동물마이크로바이옴연구회 심포지엄
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2026 Annual International Conference of KSAST

“축산 70년의 비젼과 도약:
인간, 동물, 환경이 함께 걷는
지속가능한 축산 혁신”

Vision and Leap Forward of Livestock's 70 Years:
Sustainable Innovation for Humans,
Animals, and the Environment

제주국제컨벤션 센터 (ICC JEJU)
2026년 7월 8일(수)~10일(금)

- 주최/주관 : (사)한국축산학회,(사)한국가금학회, 농림축산식품부,국립축산과학원, 축산관련단체 연합회
- 후원기관 : 한국과학기술단체총연합회, 제주특별자치도
- 후원업체 : 대한한돈협회, (사)전국한우협회, 한우자조금관리위원회, 서울우유협동조합, (주)우성사료, (주)이지홀딩스, (주)카길애그리퓨리나, BASF, 노보네시스 코리아, (주)모닝바이오, 우유자조금관리위원회, 제일사료 축산기술연구소, (주)팜스코, De Heus Korea, 농협경제지주, 낙농진흥회, 농축산용미생물산업육성지원센터, (주)다운, (주)선진, (주)솔로몬, 우리와(주), 유진바이오, 축산환경관리원, (주)파이지노믹스, (사)한국단미사료협회, (주)휘드베스트, 김유용 교수, 체리부로, (주)ACC, NH순한한우조합공동사업법인, 다산데이타, (주)마크로젠, (주)미래생명자원, 성우라이프사이언스, 에스엠티정보기술(주), (주)인프로, 정농바이오, (주)진바이오텍, 포스코리아, 한양사료주식회사, 한국반추미생물연구회, 달사랑 연구회, 고려비엔피, 나우피드, 대한사료(주), (주)대호, (주)도드람양돈서비스, (주)린도, 마리동물의학연구소, (주)솔톤바이오켐, 임실치즈&식품연구소, (주)티엔티리써치, 한국종축개량협회, 한국유가공협회, 전국낙농관련조합장협의회, 농심, 매일유업

(사) 한국축산학회
Korean Society of Animal Science and Technology

동물마이크로바이옴연구회 심포지엄

행사개요

- 주제: 안전하고 지속가능한 동물생산을 위한 반추위 마이크로바이옴 혁신 전략
- 일시 및 장소: 2026.7.9.(목) 09:30~11:30, 제주국제컨벤션 센터(ICC JEJU), 삼다홀 B

세부일정

9:00 ~ 9:30	Registration	
연사 및 제목		
Chairperson: Prof. Sejong Oh (Chonnam National University)		
09:30 ~ 10:00	Prof. Jong Nam Kim (Dongseo University)	Modulation of Rumen Microbiome and Enteric Methane Reduction by SCOBY-based Probiotics: From *In Vitro* Fermentation to TMF Application
10:00 ~ 10:30	Prof. Myunghoo Kim (Seoul National University)	Technologies to Address Climate Change and Improve the Health of Ruminants by Utilizing the Microbiome and Metabolome
10:30 ~ 11:00	Prof. Min-Jin Kwak (Kookmin University)	Characterization of Core Microbiota and Methane-Related Functional Genes associated with Methane Emissions and Growth Performance in Cattle
11:00 ~ 11:30	Prof. Younghoon Kim (Seoul National University)	Rumen-Derived Next Generation Probiotics for Sustainable Bioplastic Production and Microplastic Toxicity Mitigation

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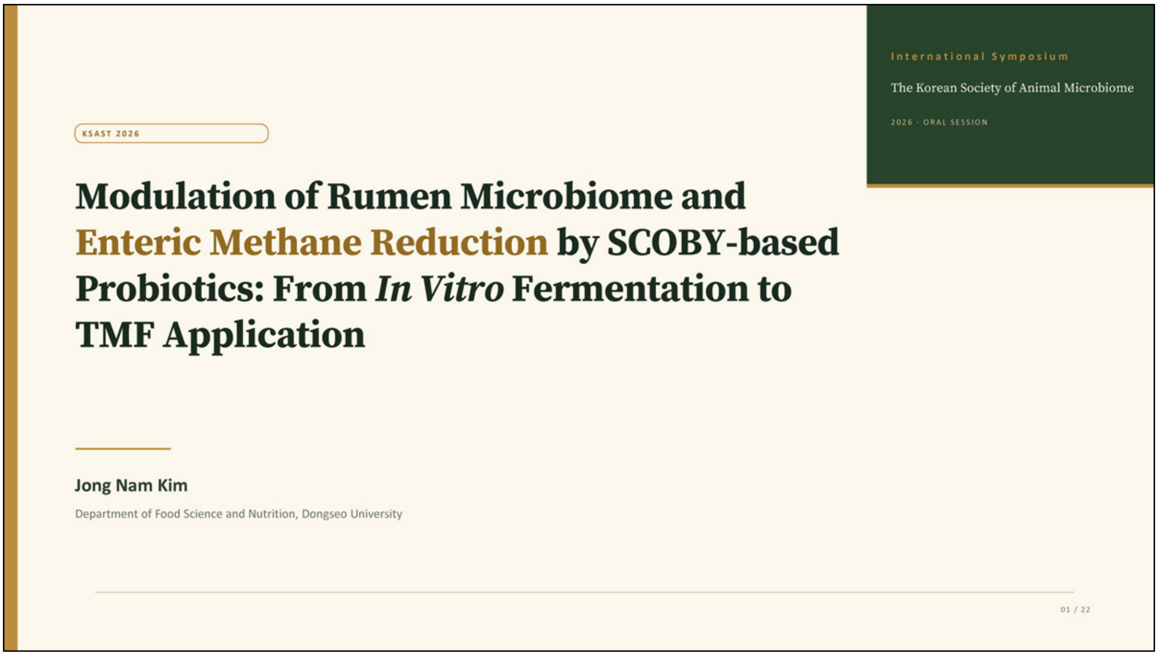
Modulation of Rumen Microbiome and Enteric Methane Reduction by SCOBY-based Probiotics: From *In Vitro* Fermentation to TMF Application

김 종 남
Kim, Jong Nam
(동서대학교)
(Dongseo University)

Curriculum Vitae

2019~현재	동서대학교 식품영양학과 부교수
2016~2019	한국농수산대학 한우학과 조교수
2016	CJ제일제당 생물자원연구소 팀장
2014~2015	이지바이오 생물자원연구소 팀장
2013~2014	충남대학교 농업과학연구소 박사후연구원
2010~2013	US FDA National Center for Toxicological Research 박사후연구원
2005~2010	일리노이대학교 축산학과 농학박사
2002~2004	성균관대학교 식품생명자원학 이학석사
1995~2002	성균관대학교 식품생명자원학 이학사



International Symposium

The Korean Society of Animal Microbiome

2026 · ORAL SESSION

KSAST 2026

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

Jong Nam Kim

Department of Food Science and Nutrition, Dongseo University

Enteric methane is the single largest agricultural greenhouse gas.

Warming Potency

28×

Global warming potential of CH_4 relative to CO_2 over a 100-year horizon.

Van Amstel, 2012 · IPCC AR6

Livestock Share

~40%

of global agricultural methane comes from enteric fermentation in ruminants.

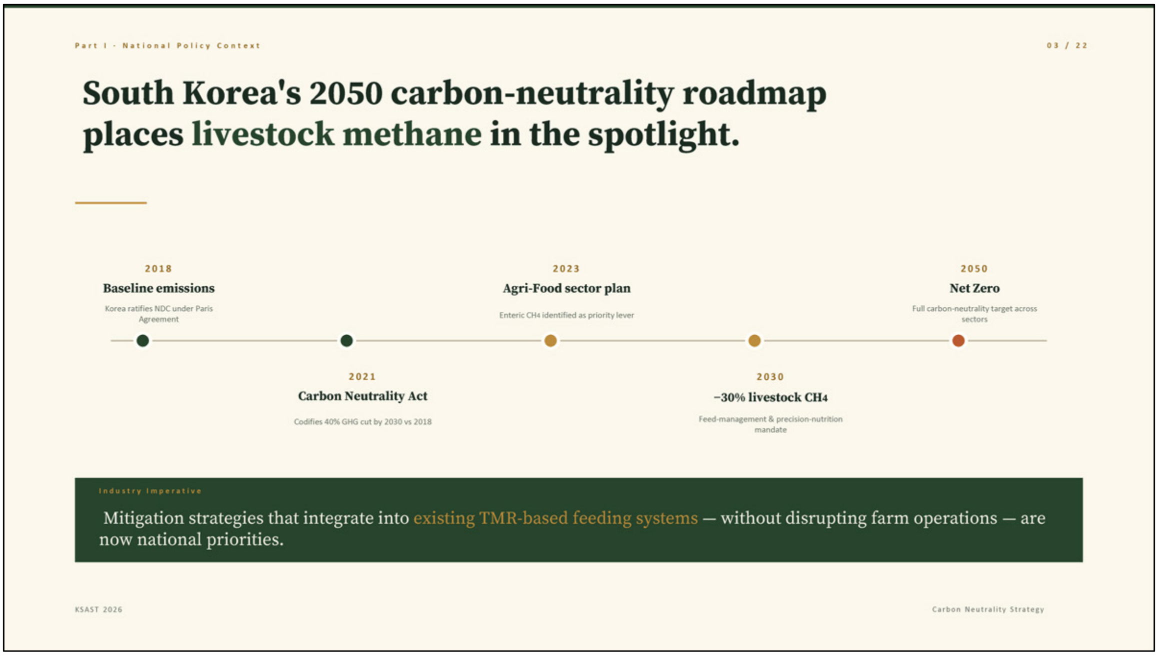
Scoones, 2023 · FAO

Energy Loss

2–12%

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

Løvendahl et al., 2018



Part I · National Policy Context

03 / 22

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

2018
Baseline emissions
Korea ratifies NDC under Paris Agreement

2021
Carbon Neutrality Act
Codifies 40% GHG cut by 2030 vs 2018

2023
Agri-Food sector plan
Enteric CH4 identified as priority lever

2030
−30% livestock CH4
Feed-management & precision-nutrition mandate

2050
Net Zero
Full carbon-neutrality target across sectors

Industry Imperative

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

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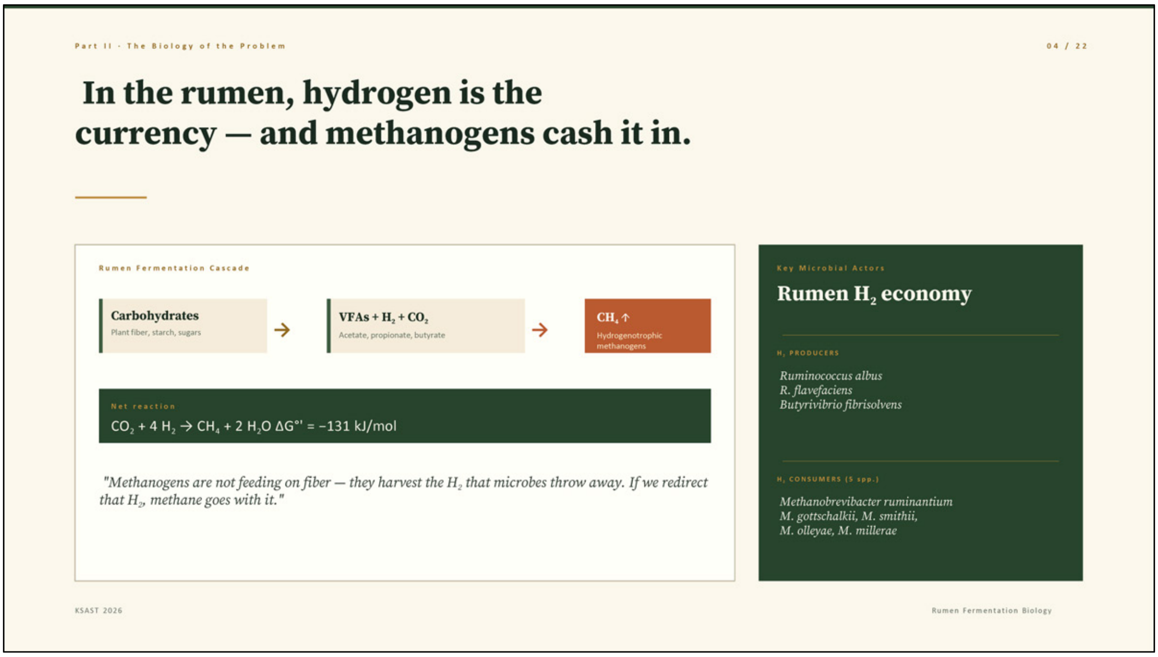
Carbon Neutrality Strategy



Part II · The Biology of the Problem

04 / 22

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

Rumen Fermentation Cascade

Carbohydrates
Plant fiber, starch, sugars

→

VFAs + H_2 + CO_2
Acetate, propionate, butyrate

→

CH_4 ↑
Hydrogenotrophic methanogens

Net reaction

$CO_2 + 4\,H_2 \rightarrow CH_4 + 2\,H_2O$ $\Delta G°' = -131$ kJ/mol

"Methanogens are not feeding on fiber — they harvest the H_2 that microbes throw away. If we redirect that H_2, methane goes with it."

Key Microbial Actors

Rumen H_2 economy

H_2 PRODUCERS

Ruminococcus albus
R. flavefaciens
Butyrivibrio fibrisolvens

H_2 CONSUMERS (5 spp.)

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

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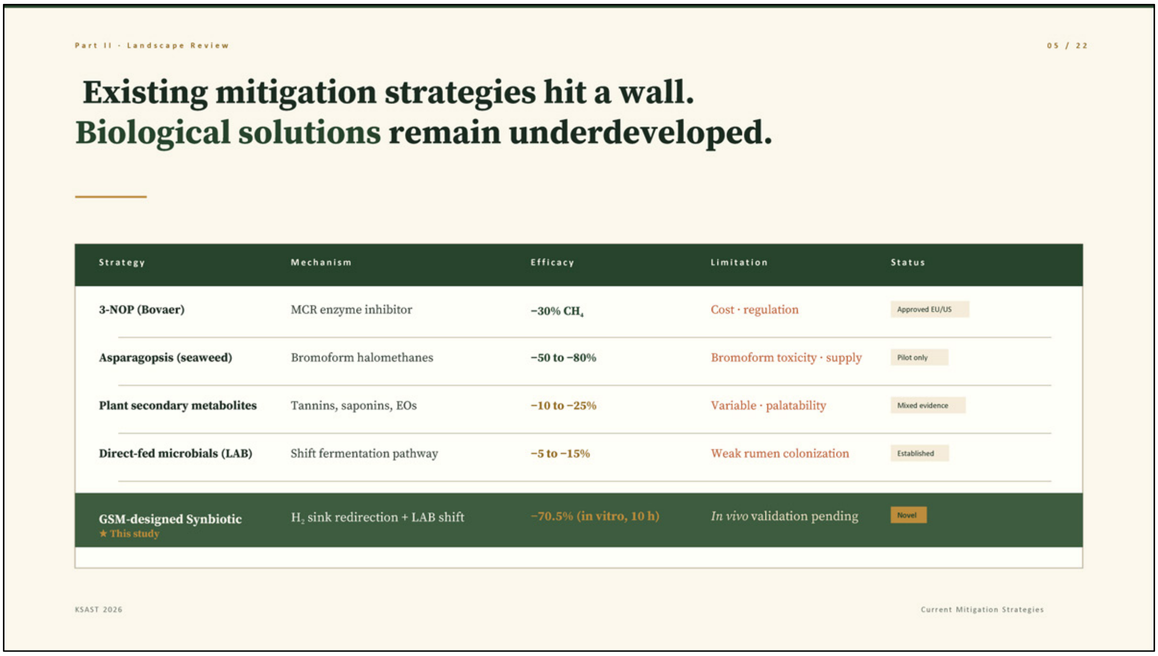
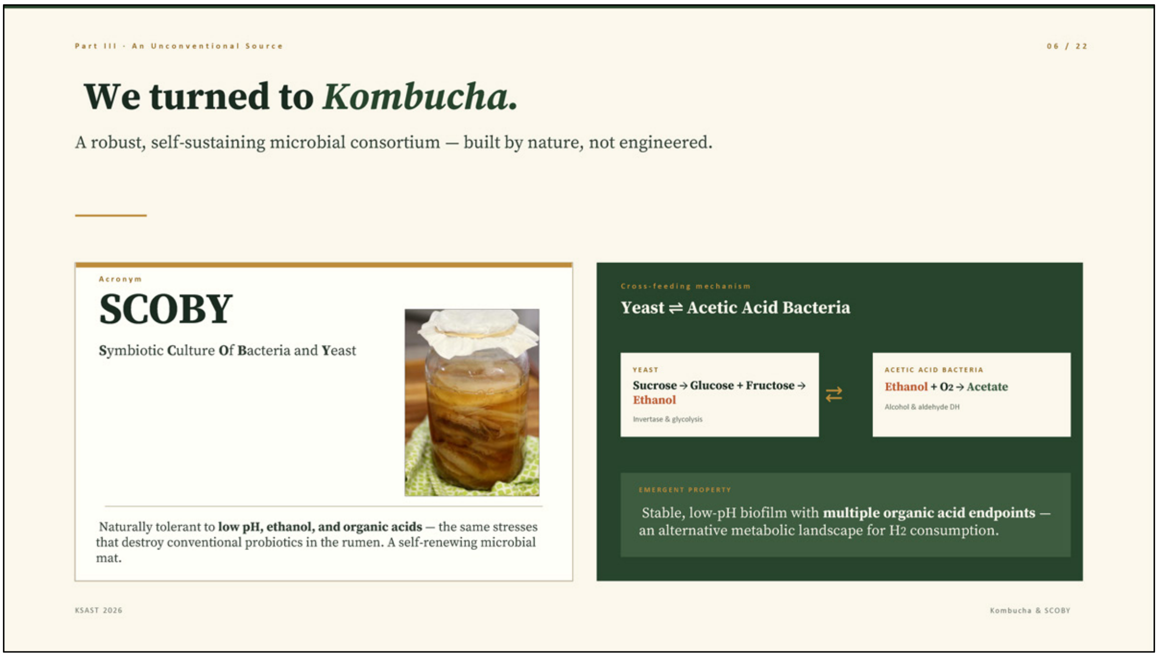
Rumen Fermentation Biology



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 **C**ulture **O**f **B**acteria and **Y**east

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

Cross-feeding mechanism

Yeast ⇌ Acetic Acid Bacteria

YEAST

Sucrose → Glucose + Fructose → Ethanol

Invertase & glycolysis

⇄

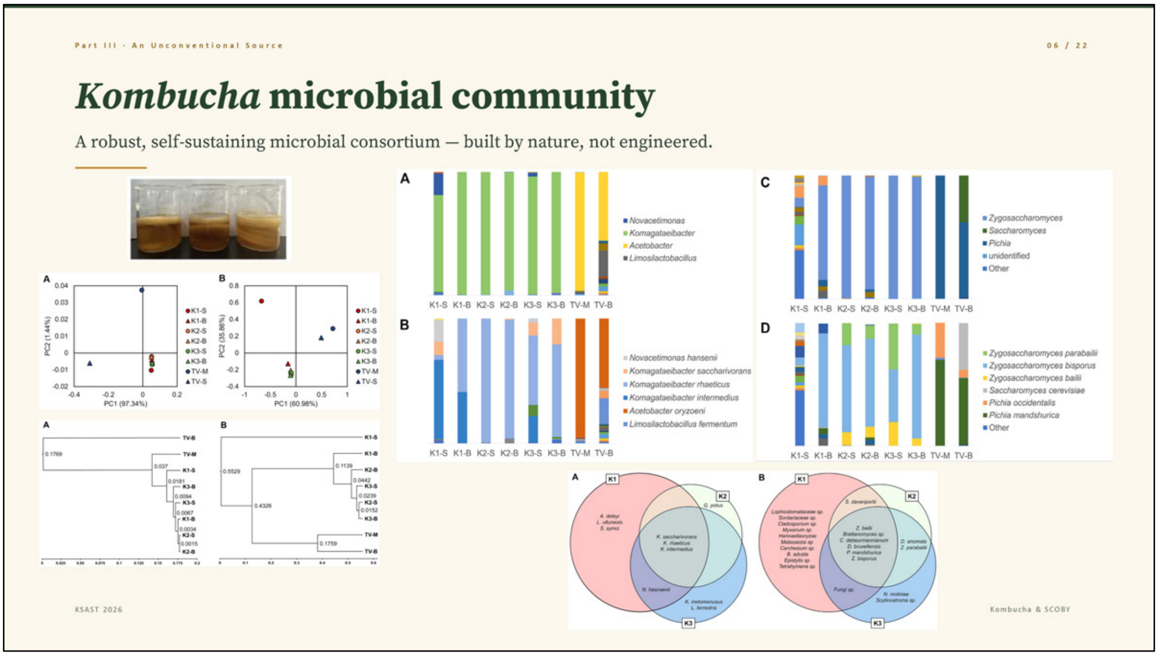
ACETIC ACID BACTERIA

Ethanol + O_2 → Acetate

Alcohol & aldehyde DH

EMERGENT PROPERTY

Stable, low-pH biofilm with **multiple organic acid endpoints** — an alternative metabolic landscape for H_2 consumption.



Part III · An Unconventional Source

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Kombucha microbial community

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

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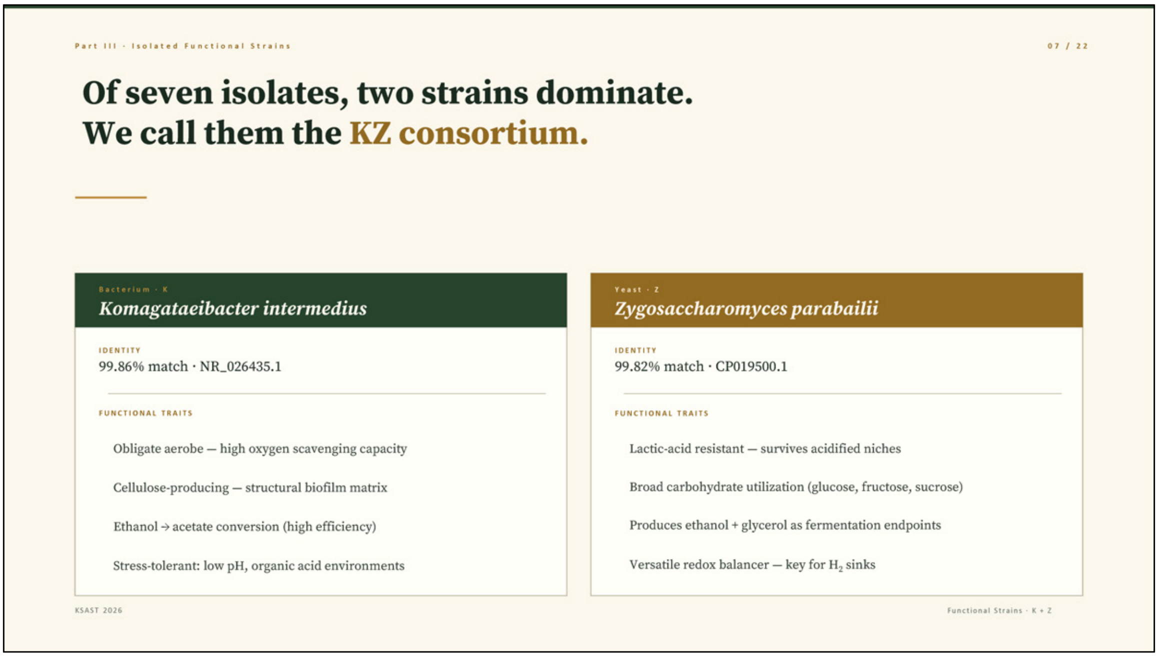
Kombucha & SCOBY



Part III · Isolated Functional Strains

07 / 22

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 parabailii

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 H_2 sinks

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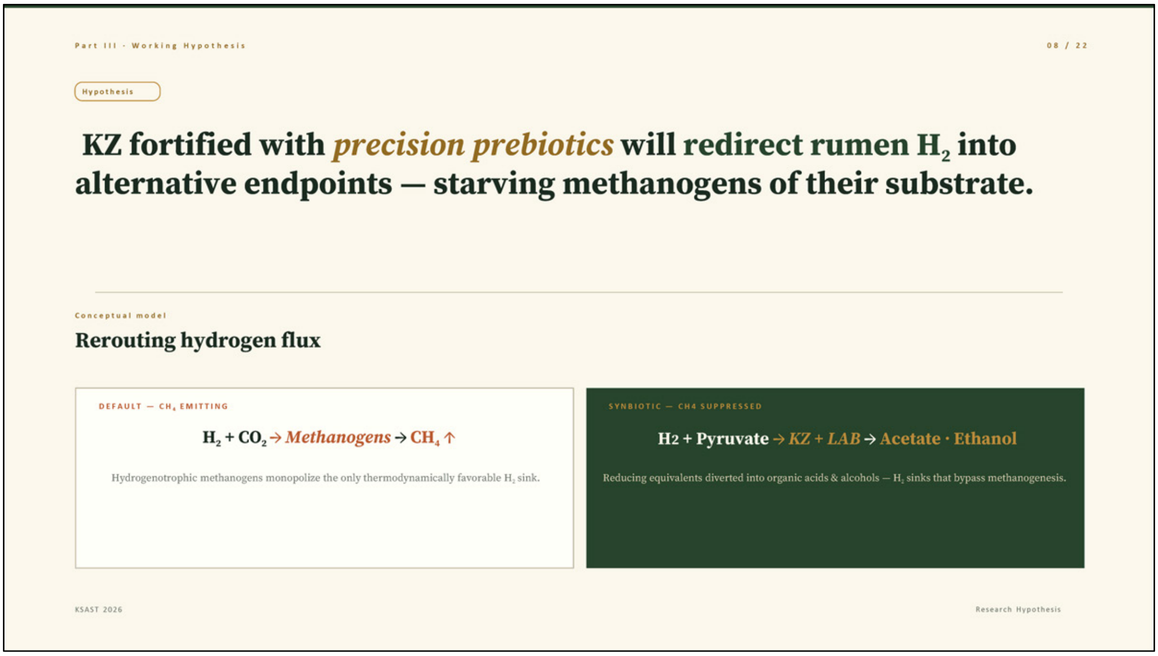
Functional Strains · K + Z



Hypothesis

KZ fortified with *precision prebiotics* will redirect rumen H_2 into alternative endpoints — starving methanogens of their substrate.

