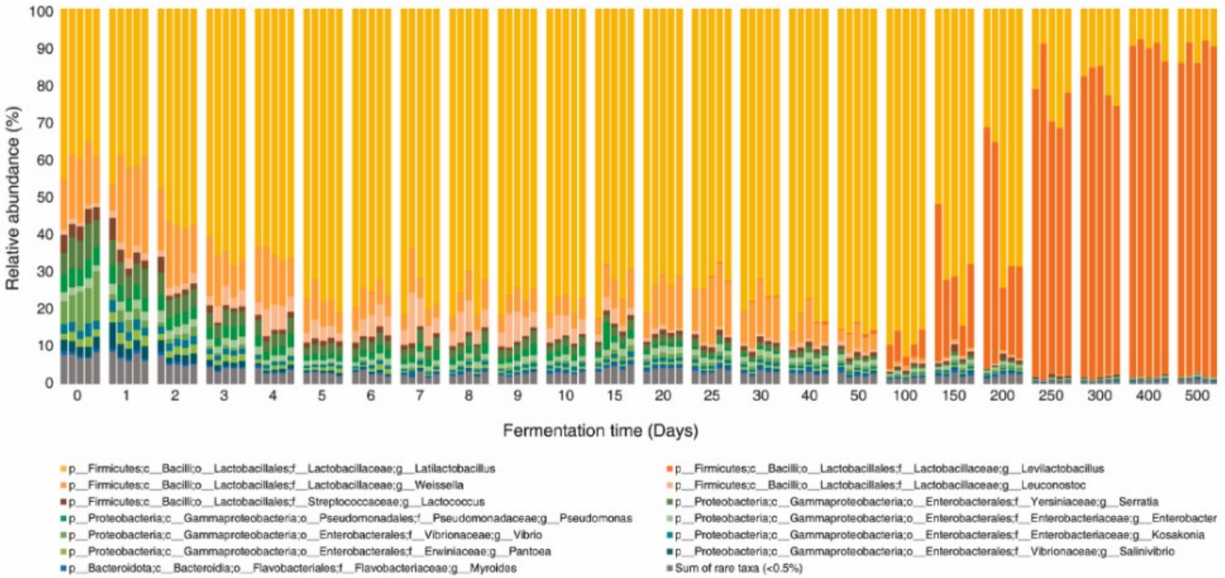
protecting intestinal barrier function from inflammatory stress.

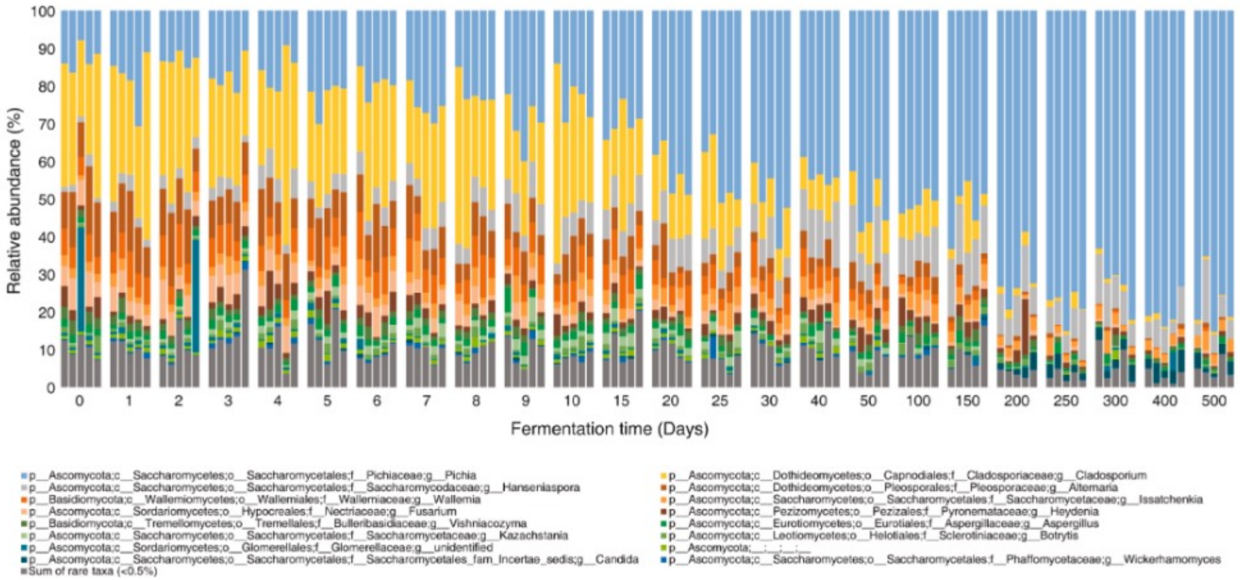
Long-term, kimchi

Long-term population dynamics of viable microbes in a closed ecosystem of fermented vegetables

Joon Yong Kim[a,c,1], Seong-Eun Park[b,1], Eun-Ju Kim[b], Seung-Ho Seo[d], Tae Woong Whon[a], Kwang-Moon Cho[e], Sun Jae Kwon[e], Seong Woon Roh[a,c,*], Hong-Seok Son[b,*]

[a] *Microbiology and Functionality Research Group, World Institute of Kimchi, Gwangju 61755, Republic of Korea*





