

Modulation of Rumen Microbiome and Enteric Methane Reduction by SCOBY-based Probiotics: From *In Vitro* Fermentation to TMF Application

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Part 1 - The Global Challenge

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Enteric methane is the single largest agricultural greenhouse gas.

Warming Potency

28_x

Global warming potential of CH₄ relative to CO₂ over a 100-year horizon.

Van Amstel, 2012 - IPCC AR6

Livestock Share

~40%

of global agricultural methane comes from enteric fermentation in ruminants.

Scopes, 2023 - FAO

Energy Loss

2-12%

of gross energy intake is lost as methane — an economic penalty for the animal.

Lovendahl et al., 2018

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The Global Methane Challenge

South Korea's 2050 carbon-neutrality roadmap places livestock methane in the spotlight.



Industry Imperative

Mitigation strategies that integrate into existing TMR-based feeding systems – without disrupting farm operations – are now national priorities.

In the rumen, hydrogen is the currency – and methanogens cash it in.

Rumen Fermentation Cascade



"Methanogens are not feeding on fiber – they harvest the H₂ that microbes throw away. If we redirect that H₂, methane goes with it."

Key Microbial Actors

Rumen H₂ economy

H₂ PRODUCERS

- Ruminococcus albus*
- R. flavifaciens*
- Butyrivibrio fibrisolvens*

H₂ CONSUMERS (spp.)

- Methanobrevibacter ruminantium*
- M. gottschalkii*, *M. smithii*,
- M. olleyae*, *M. millerae*

Existing mitigation strategies hit a wall. Biological solutions remain underdeveloped.

Strategy	Mechanism	Efficacy	Limitation	Status
3-NOP (Bovaer)	MCR enzyme inhibitor	-30% CH ₄	Cost · regulation	Approved EU/US
Asparagopsis (seaweed)	Bromoform halomethanes	-50 to -80%	Bromoform toxicity · supply	Pilot only
Plant secondary metabolites	Tannins, saponins, EOs	-10 to -25%	Variable · palatability	Mixed evidence
Direct-fed microbials (LAB)	Shift fermentation pathway	-5 to -15%	Weak rumen colonization	Established
GSM-designed Synbiotic ★ This study	H ₂ sink redirection + LAB shift	-70.5% (in vitro, 10 h)	In vivo validation pending	Novel


We turned to *Kombucha*.

A robust, self-sustaining microbial consortium — built by nature, not engineered.

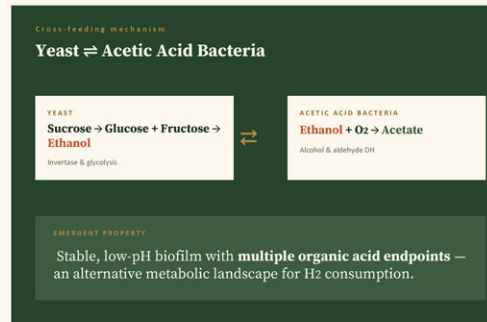
Acronym

SCOBY

Symbiotic Culture Of Bacteria and Yeast

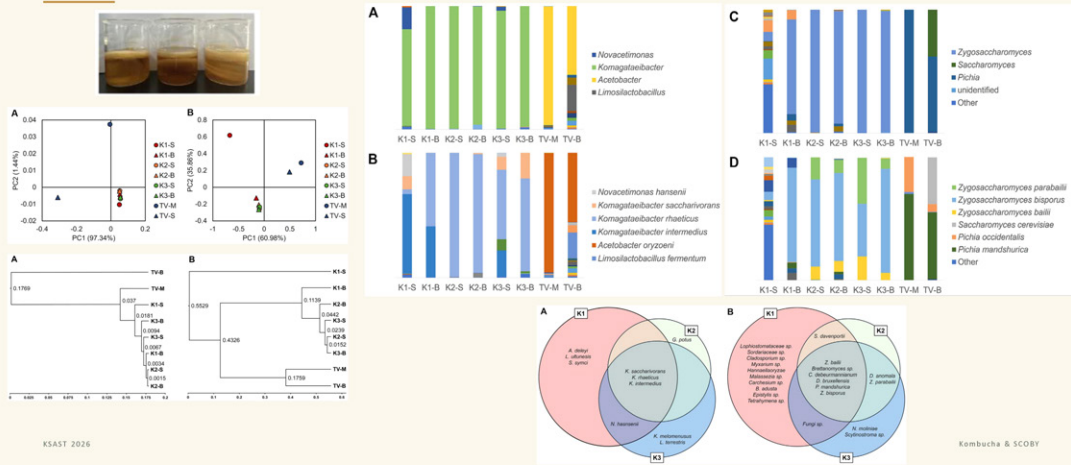
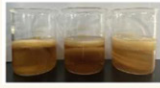


Naturally tolerant to **low pH, ethanol, and organic acids** — the same stresses that destroy conventional probiotics in the rumen. A self-renewing microbial mat.