Conceptual model

Rerouting hydrogen flux

DEFAULT — CH_4 EMITTING

$H_2 + CO_2 \rightarrow$ *Methanogens* $\rightarrow CH_4 \uparrow$

Hydrogenotrophic methanogens monopolize the only thermodynamically favorable H_2 sink.

SYNBIOTIC — CH4 SUPPRESSED

H2 + Pyruvate → *KZ + LAB* → Acetate · Ethanol

Reducing equivalents diverted into organic acids & alcohols — H_2 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 H_2-producers, 5 methanogens, 2 SCOBY). Identify substrates utilized exclusively by KZ.

02

In vitro

Validate synbiotic efficacy on rumen methanogenesis

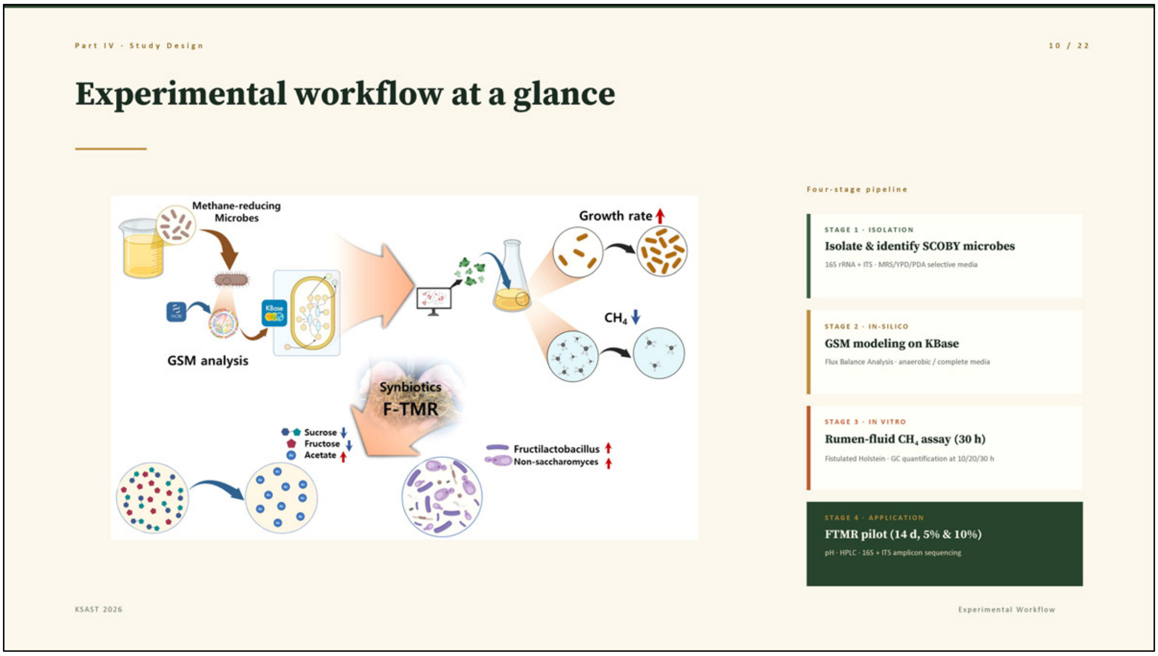
Co-culture KZ with selected prebiotics. Quantify CH4 and CO_2 via GC over a 30-hour batch rumen-fluid fermentation assay.

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

Four-stage pipeline

STAGE 1 · ISOLATION

Isolate & identify SCOBY microbes

16S rRNA + ITS · MRS/YPD/PDA selective media

STAGE 2 · IN-SILICO

GSM modeling on KBase

Flux Balance Analysis · anaerobic / complete media

STAGE 3 · IN VITRO

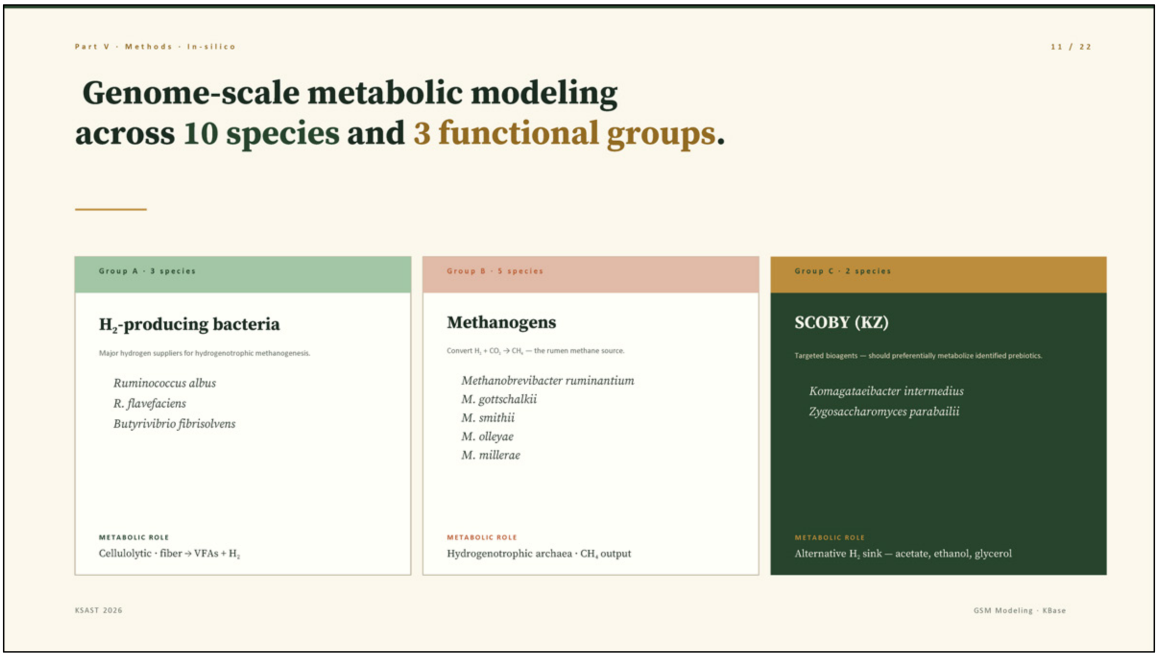
Rumen-fluid CH_4 assay (30 h)

Fistulated Holstein · GC quantification at 10/20/30 h

STAGE 4 · APPLICATION

FTMR pilot (14 d, 5% & 10%)

pH · HPLC · 16S + ITS amplicon sequencing



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

Group A · 3 species

H_2-producing bacteria

Major hydrogen suppliers for hydrogenotrophic methanogenesis.

Ruminococcus albus
R. flavefaciens
Butyrivibrio fibrisolvens

METABOLIC ROLE

Cellulolytic · fiber → VFAs + H_2

Group B · 5 species

Methanogens

Convert $H_2 + CO_2 \rightarrow CH_4$ — the rumen methane source.

Methanobrevibacter ruminantium
M. gottschalkii
M. smithii
M. olleyae
M. millerae

METABOLIC ROLE

Hydrogenotrophic archaea · CH_4 output

Group C · 2 species

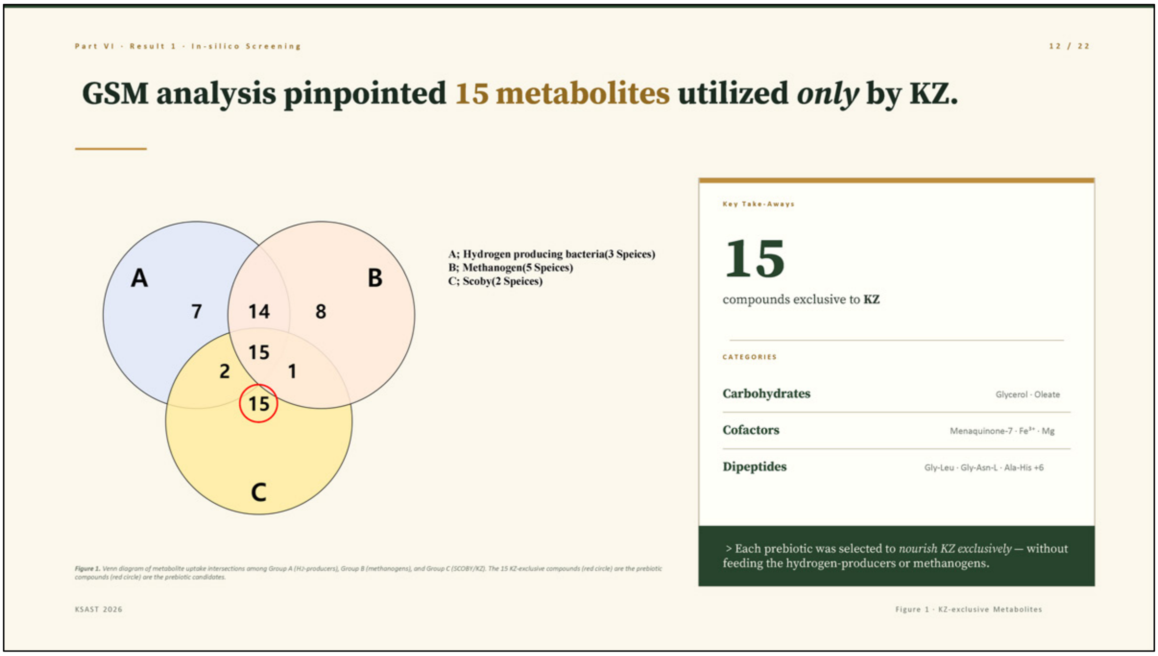
SCOBY (KZ)

Targeted bioagents — should preferentially metabolize identified prebiotics.

Komagataeibacter intermedius
Zygosaccharomyces parabailii

METABOLIC ROLE

Alternative H_2 sink — acetate, ethanol, glycerol



GSM analysis pinpointed 15 metabolites utilized *only* by KZ.

A; Hydrogen producing bacteria(3 Speices)
B; Methanogen(5 Speices)
C; Scoby(2 Speices)

Figure 1. Venn diagram of metabolite uptake intersections among Group A (H_2-producers), Group B (methanogens), and Group C (SCOBY/KZ). The 15 KZ-exclusive compounds (red circle) are the prebiotic compounds (red circle) are the prebiotic candidates.

Key Take-Aways

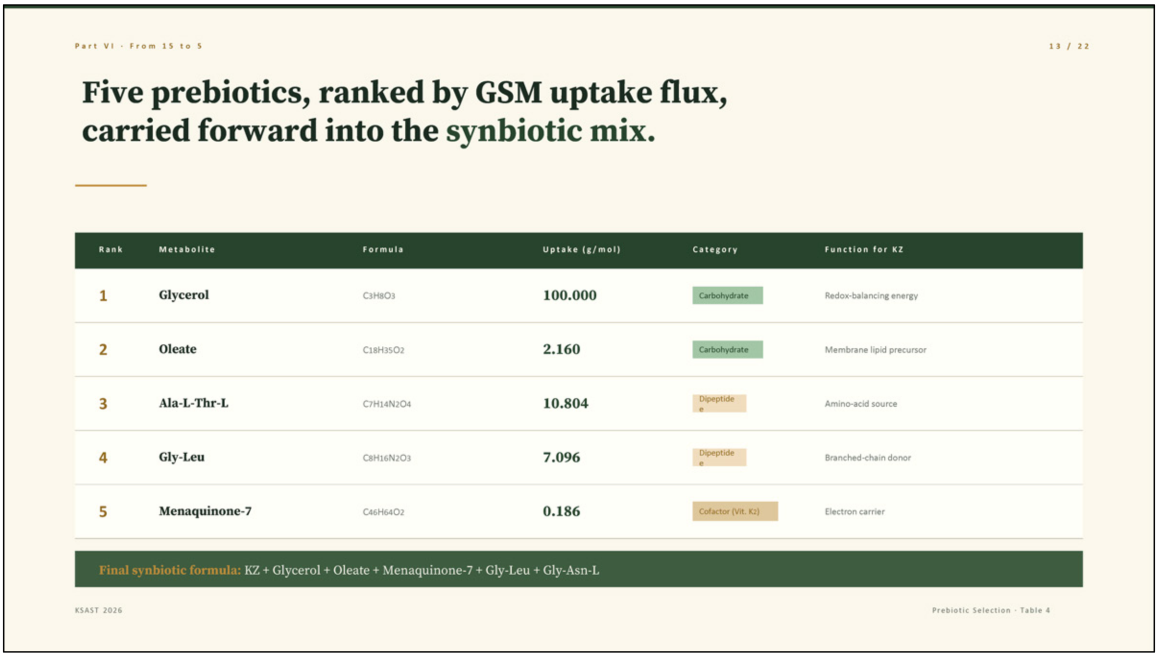
15

compounds exclusive to **KZ**

CATEGORIES

Carbohydrates	Glycerol · Oleate
Cofactors	Menaquinone-7 · Fe^{3+} · Mg
Dipeptides	Gly-Leu · Gly-Asn-L · Ala-His +6

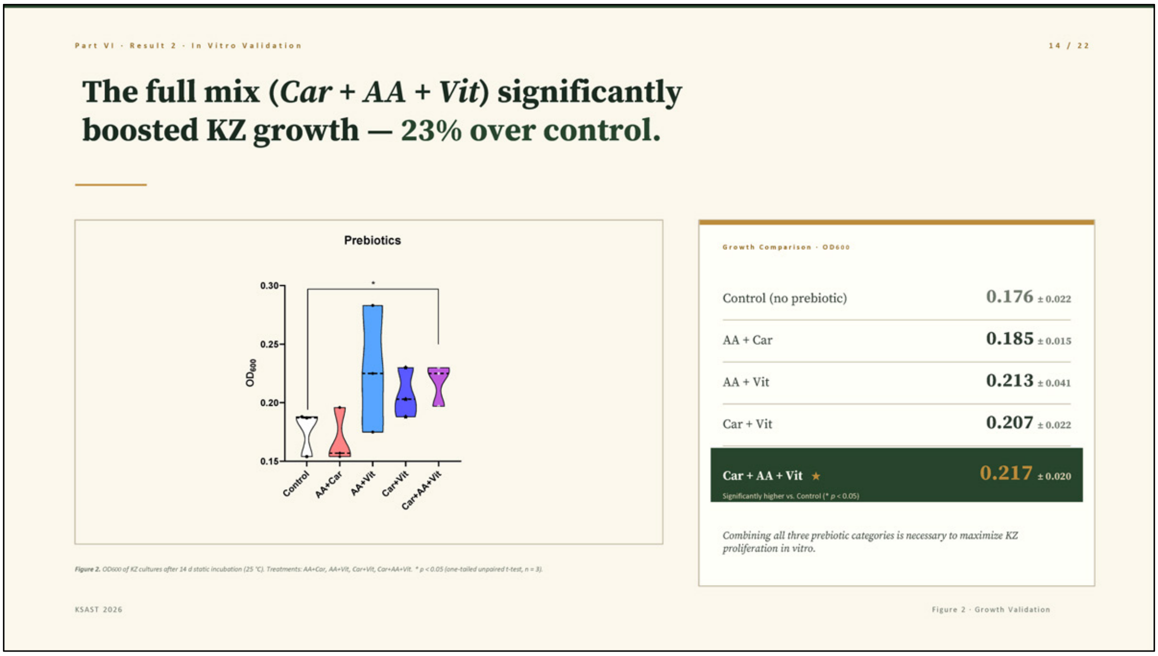
> Each prebiotic was selected to *nourish KZ exclusively* — without feeding the hydrogen-producers or methanogens.



Five prebiotics, ranked by GSM uptake flux, carried forward into the synbiotic mix.

Rank	Metabolite	Formula	Uptake (g/mol)	Category	Function for KZ
1	**Glycerol**	$C_3H_8O_3$	**100.000**	Carbohydrate	Redox-balancing energy
2	**Oleate**	$C_{18}H_{35}O_2$	**2.160**	Carbohydrate	Membrane lipid precursor
3	**Ala-L-Thr-L**	$C_7H_{14}N_2O_4$	**10.804**	Dipeptide e	Amino-acid source
4	**Gly-Leu**	$C_8H_{16}N_2O_3$	**7.096**	Dipeptide e	Branched-chain donor
5	**Menaquinone-7**	$C_{46}H_{64}O_2$	**0.186**	Cofactor (Vit. K_2)	Electron carrier

Final synbiotic formula: KZ + Glycerol + Oleate + Menaquinone-7 + Gly-Leu + Gly-Asn-L



Part VI · Result 2 · In Vitro Validation

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

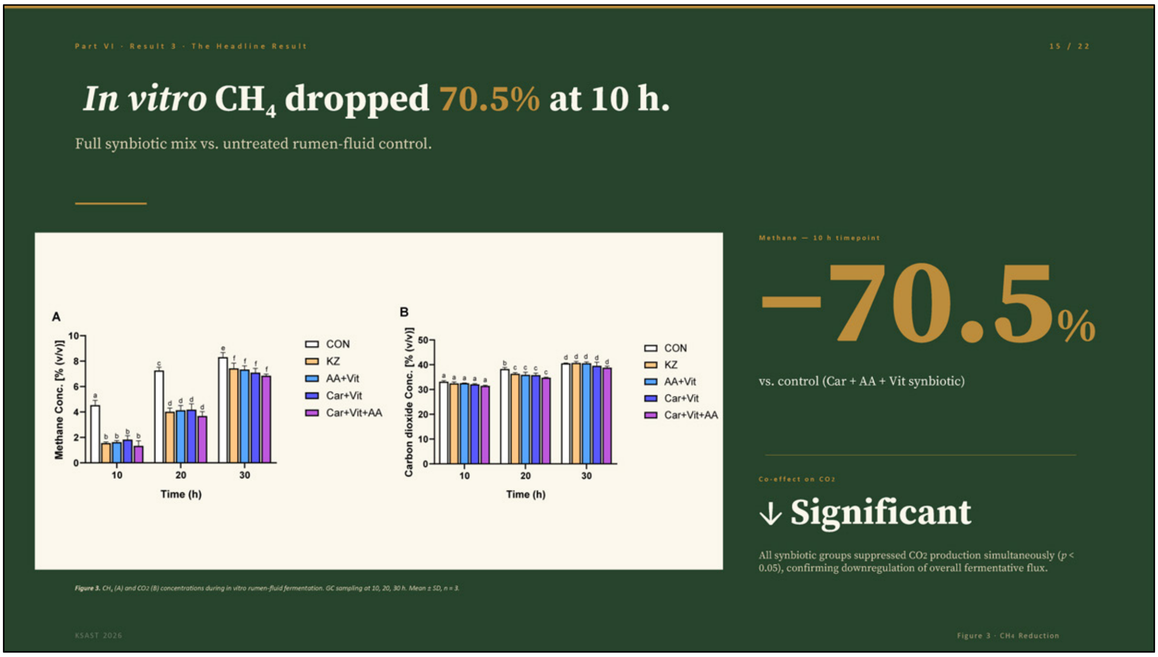
Figure 2. OD600 of KZ cultures after 14 d static incubation (25 °C). Treatments: AA+Car, AA+Vit, Car+Vit, Car+AA+Vit. * $p < 0.05$ (one-tailed unpaired t-test, $n = 3$).

Growth Comparison · OD600

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 ★ Significantly higher vs. Control (* $p < 0.05$)	**0.217** ± 0.020

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

Figure 2 · Growth Validation



Part VI · Result 3 · The Headline Result

In vitro CH_4 dropped 70.5% at 10 h.

Full synbiotic mix vs. untreated rumen-fluid control.

Figure 3. CH_4 (A) and CO_2 (B) concentrations during in vitro rumen-fluid fermentation. GC sampling at 10, 20, 30 h. Mean ± SD, $n = 3$.

Methane — 10 h timepoint

−70.5%

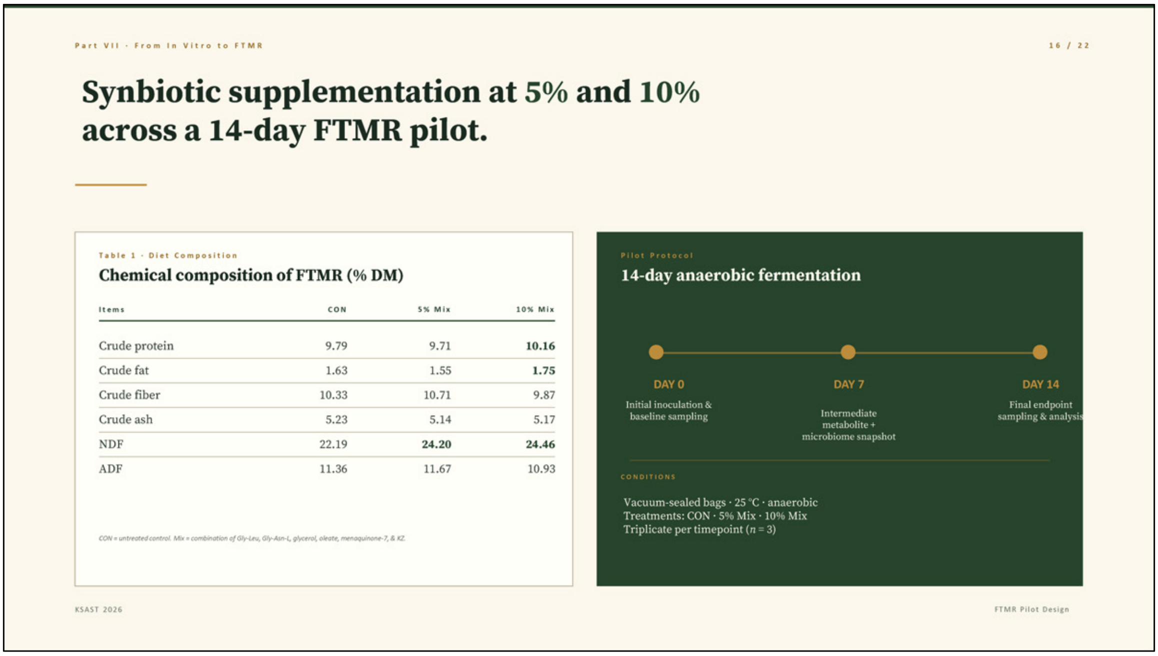
vs. control (Car + AA + Vit synbiotic)

Co-effect on CO_2

↓ Significant

All synbiotic groups suppressed CO_2 production simultaneously ($p <$ 0.05), confirming downregulation of overall fermentative flux.

Figure 3 · CH_4 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-Asn-L, glycerol, oleate, menaquinone-7, & KZ.

Pilot Protocol

14-day anaerobic fermentation

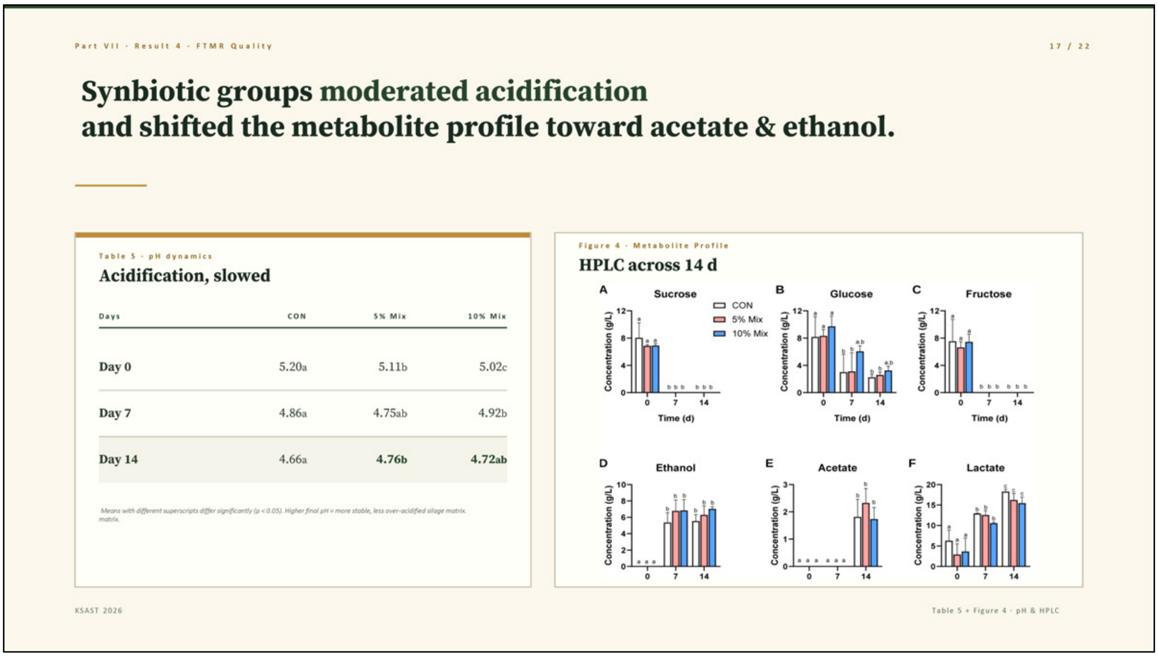
DAY 0
Initial inoculation &
baseline sampling

DAY 7
Intermediate
metabolite +
microbiome snapshot

DAY 14
Final endpoint
sampling & analysis

CONDITIONS

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



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

Table 5 · pH dynamics

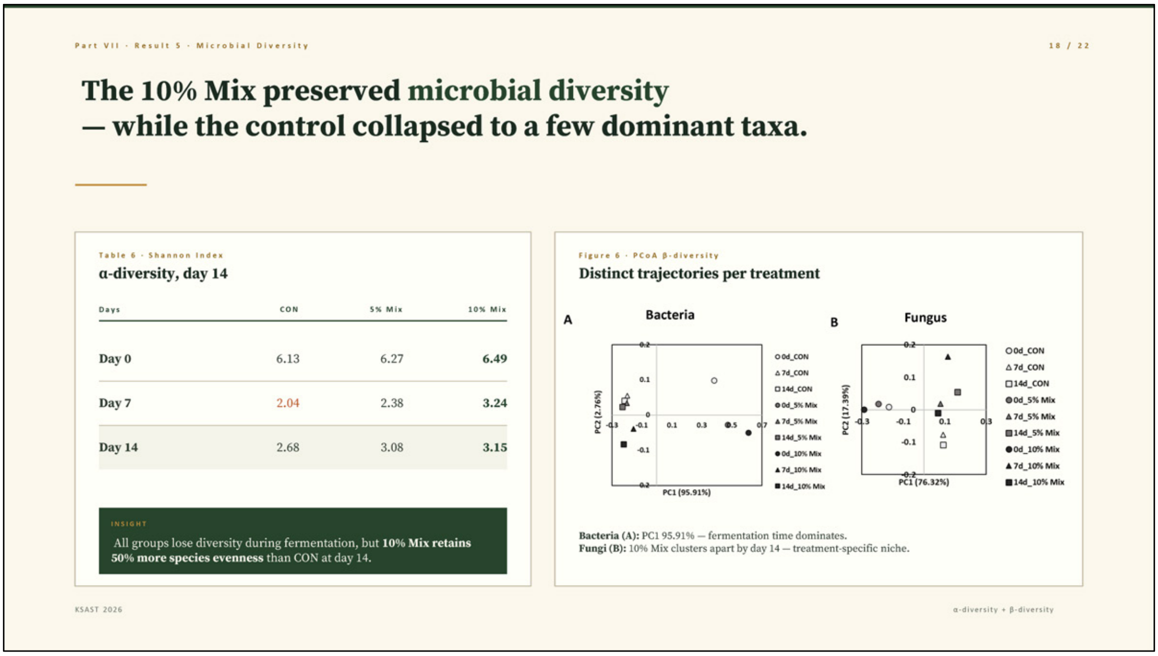
Acidification, slowed

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.