Feature	Cladosporium	Pichia	Hanseniaspora
Metabolism	Aerobic, oxidative	Respiratory/weak fermentation	Fermentative
Polysaccharides degraded	Cellulose, pectin, xylan, starch	Starch (limited), pectin (some)	Pectin (weak)
pH tolerance	pH 3-9 (wide)	pH 2.5-8 (wide)	pH 3-6 (narrow)
Alcohol tolerance	N/A	8-12%	4-8%
Organic acids degraded	Citric, malic, lactic	Citric, lactic, acetic	Limited/none
Stress tolerance	**Highest**	**Moderate-High**	**Lowest**

Leuconostoc and Weissella @ early,
Latilactobacillus and Levilactobacillus @ middle and late
Cladosporium @ early,
Pichia and Hanseniaspora @ the middle and late

>half of the metabolites after 500 days of fermentation produced @ the first 15 days

Less known about: phagome

Exploring viral diversity in fermented vegetables through viral metagenomics

Julia Cantuti Gendre [a], Claire Le Marrec [b], Stéphane Chaillou [c], Lysiane Omhover-Fougy [d], Sophie Landaud [a], Eric Dugat-Bony [a,*]

fermented vegetables harbor complex viral communities
vary depending on the vegetable used

influence of microbial succession during fermentation
potential impact on the quality of the final product

Industrial disasters – sudden
“dieouts”

Fermentation advantages

Improves digestion:

breaks sugars & proteins → less work for gut enzymes

reduces raffinose, lactose → less bloating

↓ anti-nutrients: Phytate => availability of Ca^{2+}, Fe^{2+}, Mg^{2+}

↑ bioactive compounds: cleave glycans from polyphenols

Synthesis of B-vitamins and vitamin K_2

↓ pH (organic acids) + competitive exclusion → pathogen inhibition

May reduce pro-inflammatory cytokines

Gut microbiota modulation ?

Digression: Gut microbiome

Group	Phylum	Gram	Oxygen	Plant fiber breakdown	Mucin breakdown	Main role
Firmicutes ~40–60%	Firmicutes	G+	Anaerobic / aerotolerant	❌ primary / ✅ secondary	❌	SCFA (especially butyrate), secondary fermentation
Bacteroidetes ~20–50%	Bacteroidetes	G–	Obligate anaerobic	✅ **primary**	❌	Initial degradation of dietary fiber
Proteobacteria	Proteobacteria	G–	Aerobic / facultative	❌	❌	Stress response, rapid growth, dysbiosis marker
Akkermansia muciniphila	Verrucomicrobia	G–	Obligate anaerobic	❌	✅ yes	Mucin turnover, gut barrier support

Bacs @ fibers
→ short-chain fatty acids => colon cell energy, ↓infl. cytokines, insuline sens.
→ itamins (K, B-group)

↓ pathogens
Influences:: metabolism, immune system, appetite, inflammation, mood
DIVERSITY!

Digression: Hydrogels

Polymer network type:
cross-linking type - ionic, helical, or physical

Water-holding capacity:
mesh size, hydrophilicity

Rheology:
entropic elasticity, G, E, yield stress (“strength”, “stiffness”, “damping”)
viscoelasticity

Mechanical behavior:
“chewable”, “parizer”, brittle

Processing stability:
heat (kT) and pH (electrostatic)

Transport phenomena:
diffusion, mesh size, polymer interactions: flavor release

Lead properties

Polymer

protein
polysaccharide
X vinyl polymers [−CH2−CH(R)−]n R is -OH in PVA
X acrylic polymers Vinyl backbone & carboxylic acid

Crosslinking

H-bonding	general:: X–H ··· N or O **also @ thickeners**
Helix zones (with H-bonds):	agar(2), gelatin(3)
Ionic:	alginate, LM-pectin
Covalent	proteins :: disulfide, TG-made
Hydrophobic:	proteins:: egg, soy

Heat

thermo-rev	agar, gelatin, HM-pectin, ...
thermo-irrev	Ca-alginate, formed starch, soy, egg

pH may count!

Common Food Hydrogels

Gel	Key chemistry	Best use	Heat (<130 °C)	pH stability
Tapioca starch	Entropic starch network	Chewy noodles, pearls	✓	wide range
Gluten dumpling	Protein crosslink network	Boiled dumplings, pasta	✓	acid-sensitive
Rice paper	Starch–fiber matrix	Wrapping, rolling	✓	wide range
Gelatin	Physical protein gel	Cold desserts, gummies	❌	acid-sensitive +
Agar	Ordered polysaccharide	Cuttable gels, cubes	❌	acid-stable
κ-Carrageenan	Ion-stabilized helix gel	Sliceable dairy, meats	❌	acid-sensitive
ι-Carrageenan	Ionic elastic gel	Elastic dairy desserts	❌	acid-sensitive +
Alginate	Ca^{2+} crosslinked gel	Spherification	✓	acid-sensitive +
Pectin (HM)	Sugar–acid (HM)	Soft fruit gels, jams	✓	low pH only
Soy protein (tofu)	Heat-set protein gel	Tofu, protein curds	✓	acid-sensitive

Water in hydrogels

1. Free (bulk-like) water

Similar to pure water

High mobility, weak interaction w polymer

Crystallizes to ice

2. Strongly bound water

Hydrogen bonding or ionic hydration

Low mobility

Amorphous @ freezing

- ✗ No true melting point
- ✗ Zero (no latent heat)

Thanks for your attention!