Kombucha microbial community

A robust, self-sustaining microbial consortium — built by nature, not engineered.



Of seven isolates, two strains dominate. We call them the **KZ consortium**.

Bacterium - K

Komagataeibacter intermedius

IDENTITY
99.86% match · NR_026435.1

FUNCTIONAL TRAITS

- Obligate aerobe — high oxygen scavenging capacity
- Cellulose-producing — structural biofilm matrix
- Ethanol → acetate conversion (high efficiency)
- Stress-tolerant: low pH, organic acid environments

Yeast - Z

Zygosaccharomyces parvii

IDENTITY
99.82% match · CP019500.1

FUNCTIONAL TRAITS

- Lactic-acid resistant — survives acidified niches
- Broad carbohydrate utilization (glucose, fructose, sucrose)
- Produces ethanol + glycerol as fermentation endpoints
- Versatile redox balancer — key for H₂ sinks

Hypothesis

KZ fortified with *precision prebiotics* will redirect rumen H₂ into alternative endpoints — starving methanogens of their substrate.

Conceptual model

Rerouting hydrogen flux

DEFAULT — CH₄ EMITTING

Hydrogenotrophic methanogens monopolize the only thermodynamically favorable H₂ sink.

SYMBIOTIC — CH₄ SUPPRESSED

Reducing equivalents diverted into organic acids & alcohols — H₂ sinks that bypass methanogenesis.

Three nested objectives.

From in-silico modeling to feed-system application.

01

In-silico

Identify KZ-exclusive prebiotics via GSM modeling

Construct genome-scale metabolic models for 10 representative rumen + SCOBY species (3 H₂-producers, 5 methanogens, 2 SCOBY). Identify substrates utilized exclusively by KZ.

02

In vitro

Validate synbiotic efficacy on rumen methanogenesis

Co-culture KZ with selected prebiotics. Quantify CH₄ and CO₂ via GC over a 30-hour batch rumen-fluid fermentation assay.

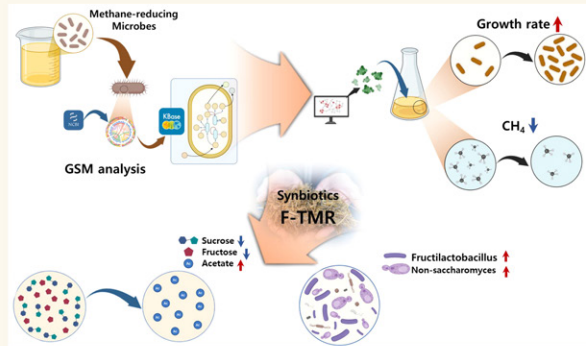
03

Application

Apply synbiotics to FTMR & assess fermentation quality

Supplement TMR at 5% and 10% (v/w). Track pH, metabolites (HPLC), and 16S+ITS microbiome shifts across 14 d anaerobic fermentation.

Experimental workflow at a glance



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Four-stage pipeline

STAGE 1 - ISOLATION
Isolate & identify SCOBY microbes
 16S rRNA + ITS - MMS/YPD/PDA selective media

STAGE 2 - IN-SILICO
GSM modeling on KBase
 Flux Balance Analysis - anaerobic / complete media

STAGE 3 - IN VITRO
Rumen-fluid CH₄ assay (30 h)
 Fistulated Holstein - GC quantification at 10/20/30 h

STAGE 4 - APPLICATION
FTMR pilot (14 d, 5% & 10%)
 pH - HPLC - 16S + ITS amplicon sequencing

Experimental Workflow

Genome-scale metabolic modeling across 10 species and 3 functional groups.



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GSM Modeling - KBase

The full mix (Car + AA + Vit) significantly boosted KZ growth — 23% over control.

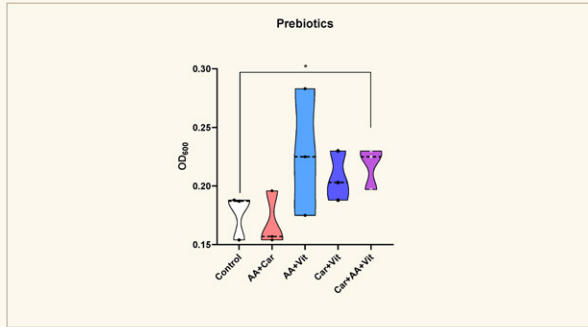


Figure 2. OD₆₀₀ of KZ cultures after 24 h static incubation (25 °C). Treatments: AA+Car, AA+Vit, Car+Vit, Car+AA+Vit. * $p < 0.05$ (one-tailed unpaired t test, $n = 3$).