Figure 4 · Metabolite Profile

HPLC across 14 d



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

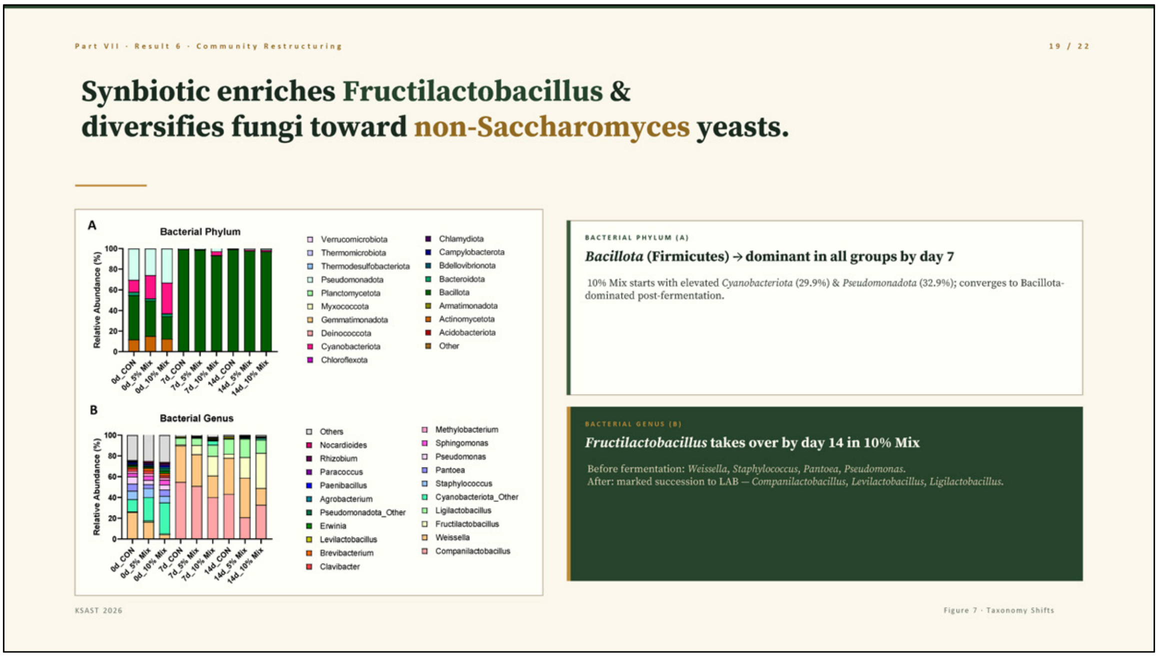
All groups lose diversity during fermentation, but **10% Mix retains 50% more species evenness** than CON at day 14.

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.

BACTERIAL PHYLUM (A)

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

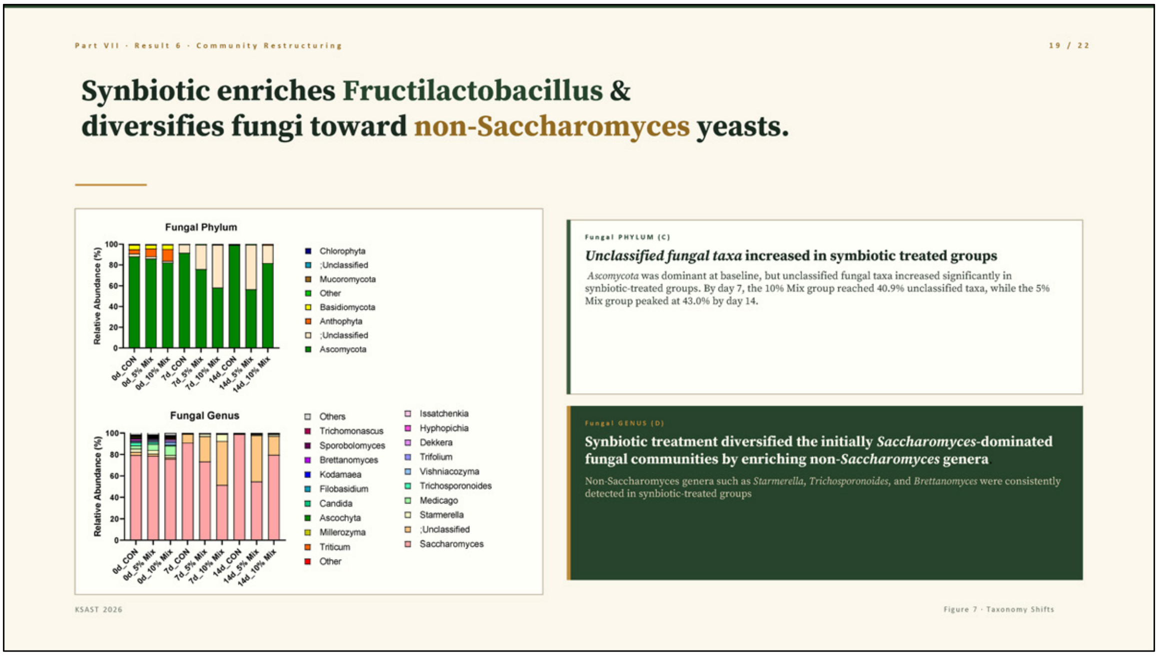
10% Mix starts with elevated *Cyanobacteriota* (29.9%) & *Pseudomonadota* (32.9%); converges to Bacillota-dominated post-fermentation.

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



Part VII · Result 6 · Community Restructuring

19 / 22

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

Fungal PHYLUM (C)

Unclassified fungal taxa increased in symbiotic treated groups

Ascomycota was dominant at baseline, but unclassified fungal taxa increased significantly in synbiotic-treated groups. By day 7, the 10% Mix group reached 40.9% unclassified taxa, while the 5% Mix group peaked at 43.0% by day 14.

Fungal GENUS (D)

Synbiotic treatment diversified the initially *Saccharomyces*-dominated fungal communities by enriching non-*Saccharomyces* genera.

Non-Saccharomyces genera such as *Starmerella*, *Trichosporonoides*, and *Brettanomyces* were consistently detected in synbiotic-treated groups

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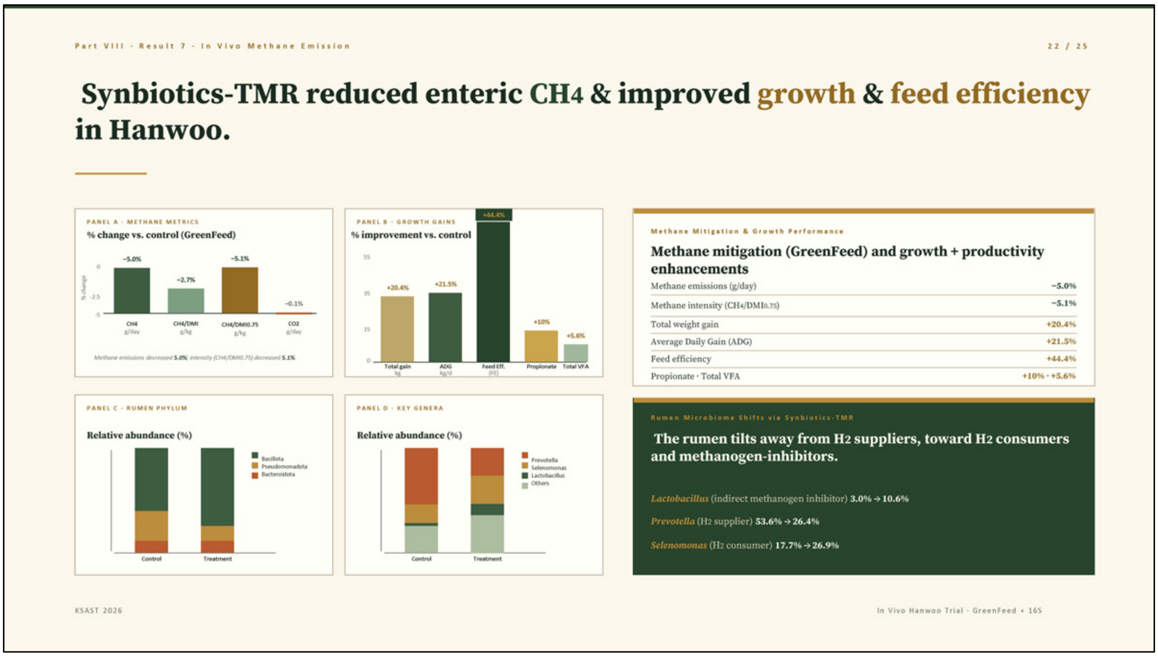
Figure 7 · Taxonomy Shifts



Part VIII · Result 7 · In Vivo Methane Emission

22 / 25

Synbiotics-TMR reduced enteric CH_4 & improved growth & feed efficiency in Hanwoo.

*Methane emissions decreased **5.0%**; intensity (CH4/DMI0.75) decreased **5.1%**.*

Methane Mitigation & Growth Performance

Methane mitigation (GreenFeed) and growth + productivity enhancements

Methane emissions (g/day)	−5.0%
Methane intensity ($CH_4/DMI_{0.75}$)	−5.1%
Total weight gain	+20.4%
Average Daily Gain (ADG)	+21.5%
Feed efficiency	+44.4%
Propionate · Total VFA	+10% · +5.6%

Rumen Microbiome Shifts via Synbiotics-TMR

The rumen tilts away from H_2 suppliers, toward H_2 consumers and methanogen-inhibitors.

Lactobacillus (indirect methanogen inhibitor) **3.0% → 10.6%**

Prevotella (H_2 supplier) **53.6% → 26.4%**

Selenomonas (H_2 consumer) **17.7% → 26.9%**

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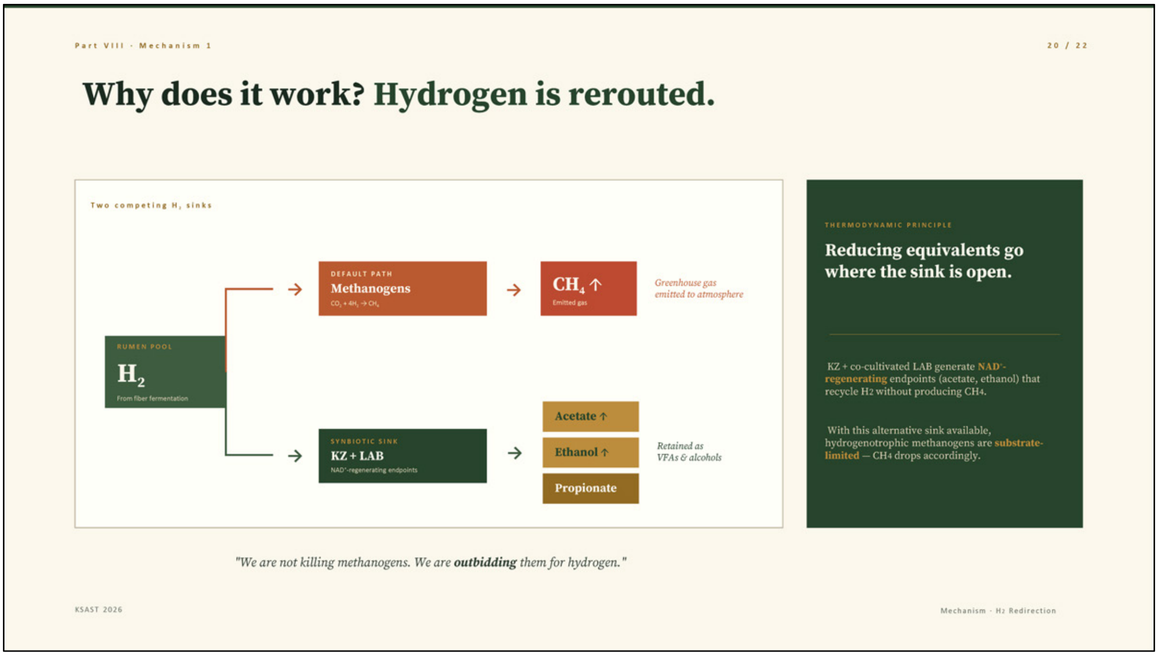
In Vivo Hanwoo Trial · GreenFeed + 16S



Why does it work? Hydrogen is rerouted.

Two competing H_2 sinks

RUMEN POOL

H_2

From fiber fermentation

DEFAULT PATH

Methanogens

$CO_2 + 4H_2 \rightarrow CH_4$

CH_4 ↑

Emitted gas

Greenhouse gas emitted to atmosphere

SYNBIOTIC SINK

KZ + LAB

NAD^+-regenerating endpoints

Acetate ↑

Ethanol ↑

Propionate

Retained as VFAs & alcohols

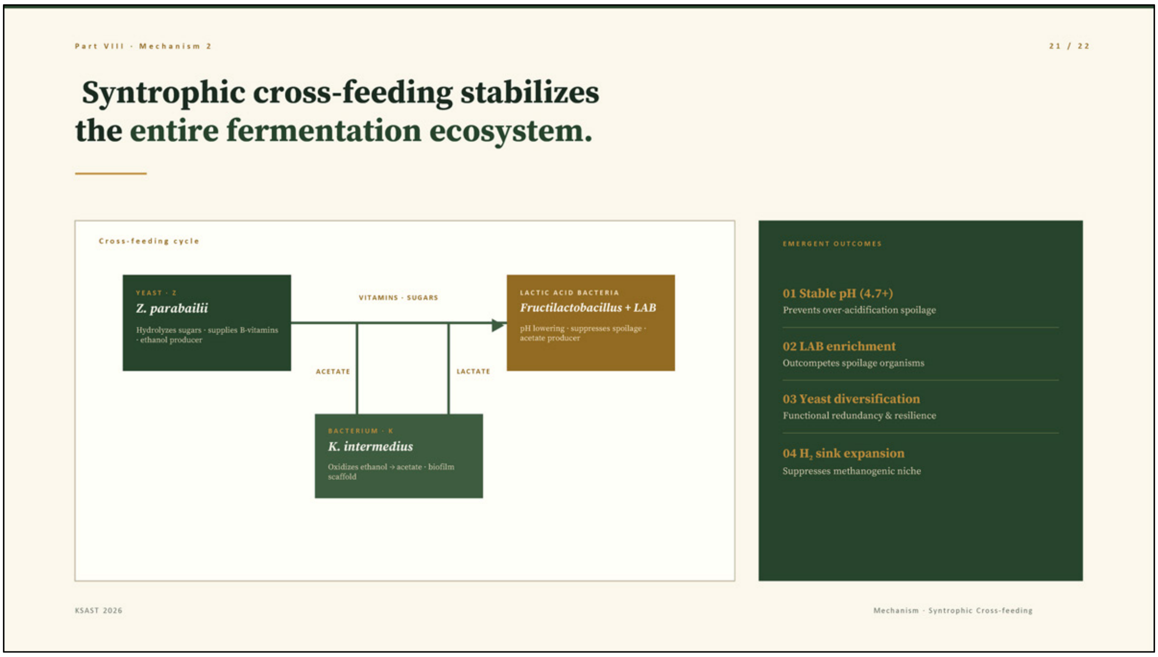
THERMODYNAMIC PRINCIPLE

Reducing equivalents go where the sink is open.

KZ + co-cultivated LAB generate **NAD^+-regenerating** endpoints (acetate, ethanol) that recycle H_2 without producing CH_4.

With this alternative sink available, hydrogenotrophic methanogens are **substrate-limited** — CH_4 drops accordingly.

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



Syntrophic cross-feeding stabilizes the entire fermentation ecosystem.

Cross-feeding cycle

YEAST · Z

Z. parabailii

Hydrolyzes sugars · supplies B-vitamins · ethanol producer

VITAMINS · SUGARS

LACTIC ACID BACTERIA

Fructilactobacillus + LAB

pH lowering · suppresses spoilage · acetate producer

ACETATE

LACTATE

BACTERIUM · K

K. intermedius

Oxidizes ethanol → acetate · biofilm scaffold

EMERGENT OUTCOMES

01 Stable pH (4.7+)
Prevents over-acidification spoilage

02 LAB enrichment
Outcompetes spoilage organisms

03 Yeast diversification
Functional redundancy & resilience

04 H_2 sink expansion
Suppresses methanogenic niche

Part IX · Conclusions & Outlook

22 / 22

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% CH_4 at 10 h.**

Significant suppression of both CH_4 and CO_2 in rumen-fluid fermentation — reproducible across triplicates.

CONCLUSION 03

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

Higher pH stability, increased acetate/ethanol — *Fructilactobacillus* dominance by day 14.

Part X · One-Slide Summary

24 / 25

From silicon to silage to live cattle — one slide.

TRACK 1 · DESIGN & IN-VITRO PROOF

In-silico

15

KZ-exclusive metabolites identified via GSM modeling on KBase

In vitro CH_4

−70.5%

Methane suppression at 10 h in rumen-fluid fermentation

FTMR pH (Day 14)

4.76

Stabilized fermentation pH vs. 4.66 in control — less over-acidification

FTMR Microbiome

3.15

Shannon diversity in 10% Mix vs. 2.68 control — richer LAB ecosystem

TRACK 2 · IN-VIVO HANWOO TRIAL

In vivo CH_4 (GreenFeed)

−5.0%

Emissions (g/day) reduced; intensity −5.1% ($CH_4/DMI^{0.75}$) vs. control

Feed Efficiency

+44.4%

Plus ADG +21.5%, total weight gain +20.4%, propionate +10%

Rumen Microbiome Shift

Lactobacillus **3.0 → 10.6%**

Prevotella **53.6 → 26.4%**

Selenomonas **17.7 → 26.9%**

GSM modeling → SCOBY synbiotic → in vitro 70.5% CH_4 ↓ → FTMR microbiome shift → in vivo Hanwoo: methane ↓ + feed efficiency ↑ — a complete bench-to-barn pipeline.

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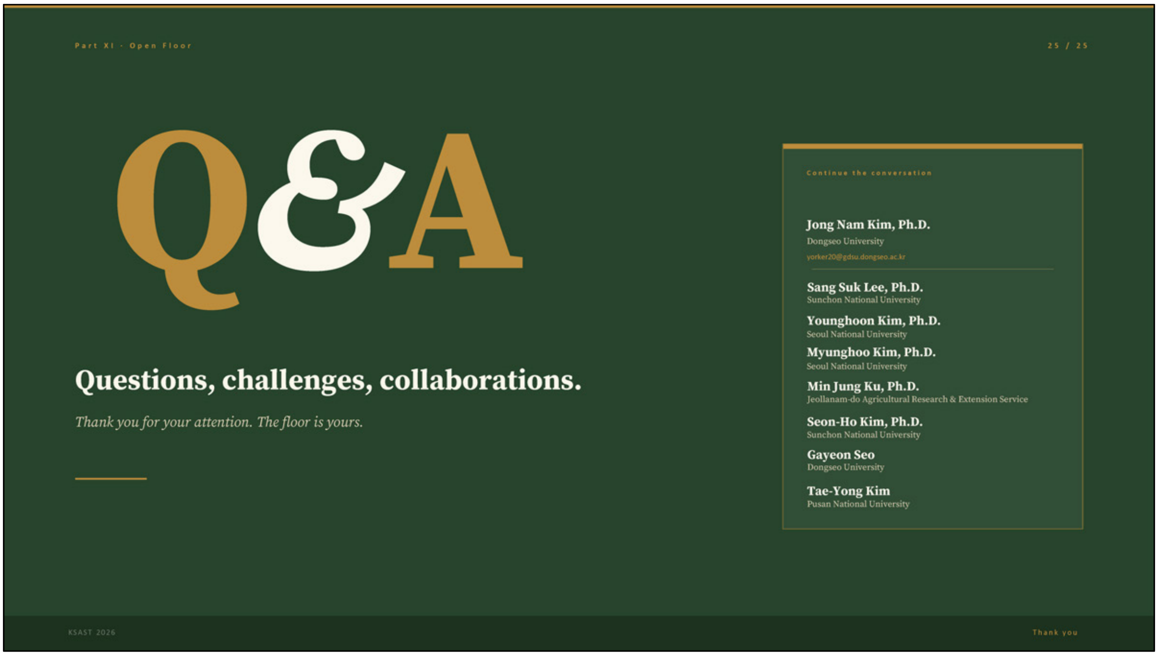
Key Takeaways · Two Tracks



Part XI · Open Floor

25 / 25

Q&A

Questions, challenges, collaborations.

Thank you for your attention. The floor is yours.