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Growth Comparison - OD ₆₀₀	
Control (no prebiotic)	0.176 ± 0.022
AA + Car	0.185 ± 0.015
AA + Vit	0.213 ± 0.041
Car + Vit	0.207 ± 0.022
Car + AA + Vit *	0.217 ± 0.020

Significantly higher vs. Control ($p < 0.05$)

Combining all three prebiotic categories is necessary to maximize KZ proliferation in vitro.

Figure 2 - Growth Validation

In vitro CH₄ dropped 70.5% at 10 h.

Full synbiotic mix vs. untreated rumen-fluid control.

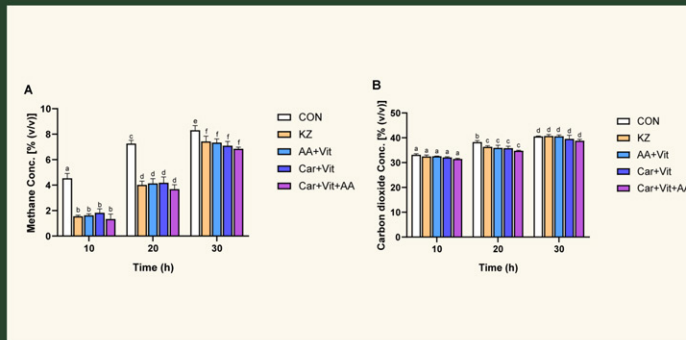


Figure 3. CH₄ (A) and CO₂ (B) concentrations during in vitro rumen-fluid fermentation. GC sampling at 10, 20, 30 h. Mean ± SD, $n = 3$.

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Methane — 10 h timepoint

-70.5%

vs. control (Car + AA + Vit synbiotic)

Co-effect on CO₂

↓ Significant

All synbiotic groups suppressed CO₂ production simultaneously ($p < 0.05$), confirming downregulation of overall fermentative flux.

Figure 3 - CH₄ Reduction

Synbiotic supplementation at 5% and 10% across a 14-day FTMR pilot.

Table 1 - Diet Composition

Chemical composition of FTMR (% DM)

Items	CON	5% Mix	10% Mix
Crude protein	9.79	9.71	10.16
Crude fat	1.63	1.55	1.75
Crude fiber	10.33	10.71	9.87
Crude ash	5.23	5.14	5.17
NDF	22.19	24.20	24.46
ADF	11.36	11.67	10.93

CON = untreated control. Mix = combination of Gly-Leu, Gly-Ala-L, glycerol, oleate, menaquinone-7, & K2

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Pilot Protocol

14-day anaerobic fermentation



CONDITIONS

Vacuum-sealed bags · 25 °C · anaerobic
Treatments: CON · 5% Mix · 10% Mix
TriPLICATE per timepoint (n = 3)

FTMR Pilot Design

Synbiotic groups moderated acidification and shifted the metabolite profile toward acetate & ethanol.

Table 5 - pH dynamics

Acidification, slowed

Days	CON	5% Mix	10% Mix
Day 0	5.20a	5.11b	5.02c
Day 7	4.86a	4.75ab	4.92b
Day 14	4.66a	4.76b	4.72ab

Means with different superscripts differ significantly (p < 0.05). Higher final pH = more stable, less over-acidified silage matrix, matrix.

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Figure 4 - Metabolite Profile

HPLC across 14 d

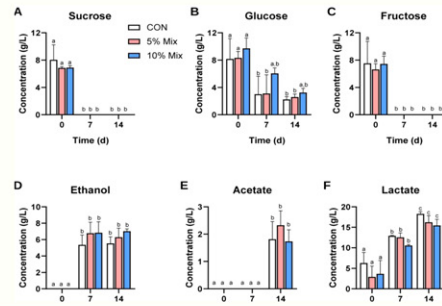


Table 5 + Figure 4 - pH & HPLC

The 10% Mix preserved microbial diversity – while the control collapsed to a few dominant taxa.

Table 6 - Shannon Index α -diversity, day 14

Days	CON	5% Mix	10% Mix
Day 0	6.13	6.27	6.49
Day 7	2.04	2.38	3.24
Day 14	2.68	3.08	3.15

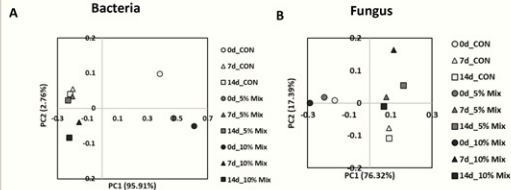
INSIGHT

All groups lose diversity during fermentation, but 10% Mix retains 50% more species evenness than CON at day 14.

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Figure 6 - PCoA β -diversity

Distinct trajectories per treatment

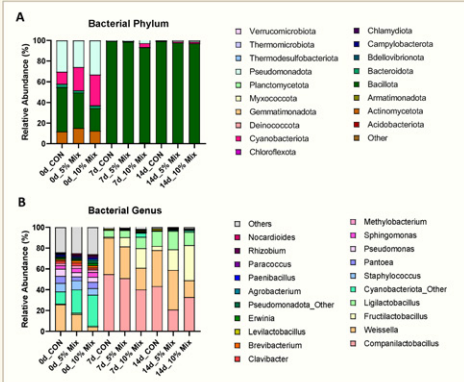


Bacteria (A): PC1 95.91% – fermentation time dominates.

Fungi (B): 10% Mix clusters apart by day 14 – treatment-specific niche.

α -diversity + β -diversity

Synbiotic enriches *Fructilactobacillus* & diversifies fungi toward non-*Saccharomyces* yeasts.



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BACTERIAL PHYLUM (A)

Bacillota (Firmicutes) → dominant in all groups by day 7

10% Mix starts with elevated *Cyanobacteriota* (29.9%) & *Pseudomonadota* (32.9%); converges to Bacillota-dominated post-fermentation.

BACTERIAL GENUS (B)

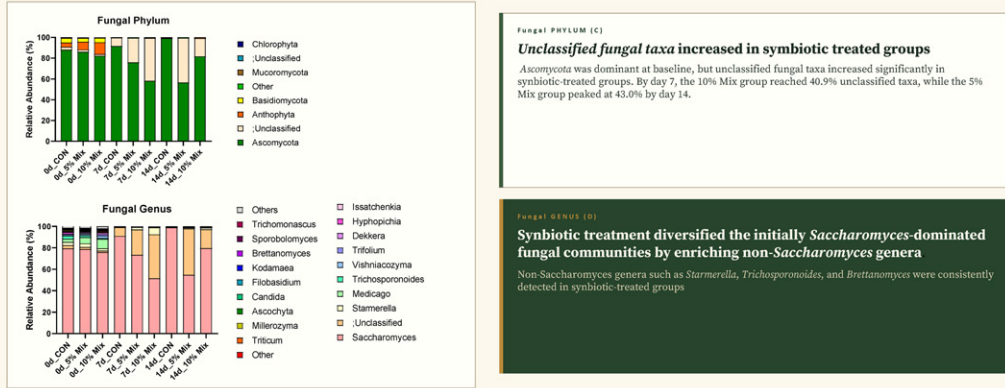
***Fructilactobacillus* takes over by day 14 in 10% Mix**

Before fermentation: *Weissella*, *Staphylococcus*, *Pantoea*, *Pseudomonas*.

After: marked succession to LAB – *Companilactobacillus*, *Levilactobacillus*, *Ligilactobacillus*.

Figure 7 - Taxonomy Shifts

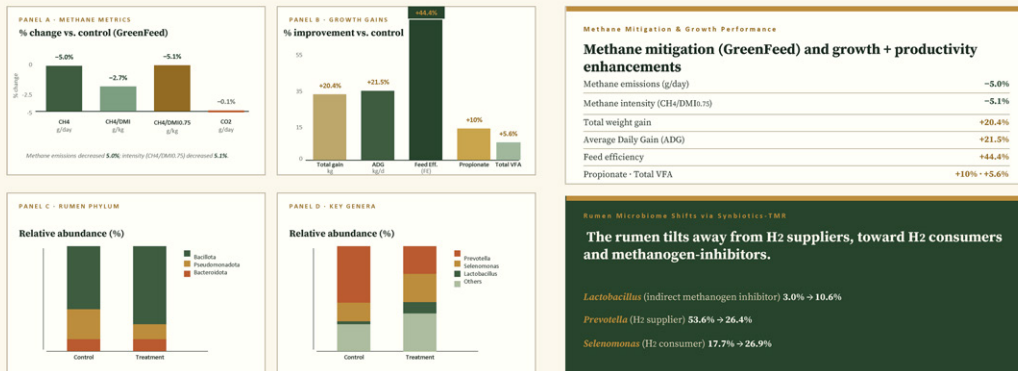
Synbiotic enriches *Fructilactobacillus* & diversifies fungi toward **non-Saccharomyces** yeasts.