Continue the conversation

Jong Nam Kim, Ph.D.
Dongseo University
yorker20@gdsu.dongseo.ac.kr

Sang Suk Lee, Ph.D.
Sunchon National University

Younghoon Kim, Ph.D.
Seoul National University

Myunghoo Kim, Ph.D.
Seoul National University

Min Jung Ku, Ph.D.
Jeollanam-do Agricultural Research & Extension Service

Seon-Ho Kim, Ph.D.
Sunchon National University

Gayeon Seo
Dongseo University

Tae-Yong Kim
Pusan National University

KSAST 2026

Thank you

Technologies to Address Climate Change and Improve the Health of Ruminants by Utilizing the Microbiome and Metabolome

김 명 후
Kim, Myunghoo
(서울대학교)
(Seoul National University)

Curriculum Vitae

- 2025~현재 서울대학교 농업생명과학대학 동물생명공학 전공 부교수
- 2022~2025 부산대학교 생명자원과학대학 동물생명자원과학과 부교수
- 2018~2022 부산대학교 생명자원과학대학 동물생명자원과학과 조교수
- 2016~2018 베일러 의과대학 박사후 연구원
- 2010~2015 퍼듀대학교 수의과대학 영양면역학 이학박사
- 2007~2009 서울대학교 농생명공학부 농학석사
- 2000~2006 서울대학교 동물생명자원과학과 농학사

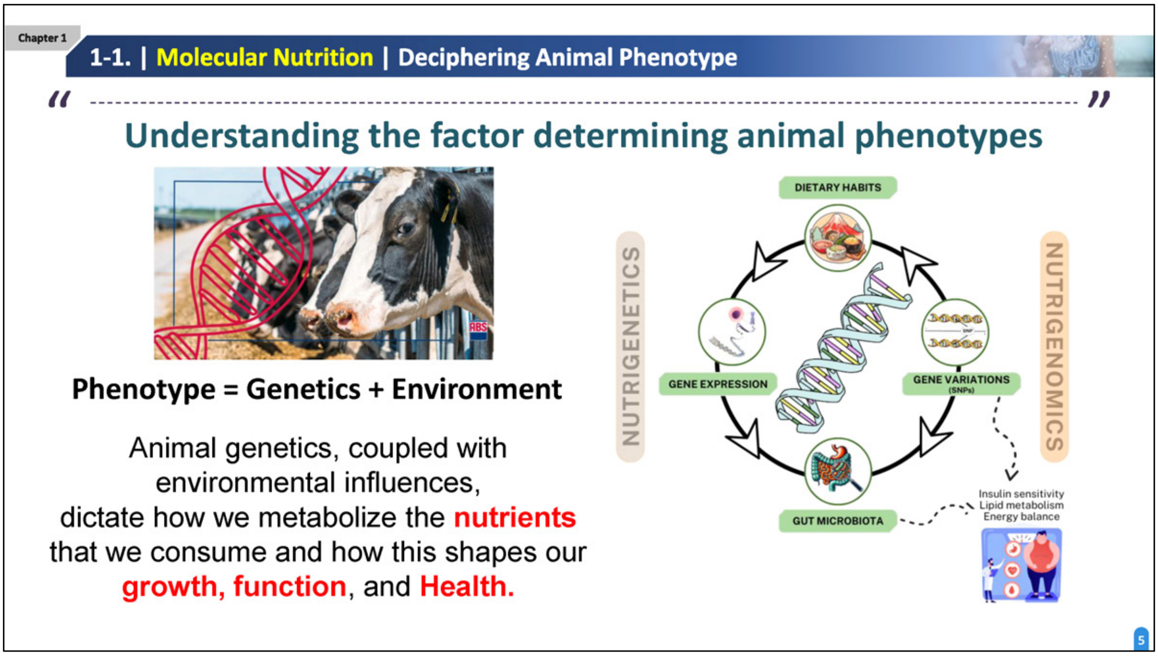
2026 KSAST
Animal Microbiome

Technologies to Address Climate Change and Improve the Health of Ruminants by Utilizing the Microbiome and Metabolome

Seoul National University
College of Agriculture and Life Science
Department of Food and Animal Biotechnology
Department of Agricultural Biotechnology
Myunghoo Kim

01

Microbiome and Metabolome in Animals



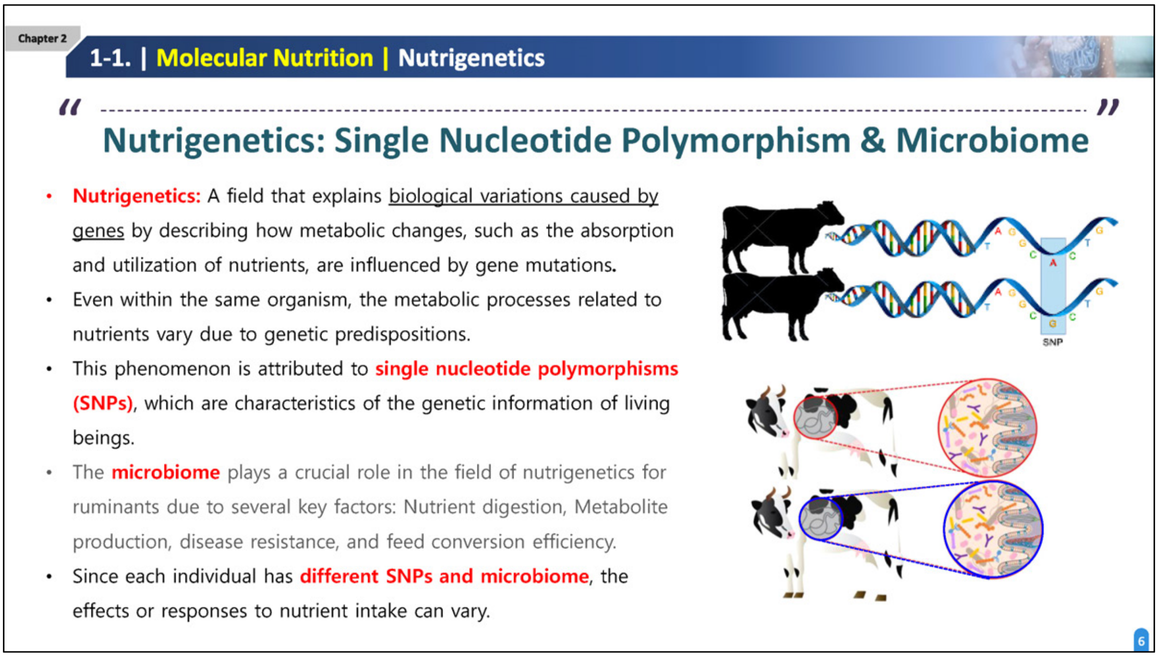
Chapter 1

1-1. | Molecular Nutrition | Deciphering Animal Phenotype

Understanding the factor determining animal phenotypes

Phenotype = Genetics + Environment

Animal genetics, coupled with environmental influences, dictate how we metabolize the **nutrients** that we consume and how this shapes our **growth, function**, and **Health.**

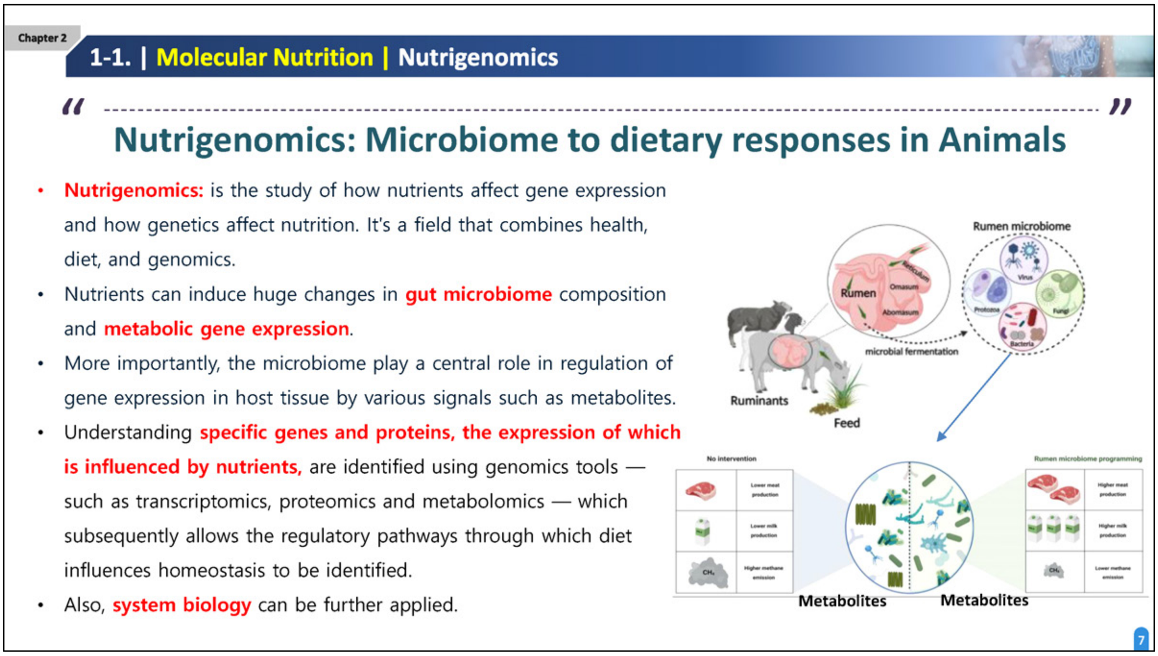


Chapter 2

1-1. | Molecular Nutrition | Nutrigenetics

Nutrigenetics: Single Nucleotide Polymorphism & Microbiome

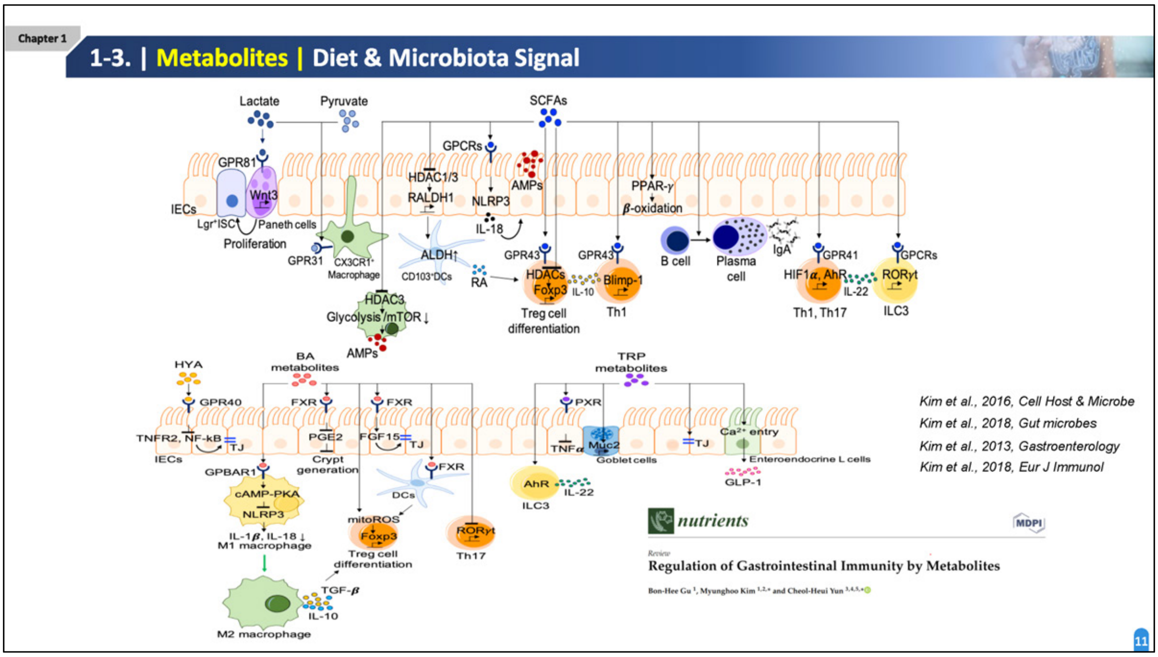
- **Nutrigenetics:** A field that explains biological variations caused by genes by describing how metabolic changes, such as the absorption and utilization of nutrients, are influenced by gene mutations.
- Even within the same organism, the metabolic processes related to nutrients vary due to genetic predispositions.
- This phenomenon is attributed to **single nucleotide polymorphisms (SNPs)**, which are characteristics of the genetic information of living beings.
- The **microbiome** plays a crucial role in the field of nutrigenetics for ruminants due to several key factors: Nutrient digestion, Metabolite production, disease resistance, and feed conversion efficiency.
- Since each individual has **different SNPs and microbiome**, the effects or responses to nutrient intake can vary.



1-1. | Molecular Nutrition | Nutrigenomics

Nutrigenomics: Microbiome to dietary responses in Animals

- **Nutrigenomics:** is the study of how nutrients affect gene expression and how genetics affect nutrition. It's a field that combines health, diet, and genomics.
- Nutrients can induce huge changes in **gut microbiome** composition and **metabolic gene expression**.
- More importantly, the microbiome play a central role in regulation of gene expression in host tissue by various signals such as metabolites.
- Understanding **specific genes and proteins, the expression of which is influenced by nutrients,** are identified using genomics tools — such as transcriptomics, proteomics and metabolomics — which subsequently allows the regulatory pathways through which diet influences homeostasis to be identified.
- Also, **system biology** can be further applied.



1-3. | Metabolites | Diet & Microbiota Signal

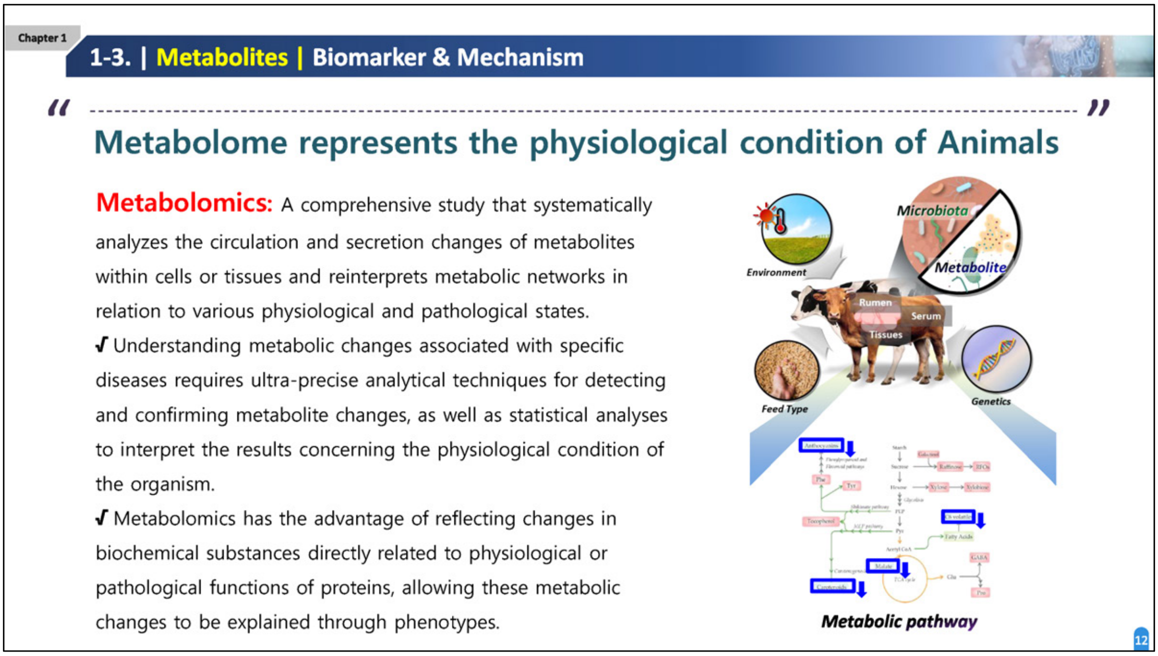
Kim et al., 2016, Cell Host & Microbe
Kim et al., 2018, Gut microbes
Kim et al., 2013, Gastroenterology
Kim et al., 2018, Eur J Immunol

nutrients MDPI

Review

Regulation of Gastrointestinal Immunity by Metabolites

Bon-Hee Gu [1], Myunghoo Kim [1,2,*] and Cheol-Heui Yun [3,4,5,*]

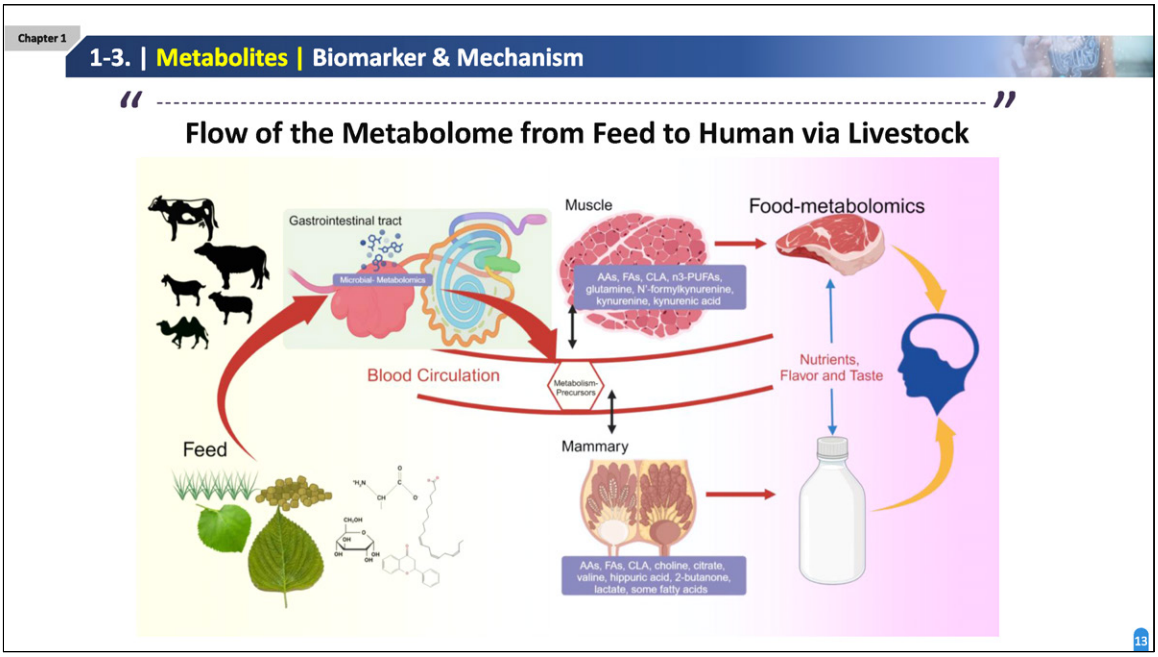


Metabolome represents the physiological condition of Animals

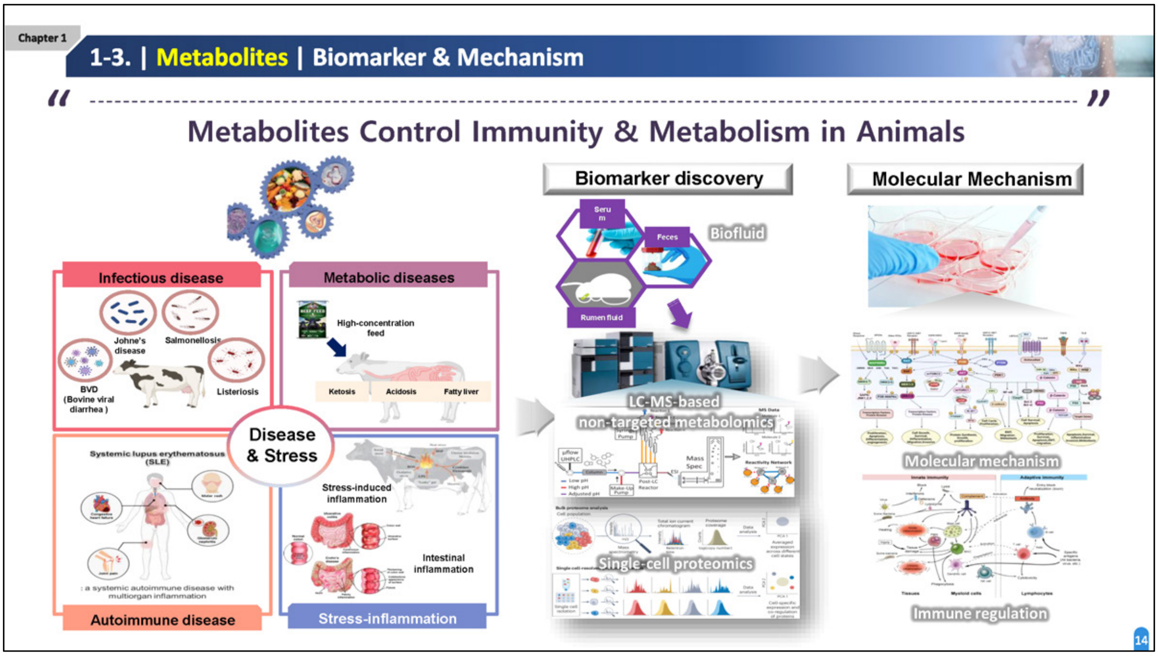
Metabolomics: A comprehensive study that systematically analyzes the circulation and secretion changes of metabolites within cells or tissues and reinterprets metabolic networks in relation to various physiological and pathological states.

√ Understanding metabolic changes associated with specific diseases requires ultra-precise analytical techniques for detecting and confirming metabolite changes, as well as statistical analyses to interpret the results concerning the physiological condition of the organism.

√ Metabolomics has the advantage of reflecting changes in biochemical substances directly related to physiological or pathological functions of proteins, allowing these metabolic changes to be explained through phenotypes.



Flow of the Metabolome from Feed to Human via Livestock



Chapter 1

1-3. | Metabolites | Biomarker & Mechanism

Metabolites Control Immunity & Metabolism in Animals

Infectious disease

Johne's disease

Salmonellosis

BVD (Bovine viral diarrhea)

Listeriosis

Metabolic diseases

High-concentration feed

Ketosis

Acidosis

Fatty liver

Disease & Stress

Systemic lupus erythematosus (SLE)

: a systemic autoimmune disease with multiorgan inflammation

Autoimmune disease

Stress-induced inflammation

Intestinal inflammation

Stress-inflammation

Biomarker discovery

Serum

Feces

Biofluid

Rumen fluid

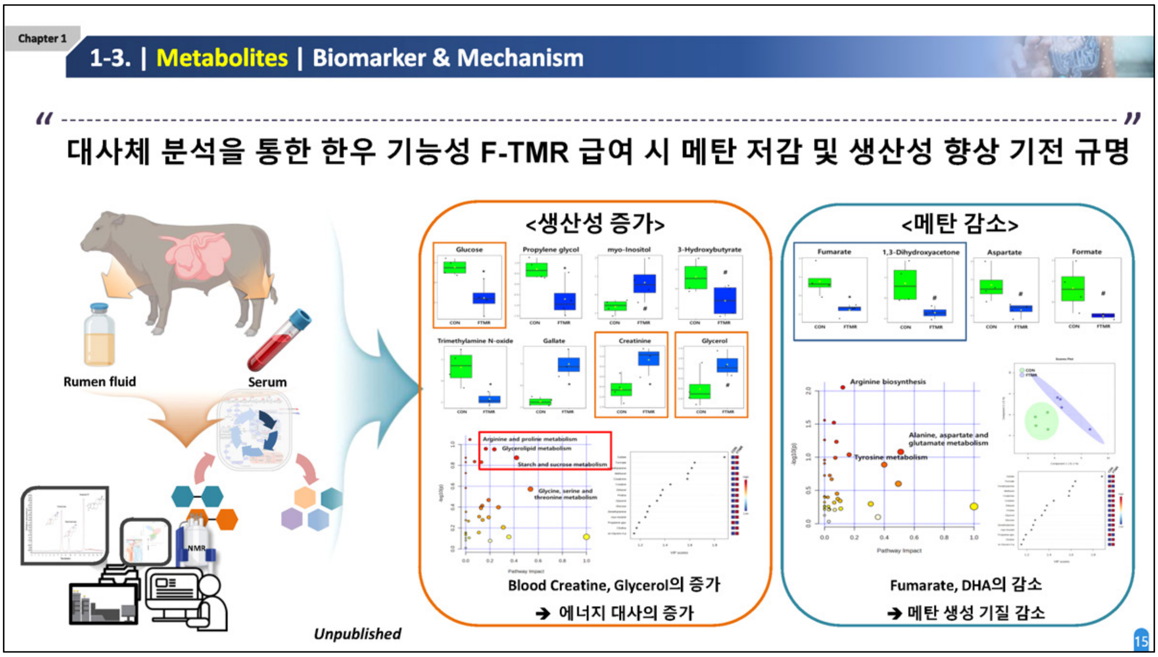
LC-MS-based non-targeted metabolomics

Single-cell proteomics

Molecular Mechanism

Molecular mechanism

Immune regulation



Chapter 1

1-3. | Metabolites | Biomarker & Mechanism

대사체 분석을 통한 한우 기능성 F-TMR 급여 시 메탄 저감 및 생산성 향상 기전 규명

Rumen fluid

Serum

NMR

<생산성 증가>

Blood Creatine, Glycerol의 증가

➔ 에너지 대사의 증가

<메탄 감소>

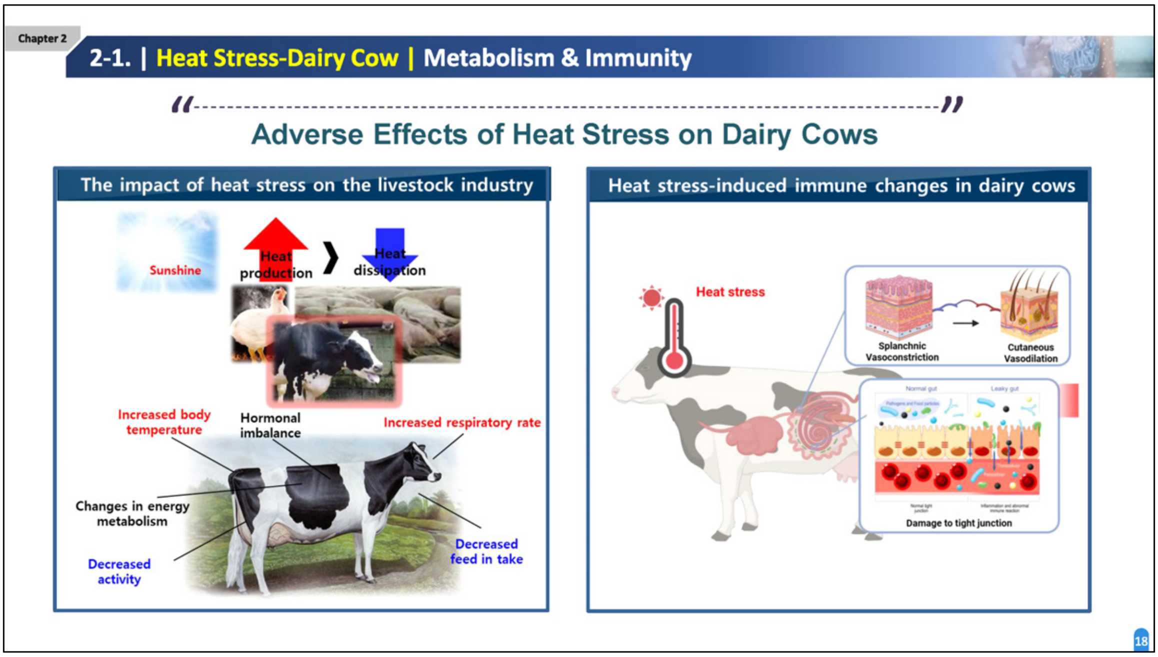
Fumarate, DHA의 감소

➔ 메탄 생성 기질 감소

Unpublished

02

Microbiome & Metabolome in Heat stressed-Dairy Cow



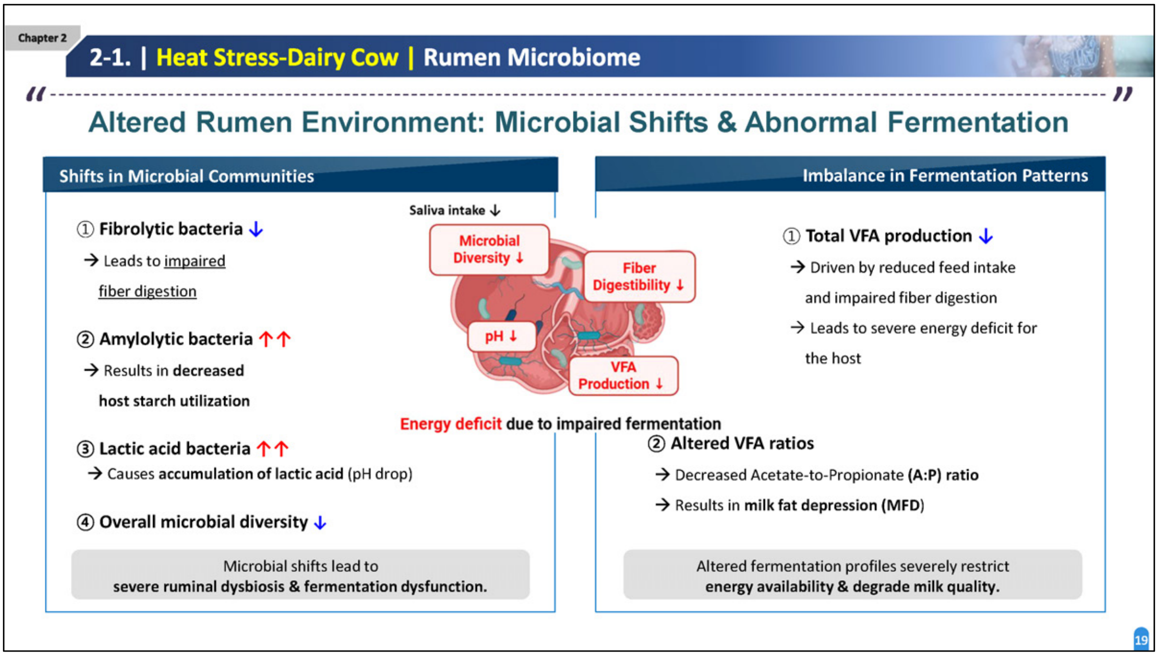
Chapter 2

2-1. | Heat Stress-Dairy Cow | Metabolism & Immunity

"Adverse Effects of Heat Stress on Dairy Cows"

The impact of heat stress on the livestock industry

Heat stress-induced immune changes in dairy cows



Chapter 2

2-1. | Heat Stress-Dairy Cow | Rumen Microbiome

Altered Rumen Environment: Microbial Shifts & Abnormal Fermentation

Shifts in Microbial Communities

① **Fibrolytic bacteria** ↓

→ Leads to impaired fiber digestion

② **Amylolytic bacteria** ↑↑

→ Results in **decreased host starch utilization**

③ **Lactic acid bacteria** ↑↑

→ Causes **accumulation of lactic acid** (pH drop)

④ **Overall microbial diversity** ↓

Microbial shifts lead to **severe ruminal dysbiosis & fermentation dysfunction.**

Saliva intake ↓

Microbial Diversity ↓

Fiber Digestibility ↓

pH ↓

VFA Production ↓

Energy deficit due to impaired fermentation

Imbalance in Fermentation Patterns

① **Total VFA production** ↓

→ Driven by reduced feed intake and impaired fiber digestion

→ Leads to severe energy deficit for the host

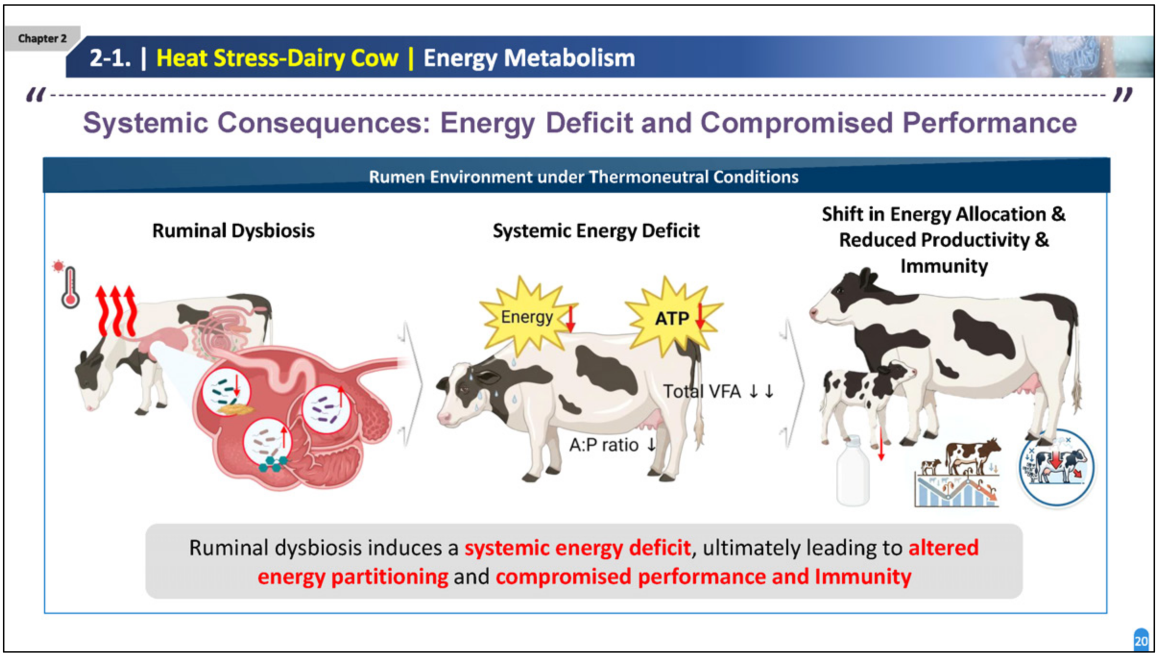
② **Altered VFA ratios**

→ Decreased Acetate-to-Propionate **(A:P) ratio**

→ Results in **milk fat depression (MFD)**

Altered fermentation profiles severely restrict **energy availability & degrade milk quality.**

19



Chapter 2

2-1. | Heat Stress-Dairy Cow | Energy Metabolism

Systemic Consequences: Energy Deficit and Compromised Performance

Rumen Environment under Thermoneutral Conditions

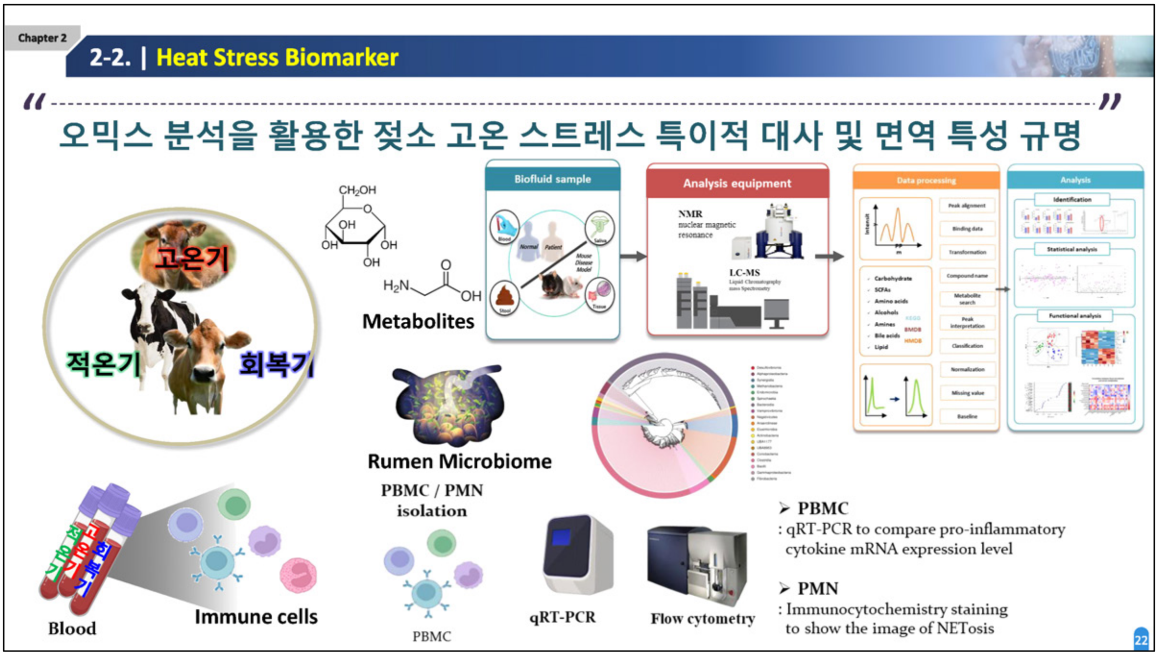
Ruminal Dysbiosis

Systemic Energy Deficit

Shift in Energy Allocation & Reduced Productivity & Immunity

Ruminal dysbiosis induces a **systemic energy deficit**, ultimately leading to **altered energy partitioning** and **compromised performance and Immunity**

20

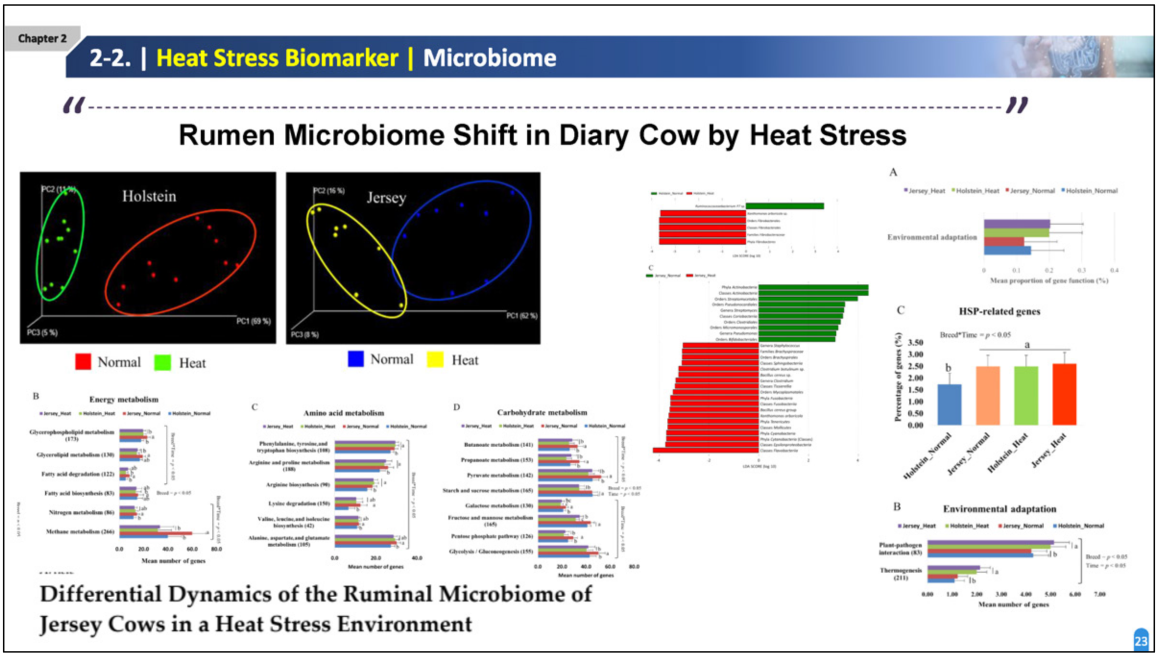


2-2. | Heat Stress Biomarker

“오믹스 분석을 활용한 젖소 고온 스트레스 특이적 대사 및 면역 특성 규명”

- **PBMC**
: qRT-PCR to compare pro-inflammatory cytokine mRNA expression level

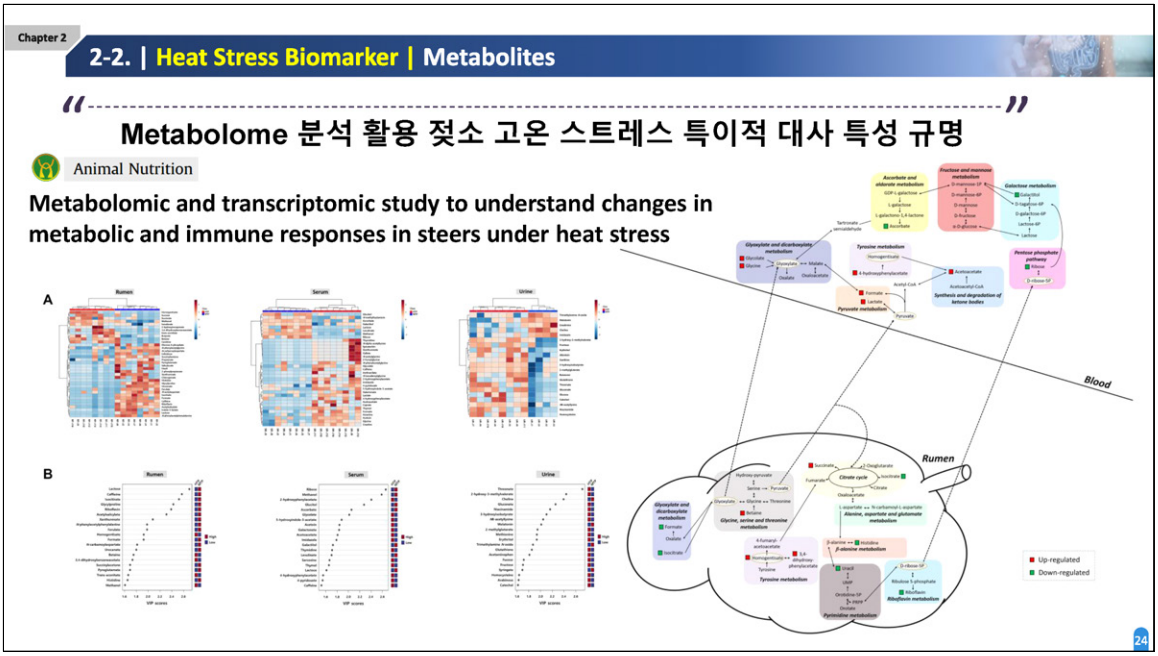
- **PMN**
: Immunocytochemistry staining to show the image of NETosis



2-2. | Heat Stress Biomarker | Microbiome

“Rumen Microbiome Shift in Diary Cow by Heat Stress”

Differential Dynamics of the Ruminal Microbiome of Jersey Cows in a Heat Stress Environment



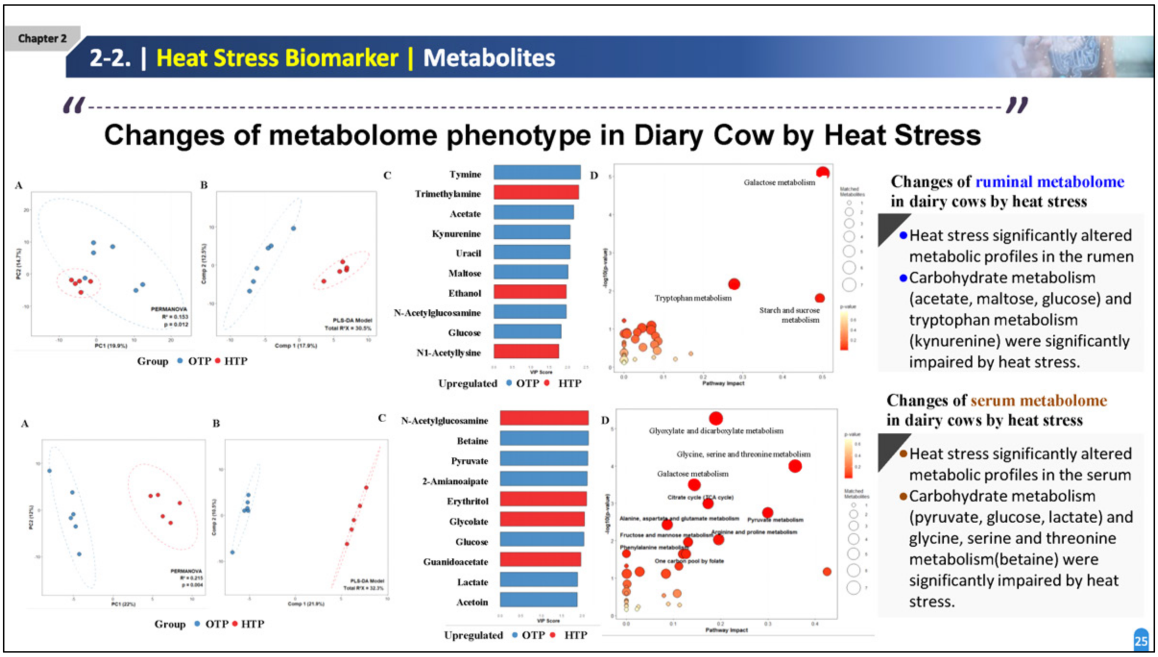
Chapter 2

2-2. | Heat Stress Biomarker | Metabolites

Metabolome 분석 활용 젖소 고온 스트레스 특이적 대사 특성 규명

Animal Nutrition

Metabolomic and transcriptomic study to understand changes in metabolic and immune responses in steers under heat stress



Chapter 2

2-2. | Heat Stress Biomarker | Metabolites

Changes of metabolome phenotype in Diary Cow by Heat Stress

Changes of ruminal metabolome in dairy cows by heat stress

- Heat stress significantly altered metabolic profiles in the rumen
- Carbohydrate metabolism (acetate, maltose, glucose) and tryptophan metabolism (kynurenine) were significantly impaired by heat stress.

Changes of serum metabolome in dairy cows by heat stress

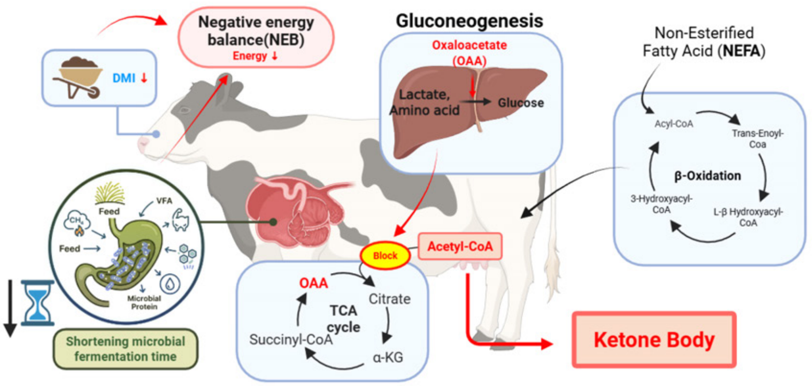
- Heat stress significantly altered metabolic profiles in the serum
- Carbohydrate metabolism (pyruvate, glucose, lactate) and glycine, serine and threonine metabolism(betaine) were significantly impaired by heat stress.

Chapter 2

2-2. | Heat Stress Biomarker | Metabolites

Shifts in Host Metabolism and Negative Energy Balance

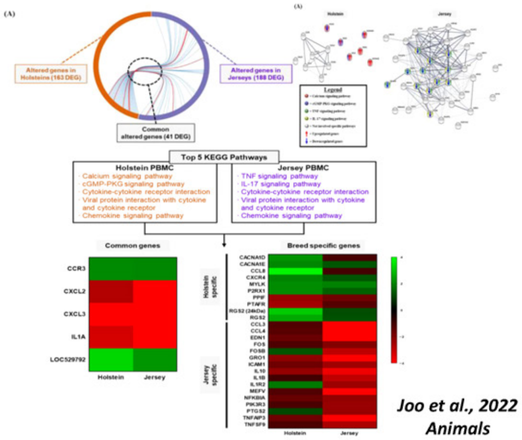
Heat Stress-Driven Negative Energy Balance and Systemic Cascades



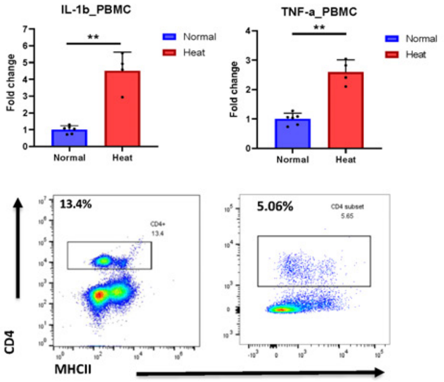
Chapter 2

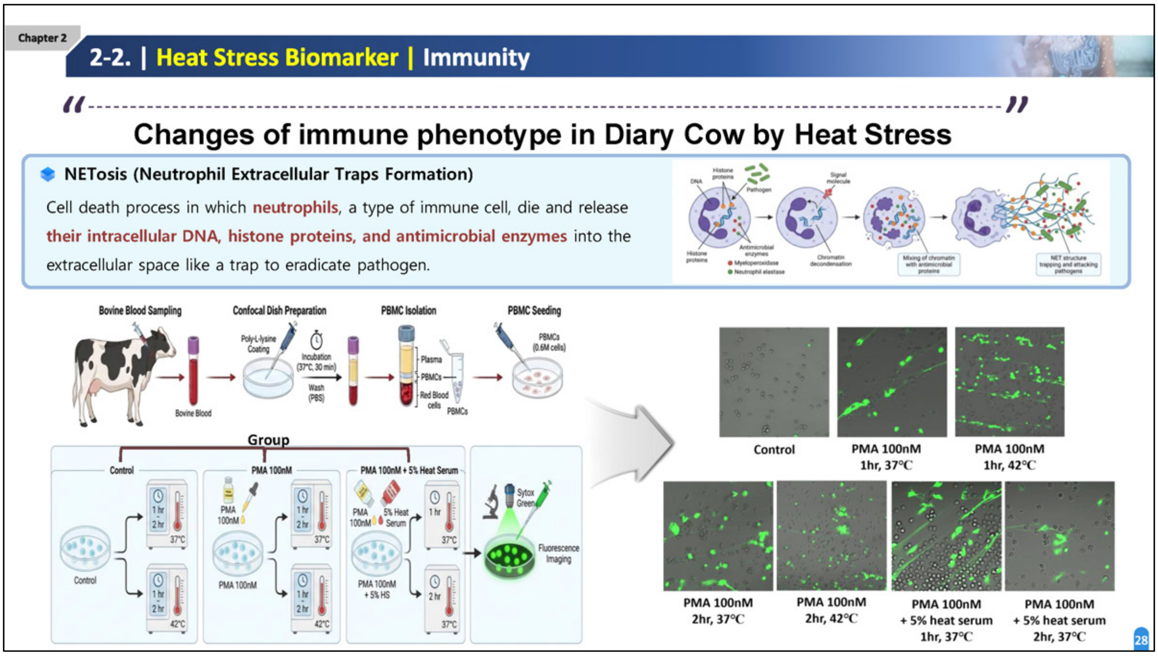
2-2. | Heat Stress Biomarker | Immunity

Changes of immune phenotype in Diary Cow by Heat Stress



Joo et al., 2022
Animals





Chapter 2

2-2. | Heat Stress Biomarker | Immunity

Changes of immune phenotype in Diary Cow by Heat Stress

NETosis (Neutrophil Extracellular Traps Formation)

Cell death process in which **neutrophils**, a type of immune cell, die and release **their intracellular DNA, histone proteins, and antimicrobial enzymes** into the extracellular space like a trap to eradicate pathogen.

Control

PMA 100nM 1hr, 37℃

PMA 100nM 1hr, 42℃

PMA 100nM 2hr, 37℃

PMA 100nM 2hr, 42℃

PMA 100nM + 5% heat serum 1hr, 37℃

PMA 100nM + 5% heat serum 2hr, 37℃

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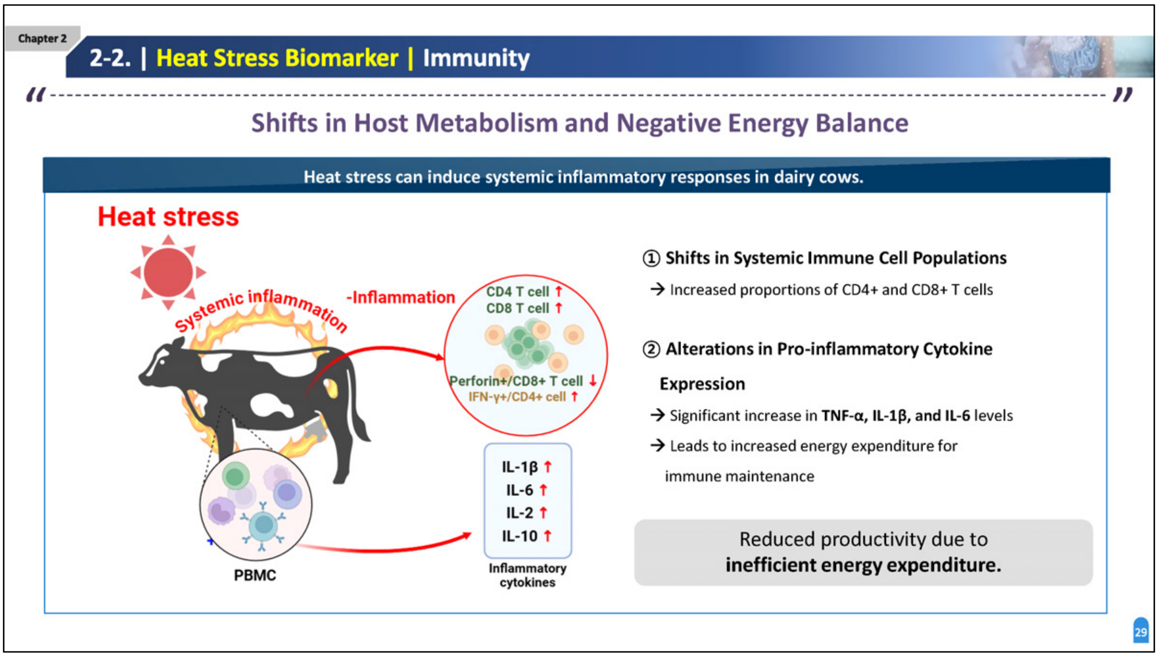

Chapter 2

2-2. | Heat Stress Biomarker | Immunity

Shifts in Host Metabolism and Negative Energy Balance

Heat stress can induce systemic inflammatory responses in dairy cows.