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Figure 7 - Taxonomy Shifts

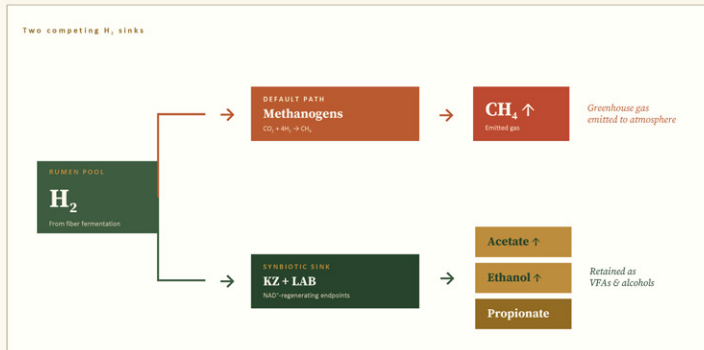
Synbiotics-TMR reduced enteric CH₄ & improved **growth & feed efficiency** in Hanwoo.



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In Vivo Hanwoo Trial - GreenFeed + 165

Why does it work? Hydrogen is rerouted.



Thermodynamic Principle

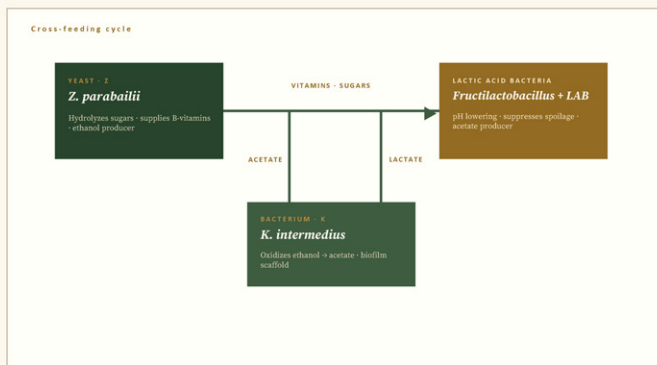
Reducing equivalents go where the sink is open.

KZ + co-cultivated LAB generate NAD⁺-regenerating endpoints (acetate, ethanol) that recycle H₂ without producing CH₄.

With this alternative sink available, hydrogenotrophic methanogens are substrate-limited — CH₄ drops accordingly.

"We are not killing methanogens. We are **outbidding** them for hydrogen."

Syntrophic cross-feeding stabilizes the entire fermentation ecosystem.



Emergent Outcomes

- 01 Stable pH (4.7+)**
Prevents over-acidification spoilage
- 02 LAB enrichment**
Outcompetes spoilage organisms
- 03 Yeast diversification**
Functional redundancy & resilience
- 04 H₂ sink expansion**
Suppresses methanogenic niche

Precision microbiome design, from *silicon* to *silage*.

CONCLUSION 01

GSM modeling delivers precision prebiotic design.

15 KZ-exclusive metabolites identified in silico; 5 carried forward into a functional synbiotic.

CONCLUSION 02

In vitro: -70.5% CH₄ at 10 h.

Significant suppression of both CH₄ and CO₂ in rumen-fluid fermentation – reproducible across triplicates.

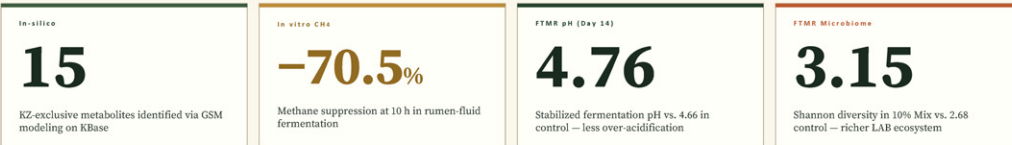
CONCLUSION 03

FTMR microbiome shifted toward LAB & non-Sacc. yeasts.

Higher pH stability, increased acetate/ethanol – *Pracilactobacillus* dominance by day 14.

From silicon to silage to live cattle – one slide.

TRACK 1 - DESIGN & IN-VITRO PROOF



TRACK 2 - IN-VIVO HANWOO TRIAL



GSM modeling → SCOBY synbiotic → in vitro 70.5% CH₄ ↓ → FTMR microbiome shift → in vivo Hanwoo: methane ↓ + feed efficiency ↑ – a complete bench-to-barn pipeline.

Q&A

Questions, challenges, collaborations.

Thank you for your attention. The floor is yours.



Continue the conversation

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