① Shifts in Systemic Immune Cell Populations

→ Increased proportions of CD4+ and CD8+ T cells

② Alterations in Pro-inflammatory Cytokine Expression

→ Significant increase in **TNF-α, IL-1β, and IL-6** levels

→ Leads to increased energy expenditure for immune maintenance

Reduced productivity due to **inefficient energy expenditure.**

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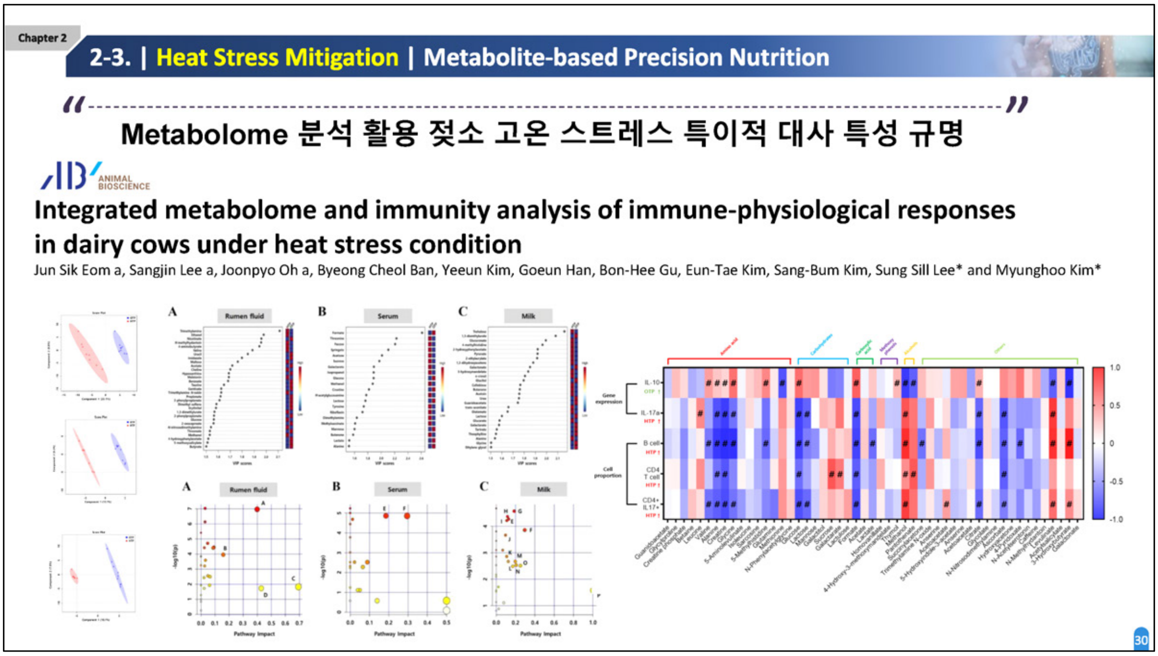


Chapter 2

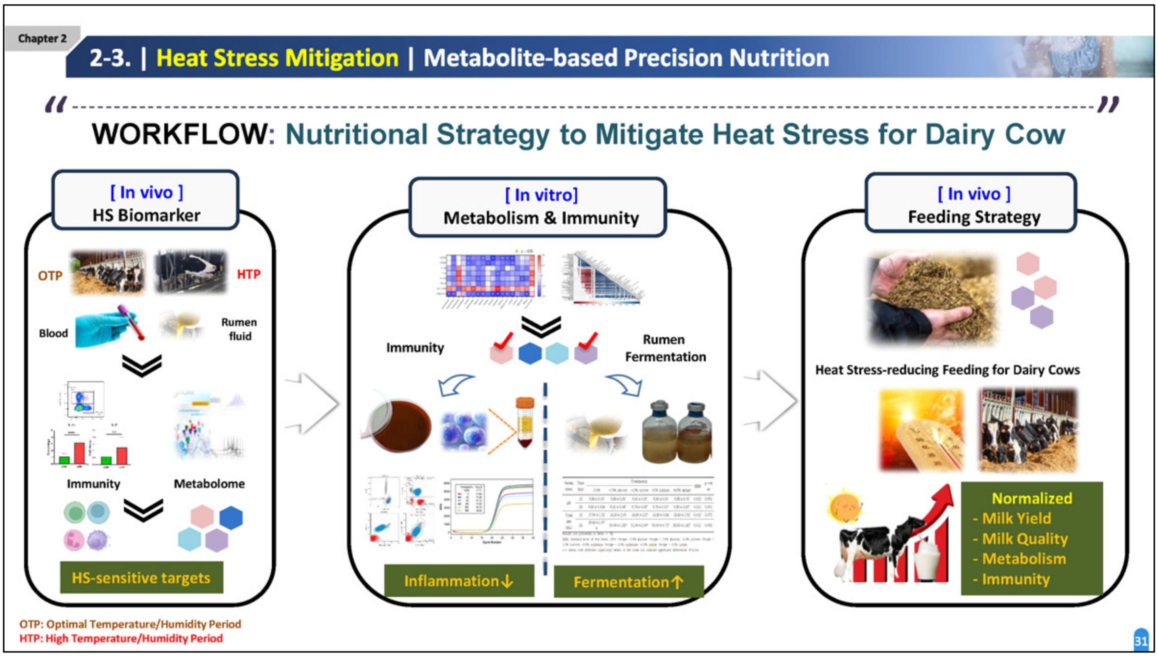
2-3. | Heat Stress Mitigation | Metabolite-based Precision Nutrition

“ Metabolome 분석 활용 젖소 고온 스트레스 특이적 대사 특성 규명 ”

ANIMAL BIOSCIENCE

Integrated metabolome and immunity analysis of immune-physiological responses in dairy cows under heat stress condition

Jun Sik Eom a, Sangjin Lee a, Joonpyo Oh a, Byeong Cheol Ban, Yeeun Kim, Goeun Han, Bon-Hee Gu, Eun-Tae Kim, Sang-Bum Kim, Sung Sill Lee* and Myunghoo Kim*

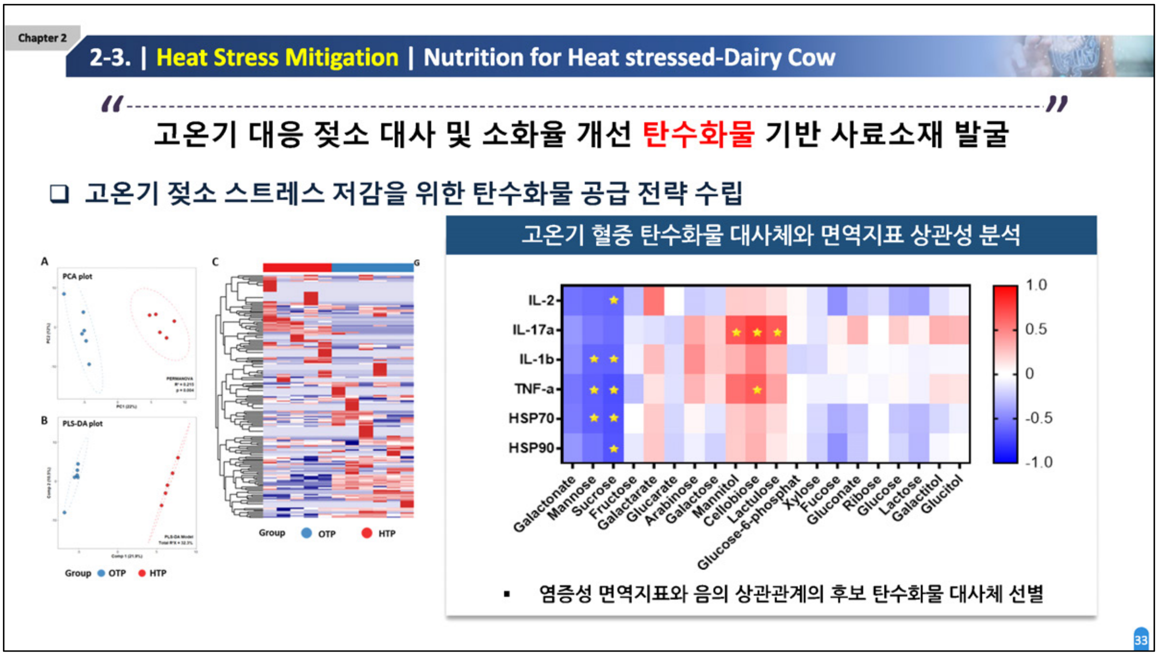


Chapter 2

2-3. | Heat Stress Mitigation | Metabolite-based Precision Nutrition

“ **WORKFLOW**: Nutritional Strategy to Mitigate Heat Stress for Dairy Cow ”

OTP: Optimal Temperature/Humidity Period
HTP: High Temperature/Humidity Period

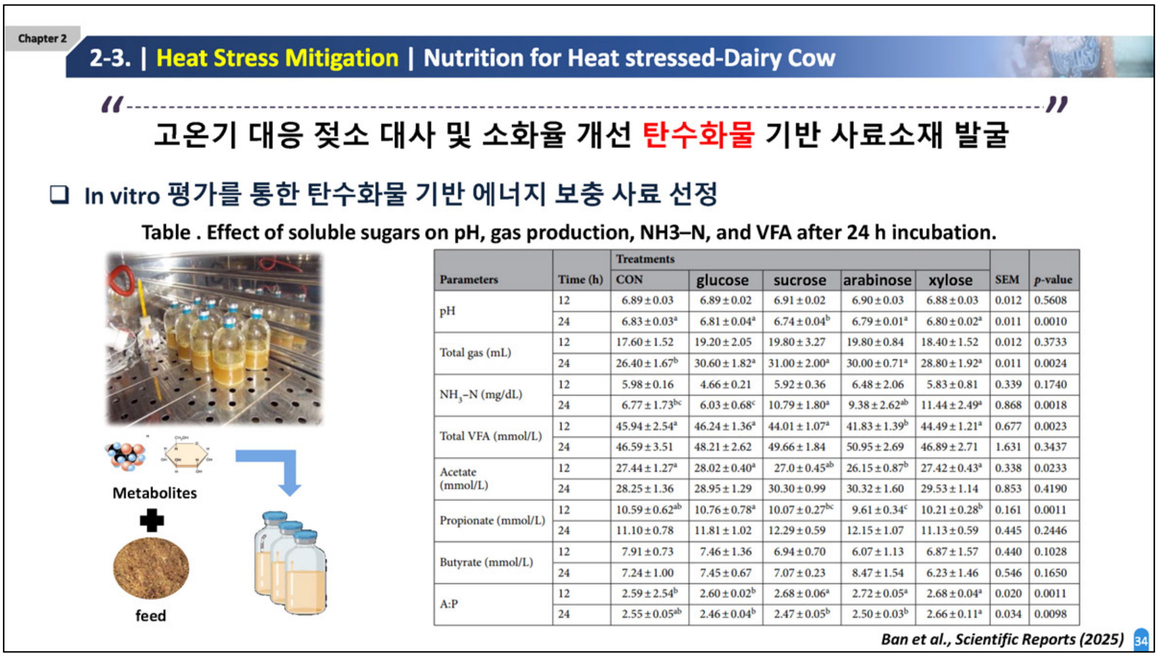


고온기 대응 젖소 대사 및 소화율 개선 탄수화물 기반 사료소재 발굴

☐ 고온기 젖소 스트레스 저감을 위한 탄수화물 공급 전략 수립

고온기 혈중 탄수화물 대사체와 면역지표 상관성 분석

- 염증성 면역지표와 음의 상관관계의 후보 탄수화물 대사체 선별



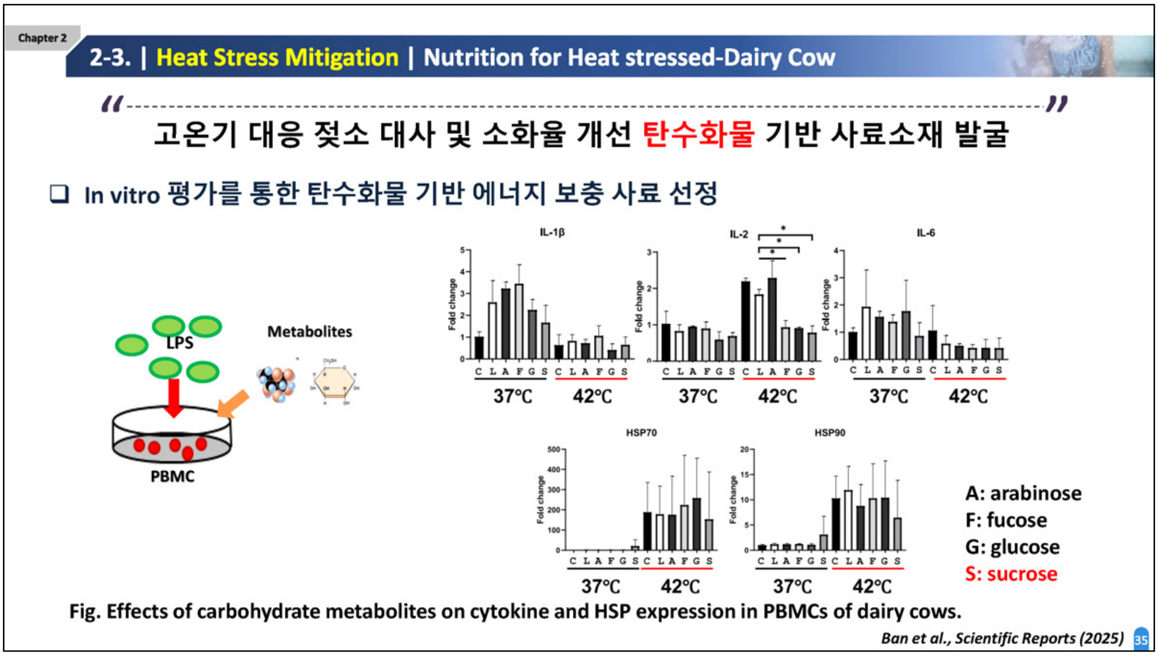
고온기 대응 젖소 대사 및 소화율 개선 탄수화물 기반 사료소재 발굴

☐ In vitro 평가를 통한 탄수화물 기반 에너지 보충 사료 선정

Table . Effect of soluble sugars on pH, gas production, NH3–N, and VFA after 24 h incubation.

Parameters	Time (h)	Treatments					SEM	*p*-value
		CON	glucose	sucrose	arabinose	xylose		
pH	12	6.89±0.03	6.89±0.02	6.91±0.02	6.90±0.03	6.88±0.03	0.012	0.5608
	24	6.83±0.03^{a}	6.81±0.04^{a}	6.74±0.04^{b}	6.79±0.01^{a}	6.80±0.02^{a}	0.011	0.0010
Total gas (mL)	12	17.60±1.52	19.20±2.05	19.80±3.27	19.80±0.84	18.40±1.52	0.012	0.3733
	24	26.40±1.67^{b}	30.60±1.82^{a}	31.00±2.00^{a}	30.00±0.71^{a}	28.80±1.92^{a}	0.011	0.0024
NH_3-N (mg/dL)	12	5.98±0.16	4.66±0.21	5.92±0.36	6.48±2.06	5.83±0.81	0.339	0.1740
	24	6.77±1.73^{bc}	6.03±0.68^{c}	10.79±1.80^{a}	9.38±2.62^{ab}	11.44±2.49^{a}	0.868	0.0018
Total VFA (mmol/L)	12	45.94±2.54^{a}	46.24±1.36^{a}	44.01±1.07^{a}	41.83±1.39^{b}	44.49±1.21^{a}	0.677	0.0023
	24	46.59±3.51	48.21±2.62	49.66±1.84	50.95±2.69	46.89±2.71	1.631	0.3437
Acetate (mmol/L)	12	27.44±1.27^{a}	28.02±0.40^{a}	27.0±0.45^{ab}	26.15±0.87^{b}	27.42±0.43^{a}	0.338	0.0233
	24	28.25±1.36	28.95±1.29	30.30±0.99	30.32±1.60	29.53±1.14	0.853	0.4190
Propionate (mmol/L)	12	10.59±0.62^{ab}	10.76±0.78^{a}	10.07±0.27^{bc}	9.61±0.34^{c}	10.21±0.28^{b}	0.161	0.0011
	24	11.10±0.78	11.81±1.02	12.29±0.59	12.15±1.07	11.13±0.59	0.445	0.2446
Butyrate (mmol/L)	12	7.91±0.73	7.46±1.36	6.94±0.70	6.07±1.13	6.87±1.57	0.440	0.1028
	24	7.24±1.00	7.45±0.67	7.07±0.23	8.47±1.54	6.23±1.46	0.546	0.1650
A:P	12	2.59±2.54^{b}	2.60±0.02^{b}	2.68±0.06^{a}	2.72±0.05^{a}	2.68±0.04^{a}	0.020	0.0011
	24	2.55±0.05^{ab}	2.46±0.04^{b}	2.47±0.05^{b}	2.50±0.03^{b}	2.66±0.11^{a}	0.034	0.0098

Ban et al., Scientific Reports (2025)



Chapter 2

2-3. | Heat Stress Mitigation | Nutrition for Heat stressed-Dairy Cow

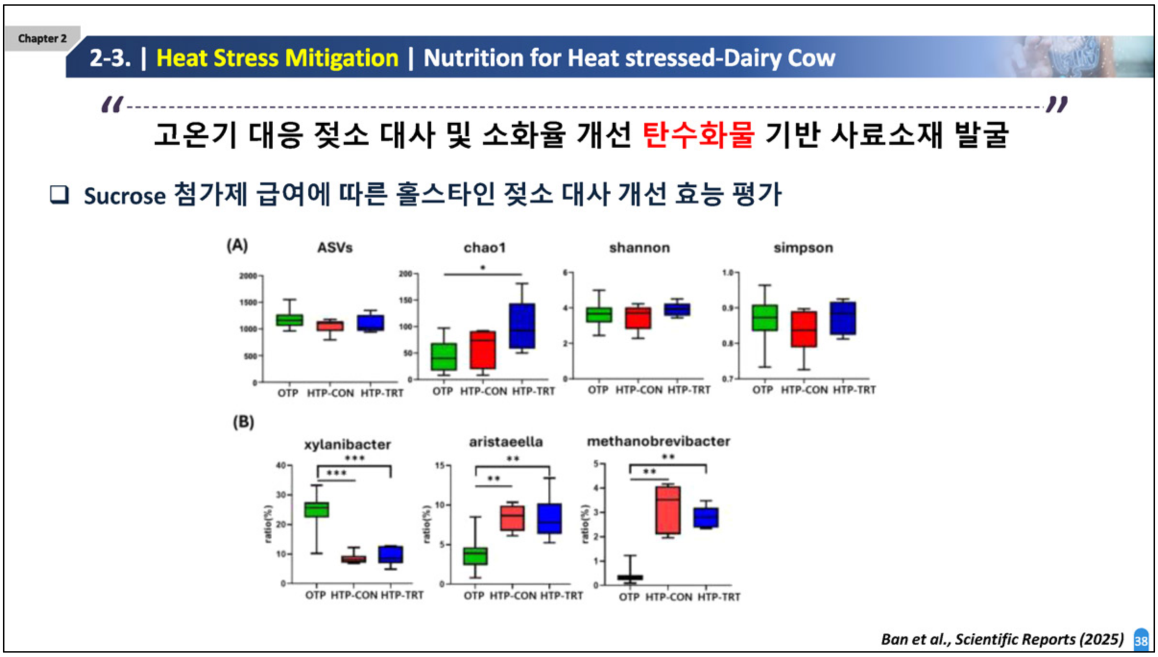
"고온기 대응 젖소 대사 및 소화율 개선 탄수화물 기반 사료소재 발굴"

❑ In vitro 평가를 통한 탄수화물 기반 에너지 보충 사료 선정

A: arabinose
F: fucose
G: glucose
S: sucrose

Fig. Effects of carbohydrate metabolites on cytokine and HSP expression in PBMCs of dairy cows.

Ban et al., Scientific Reports (2025)



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2-3. | Heat Stress Mitigation | Nutrition for Heat stressed-Dairy Cow

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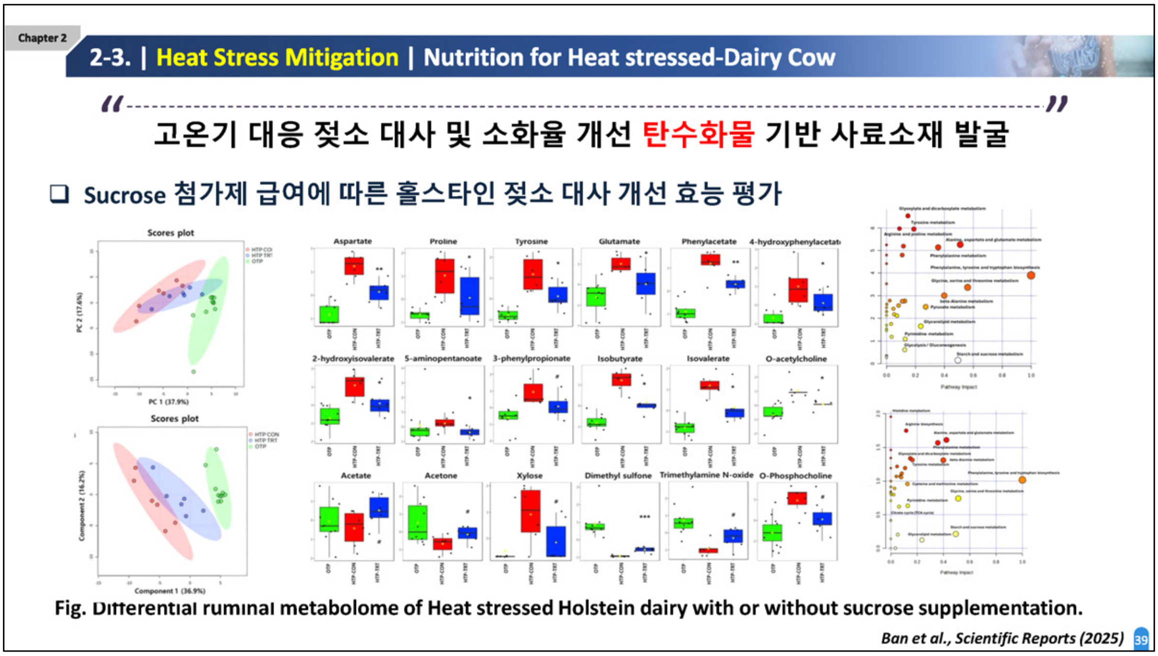
❑ Sucrose 첨가제 급여에 따른 홀스타인 젖소 대사 개선 효능 평가

Ban et al., Scientific Reports (2025)



Chapter 2

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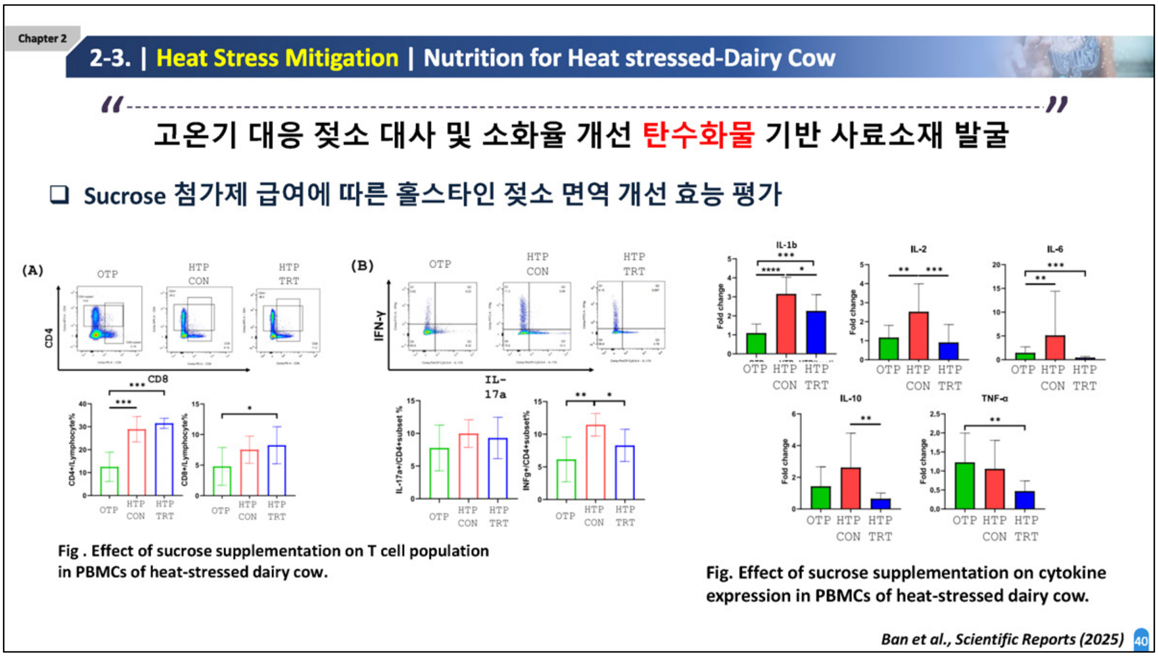
"고온기 대응 젖소 대사 및 소화율 개선 **탄수화물** 기반 사료소재 발굴"

❑ Sucrose 첨가제 급여에 따른 홀스타인 젖소 대사 개선 효능 평가

Fig. Differential ruminal metabolome of Heat stressed Holstein dairy with or without sucrose supplementation.

Ban et al., Scientific Reports (2025) 39



Chapter 2

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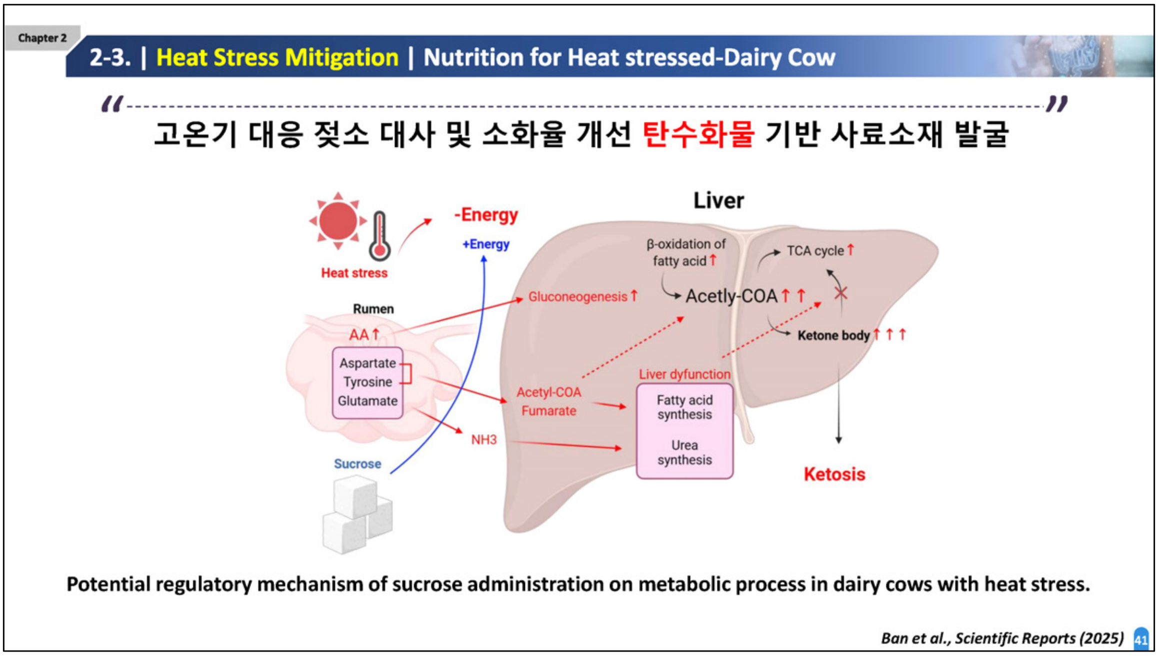
❑ Sucrose 첨가제 급여에 따른 홀스타인 젖소 면역 개선 효능 평가

Fig . Effect of sucrose supplementation on T cell population in PBMCs of heat-stressed dairy cow.

Fig. Effect of sucrose supplementation on cytokine expression in PBMCs of heat-stressed dairy cow.

Ban et al., Scientific Reports (2025) 40



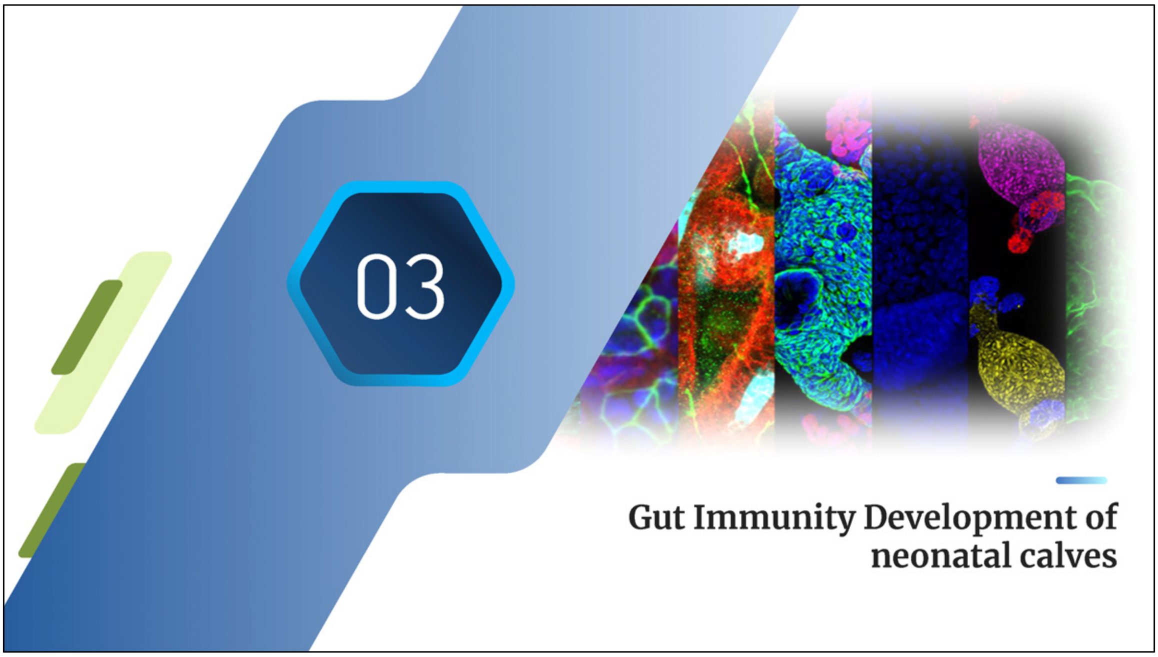
Chapter 2

2-3. | Heat Stress Mitigation | Nutrition for Heat stressed-Dairy Cow

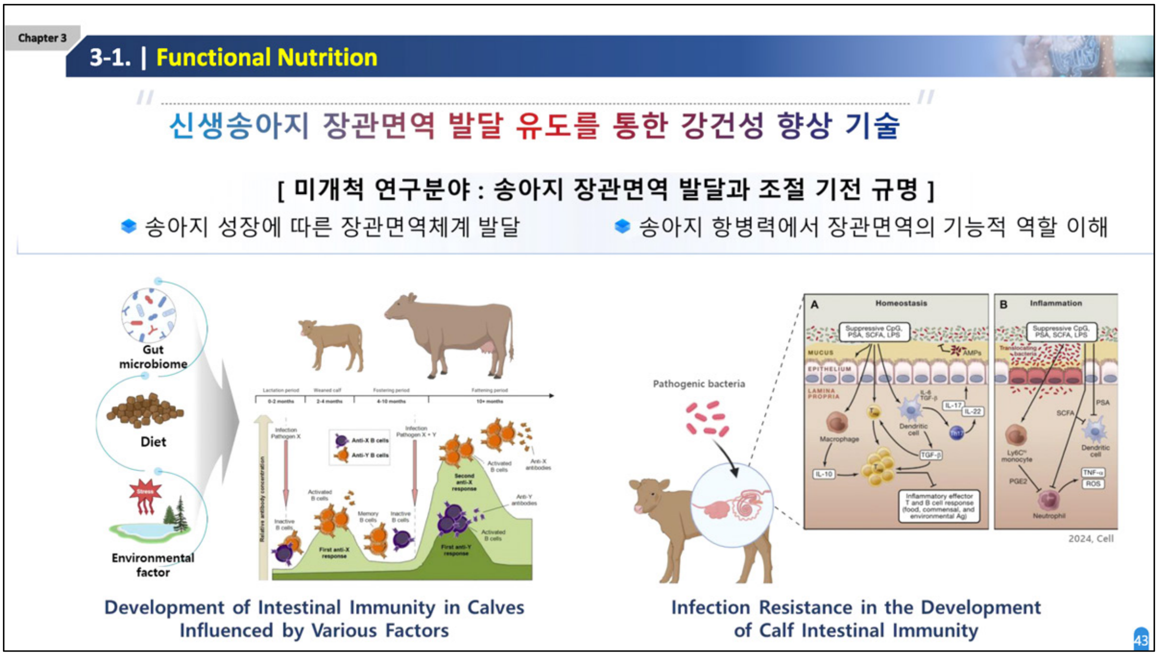
"고온기 대응 젖소 대사 및 소화율 개선 탄수화물 기반 사료소재 발굴"

Potential regulatory mechanism of sucrose administration on metabolic process in dairy cows with heat stress.

Ban et al., Scientific Reports (2025)



Gut Immunity Development of neonatal calves



Chapter 3

3-1. | **Functional Nutrition**

신생송아지 장관면역 발달 유도를 통한 강건성 향상 기술

[미개척 연구분야 : 송아지 장관면역 발달과 조절 기전 규명]

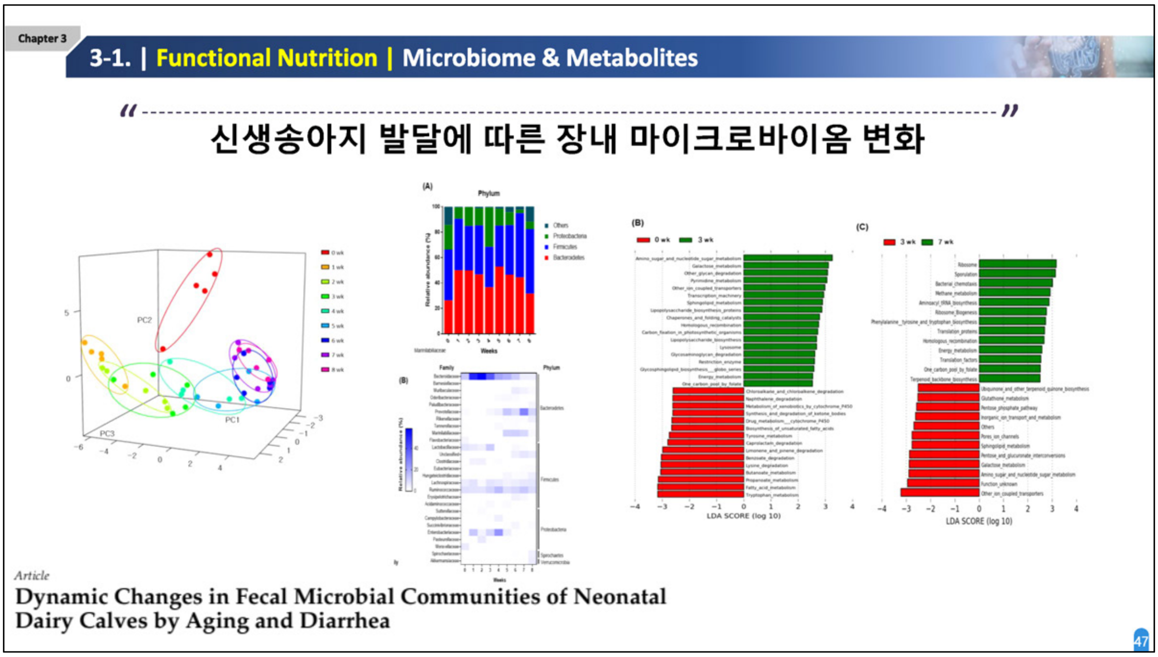
- 송아지 성장에 따른 장관면역체계 발달
- 송아지 항병력에서 장관면역의 기능적 역할 이해

2024, Cell

Development of Intestinal Immunity in Calves Influenced by Various Factors

Infection Resistance in the Development of Calf Intestinal Immunity

43



Chapter 3

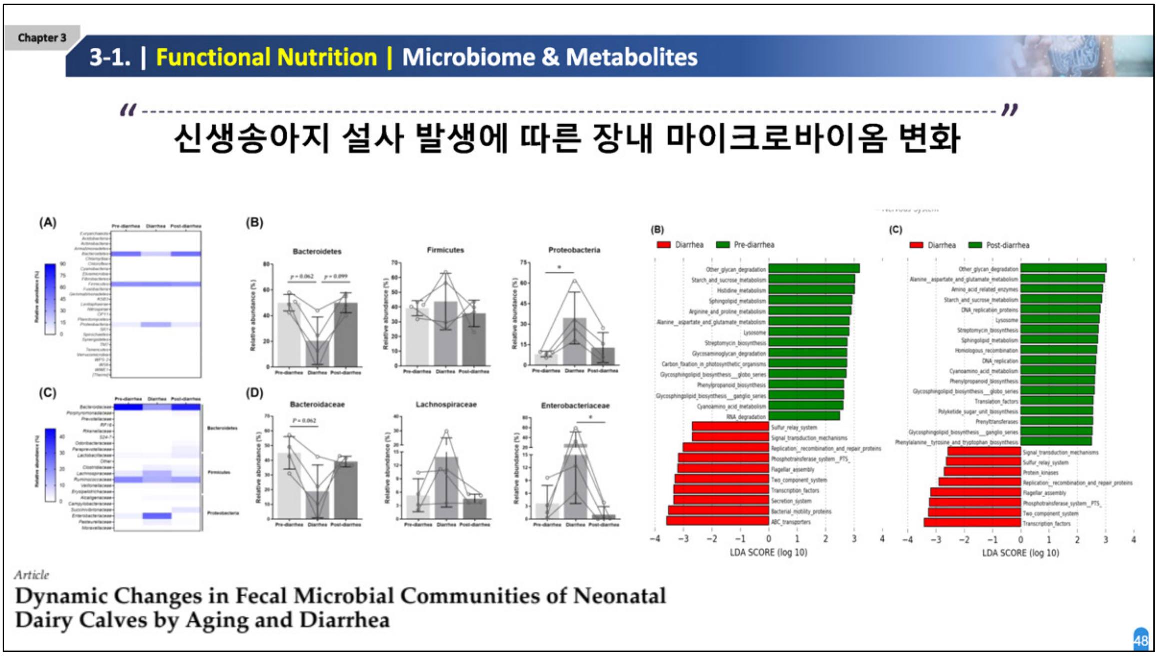
3-1. | **Functional Nutrition** | **Microbiome & Metabolites**

신생송아지 발달에 따른 장내 마이크로바이옴 변화

Article

Dynamic Changes in Fecal Microbial Communities of Neonatal Dairy Calves by Aging and Diarrhea

47



Chapter 3

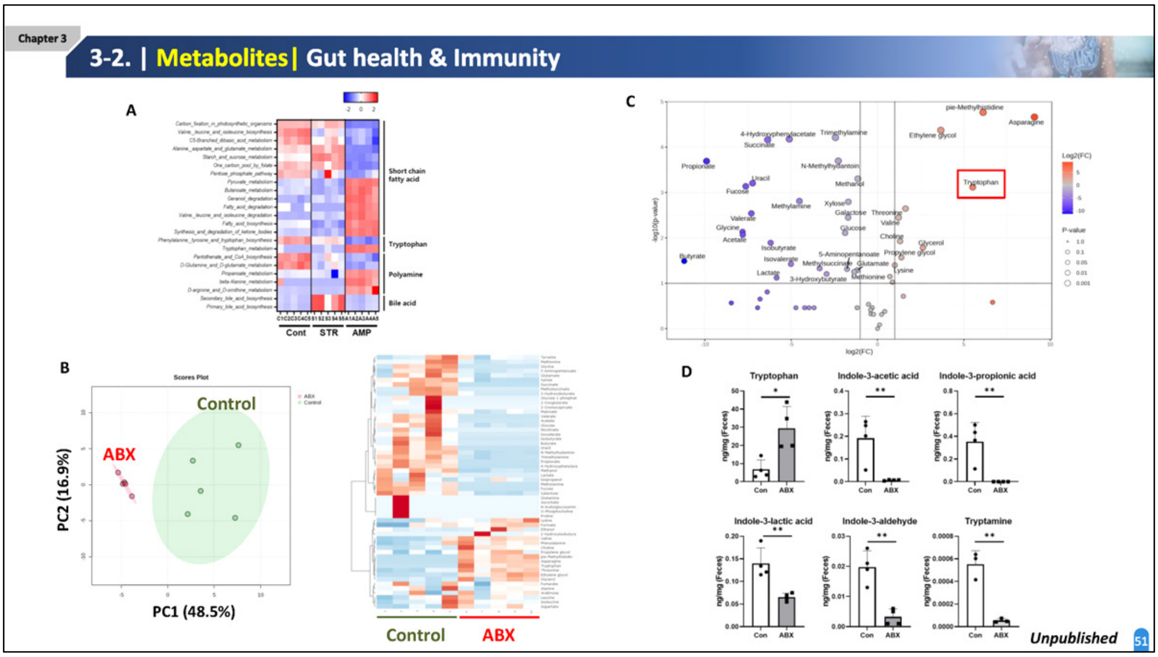
3-1. | Functional Nutrition | Microbiome & Metabolites

신생송아지 설사 발생에 따른 장내 마이크로바이옴 변화

Article

Dynamic Changes in Fecal Microbial Communities of Neonatal Dairy Calves by Aging and Diarrhea

48

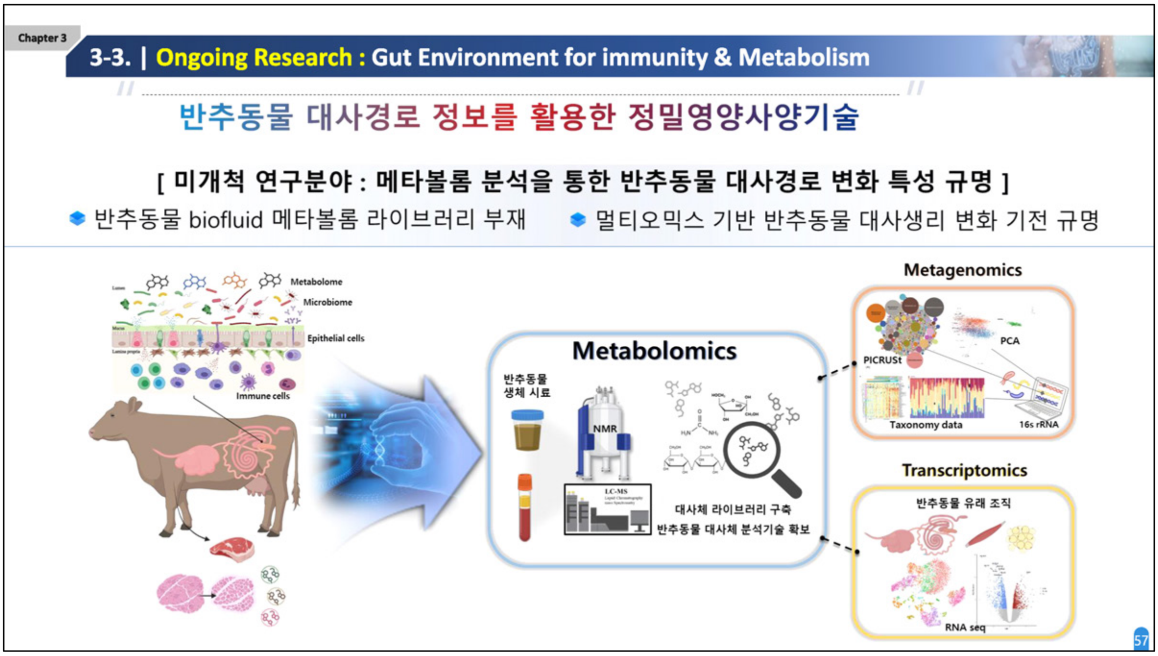


Chapter 3

3-2. | Metabolites | Gut health & Immunity

Unpublished

51

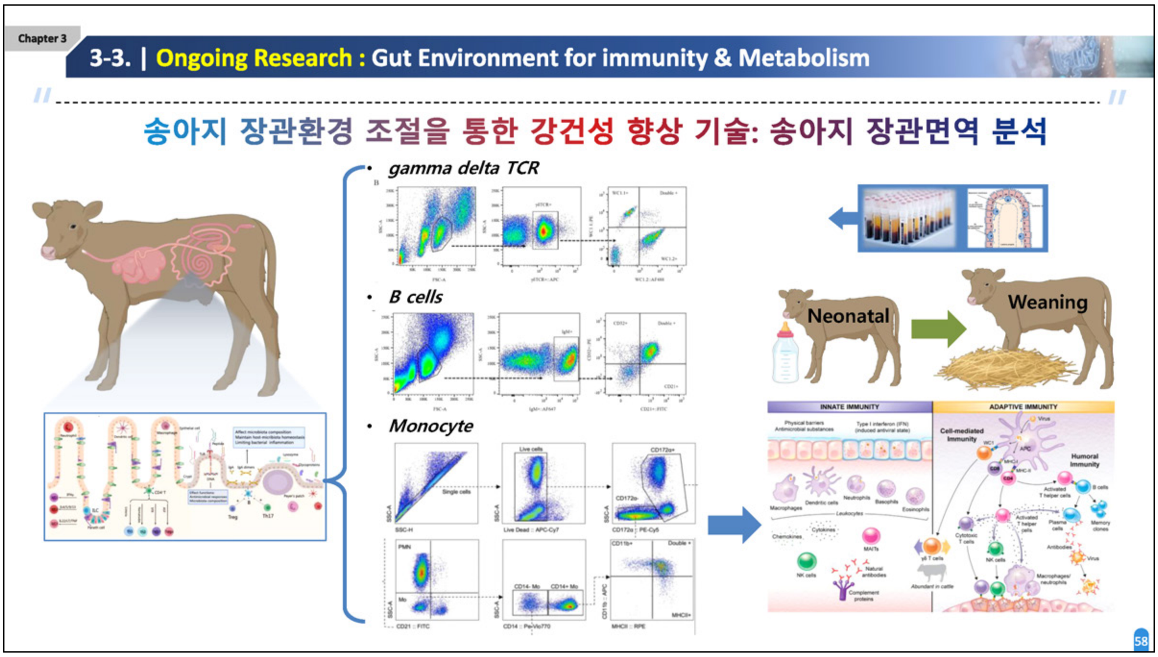


3-3. | Ongoing Research : Gut Environment for immunity & Metabolism

반추동물 대사경로 정보를 활용한 정밀영양사양기술

[미개척 연구분야 : 메타볼롬 분석을 통한 반추동물 대사경로 변화 특성 규명]

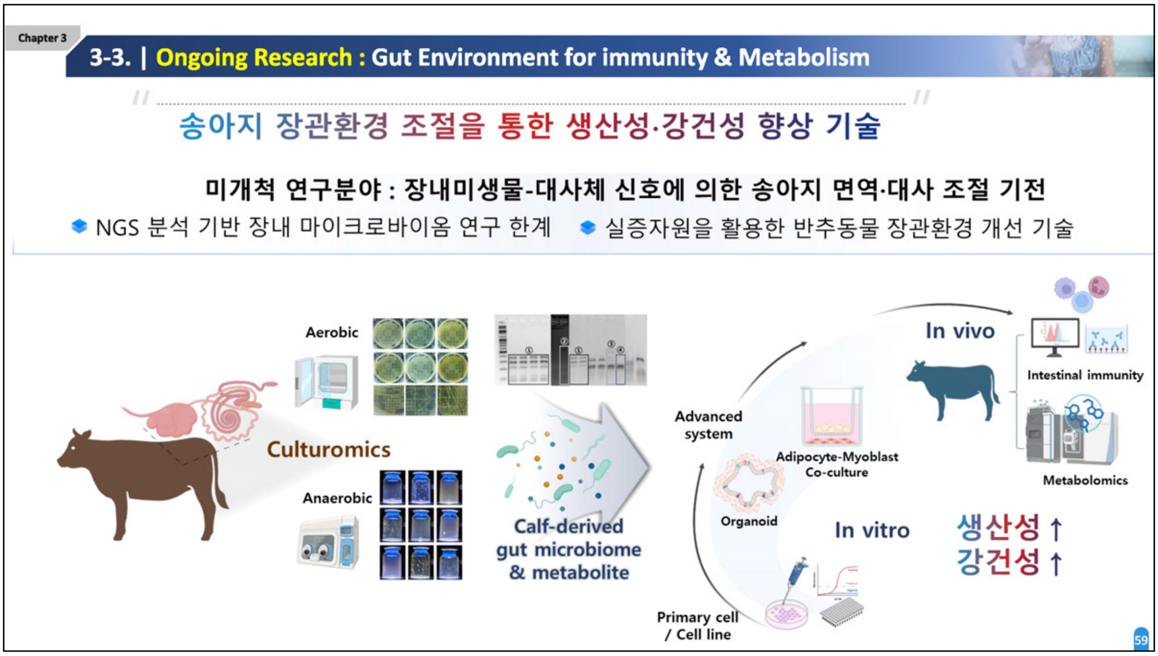
- 반추동물 biofluid 메타볼롬 라이브러리 부재
- 멀티오믹스 기반 반추동물 대사생리 변화 기전 규명



3-3. | Ongoing Research : Gut Environment for immunity & Metabolism

송아지 장관환경 조절을 통한 강건성 향상 기술: 송아지 장관면역 분석

- *gamma delta TCR*
- *B cells*
- *Monocyte*



Chapter 3

3-3. | Ongoing Research : Gut Environment for immunity & Metabolism

송아지 장관환경 조절을 통한 생산성·강건성 향상 기술

미개척 연구분야 : 장내미생물-대사체 신호에 의한 송아지 면역·대사 조절 기전

- NGS 분석 기반 장내 마이크로바이옴 연구 한계
- 실증자원을 활용한 반추동물 장관환경 개선 기술

Characterization of Core Microbiota and Methane-Related Functional Genes associated with Methane Emissions and Growth Performance in Cattle

곽 민 진
Kwak, Min-Jin
(국민대학교)
(Kookmin University)

Curriculum Vitae

- 2025~현재 국민대학교 임산생명공학과 조교수
- 2024~2025 University of Florida 박사 후 연구원
- 2022~2024 서울대학교 연구조교수
- 2021~2022 고려대학교 연구교수
- 2015~2021 고려대학교 생명공학과 석박사통합과정
- 2008~2015 고려대학교 생명공학부 이학사

Characterization of Core Microbiota and Methane-Related Functional Genes Associated with Methane Emissions and Growth Performance in Cattle

Min-Jin Kwak, Ph. D.
Department of Forest Products and Biotechnology
Kookmin University

Introduction

Global warming

❑ **Fire disaster**

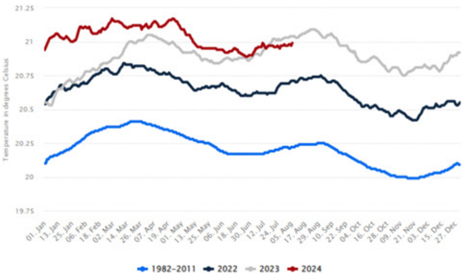
Global warming breached 1.5°C threshold in 2024

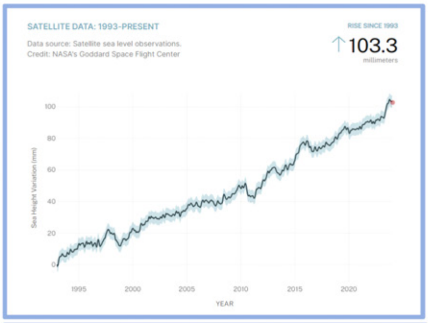
Annual global average temperatures, compared to the 1850-1900 pre-industrial period. °C

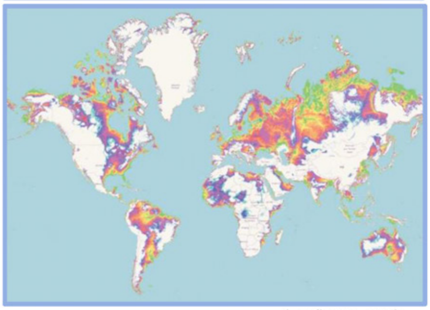
By Kate Abnett • Source: Copernicus Climate Change Service

Global warming

❑ **Flood disaster**



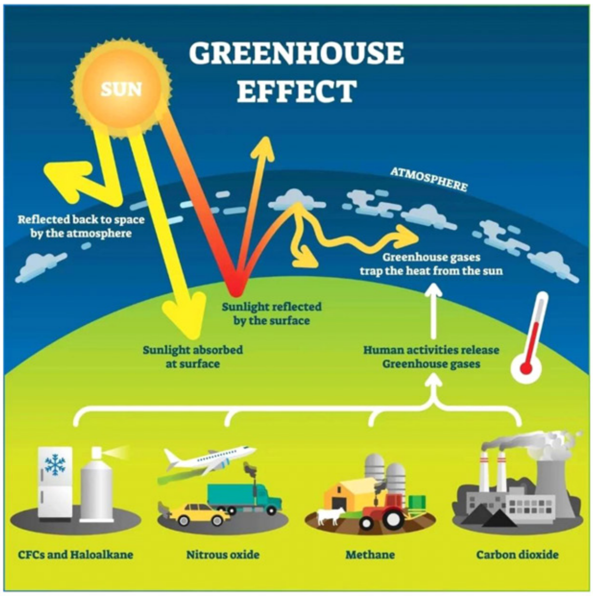


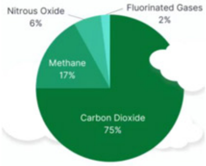


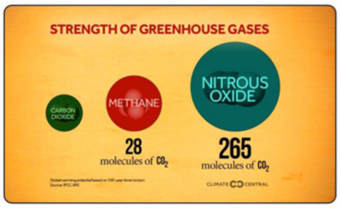
(España et al., 2024)

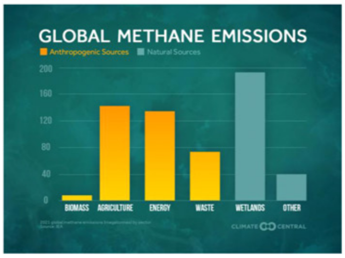
Greenhouse gases

- **Greenhouse gases**: gases in the earth's atmosphere that trap heat



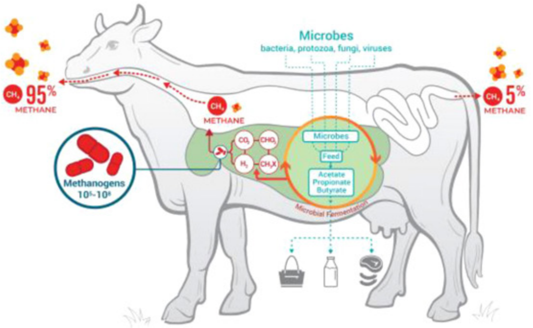






Global Methane Pledge in livestock

- Reduce **methane emissions by 30%** below 2020 levels **until 2030**
- If successful, warming is projected to **decrease by 0.2°C by 2050**
- **160 countries** signed up to GMP



ANIMAL & FEED MANAGEMENT

- Feed processing
- Genetic selection
- Improving animal health
- Improving pasture management
- Increasing feeding level
- Increasing forage quality
- Optimizing temperature
- TMR feeding

DIET FORMULATION

- By-products
- Decreasing forage-to-concentrate ratios
- Minerals and salts
- Oils and fats
- Oilseeds
- Increasing protein
- Tanniferous forages
- Urea

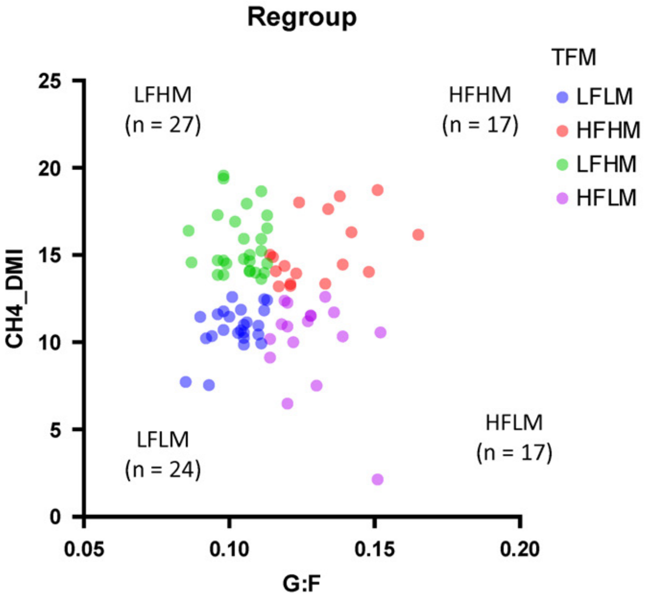
RUMEN MANIPULATION

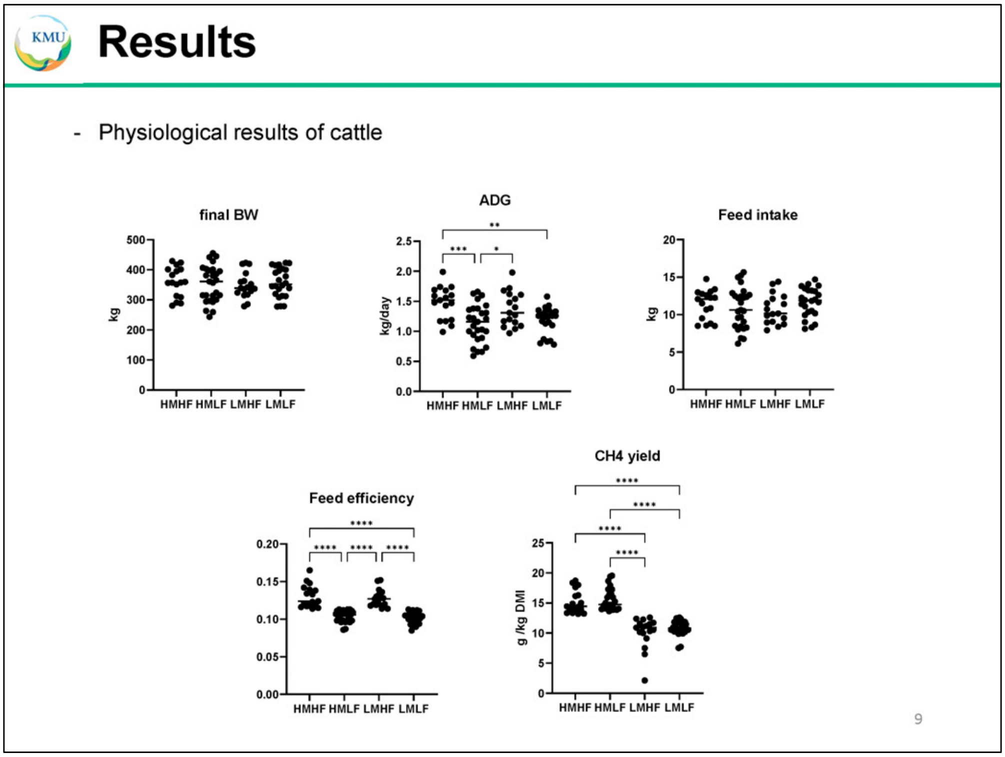
- Additives
- Defaunation
- Electron sinks

Results

Results

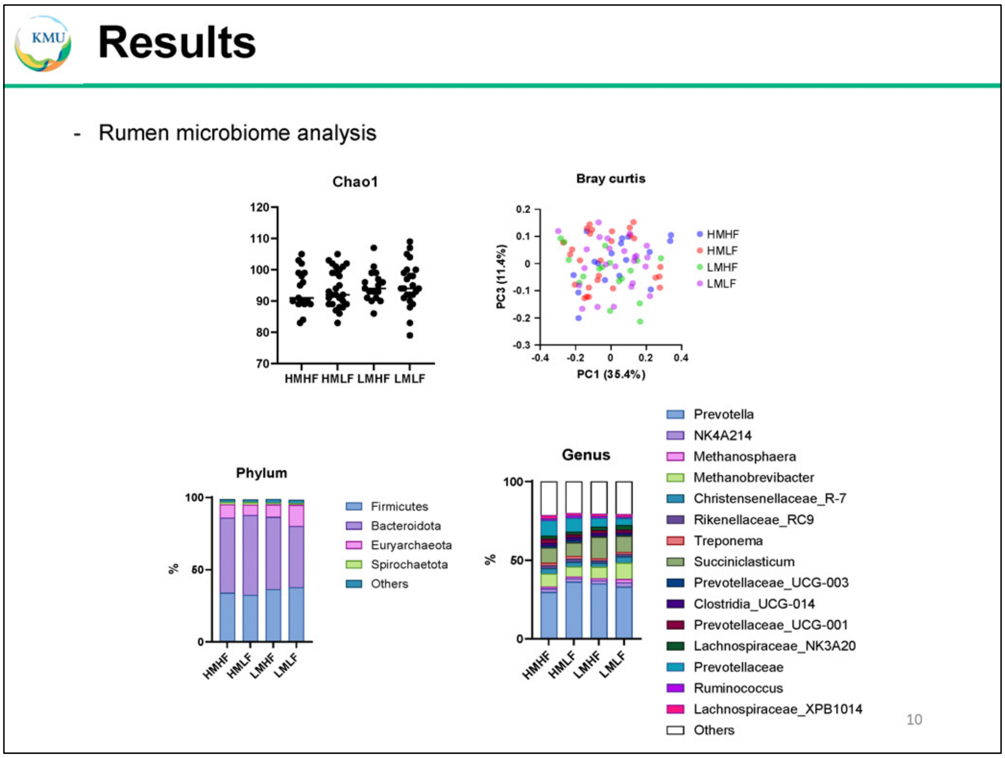
- Characterization of experimental treatments based on FE and Methane yields





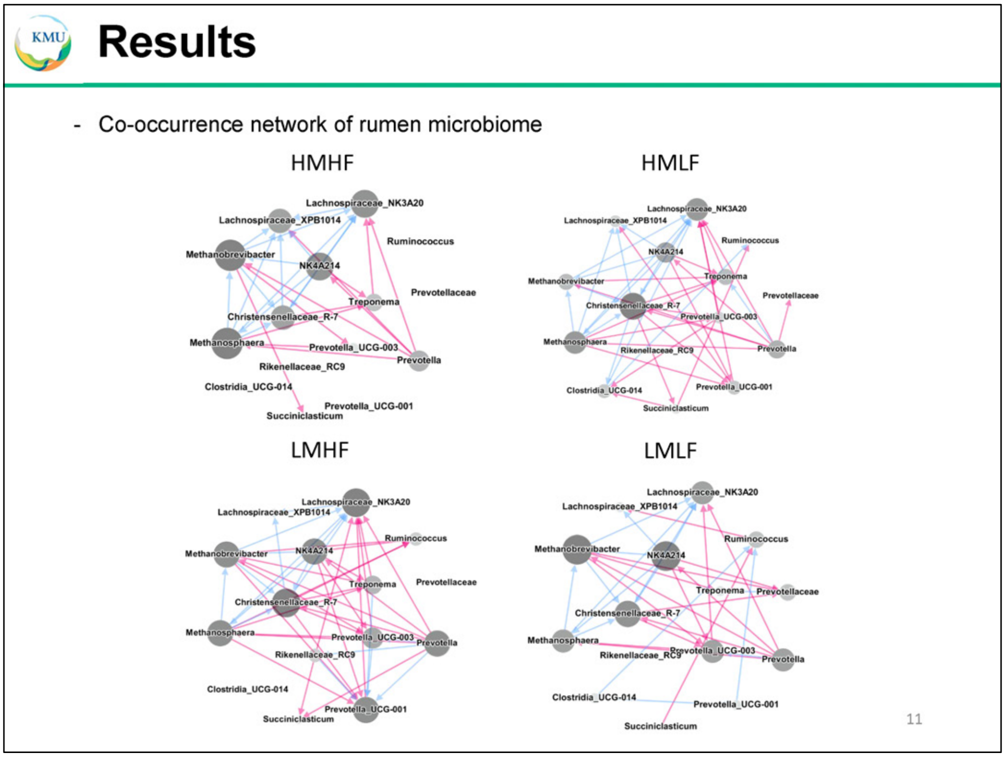
Results

- Physiological results of cattle



Results

- Rumen microbiome analysis

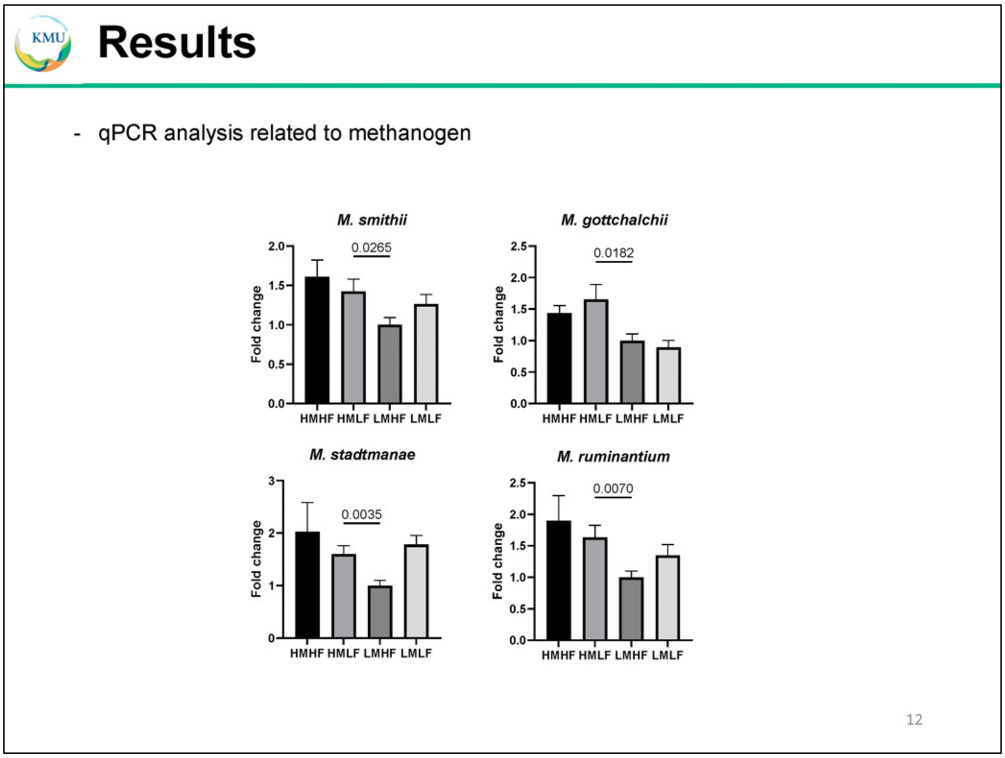


KMU

Results

- Co-occurrence network of rumen microbiome

11

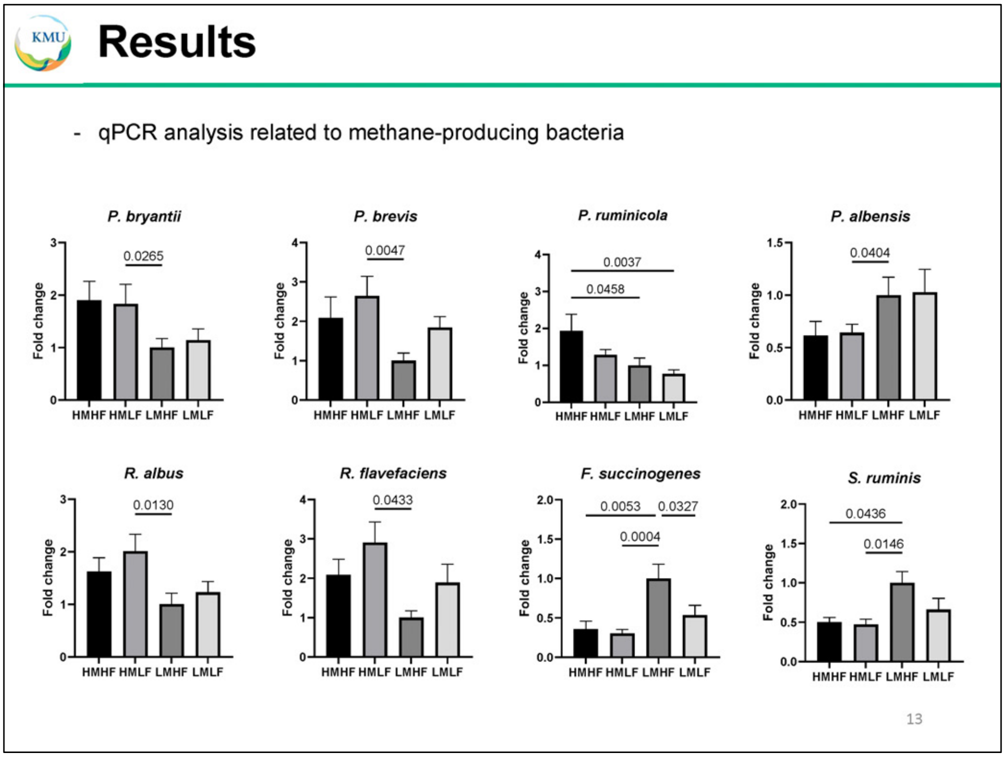


KMU

Results

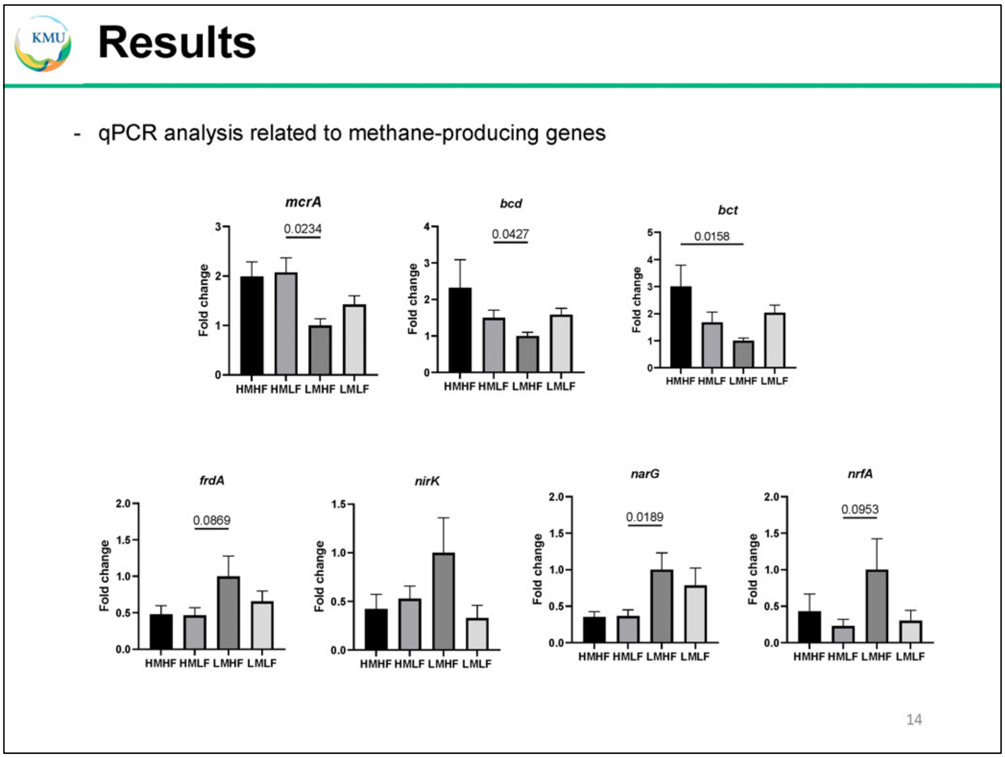
- qPCR analysis related to methanogen

12



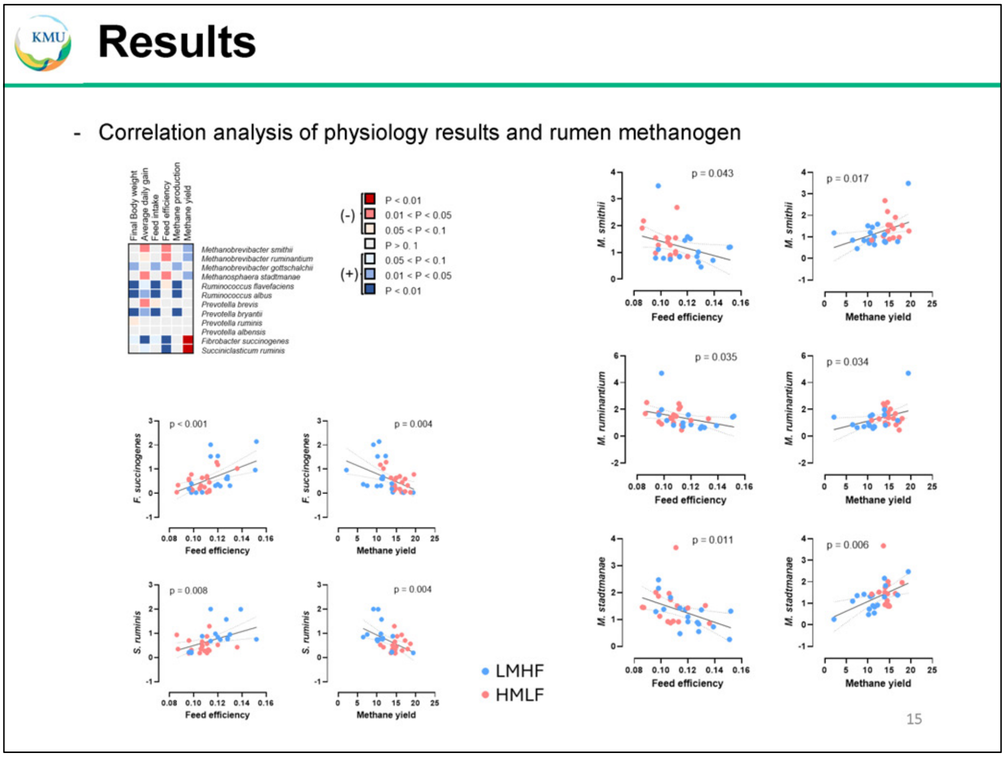
Results

- qPCR analysis related to methane-producing bacteria



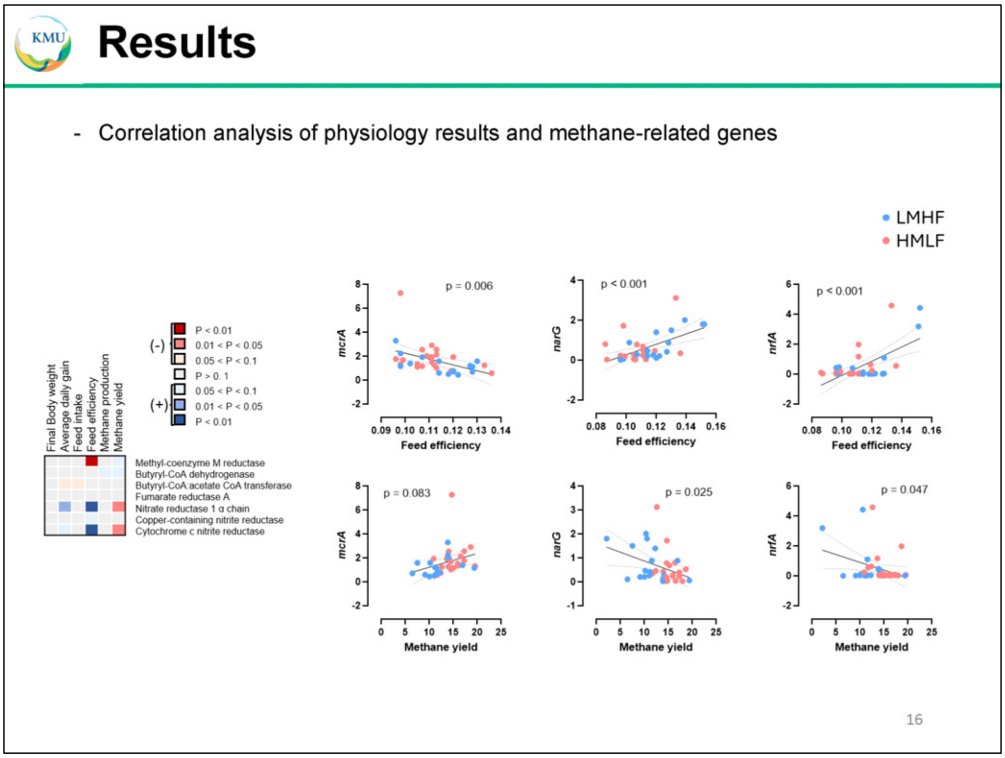
Results

- qPCR analysis related to methane-producing genes



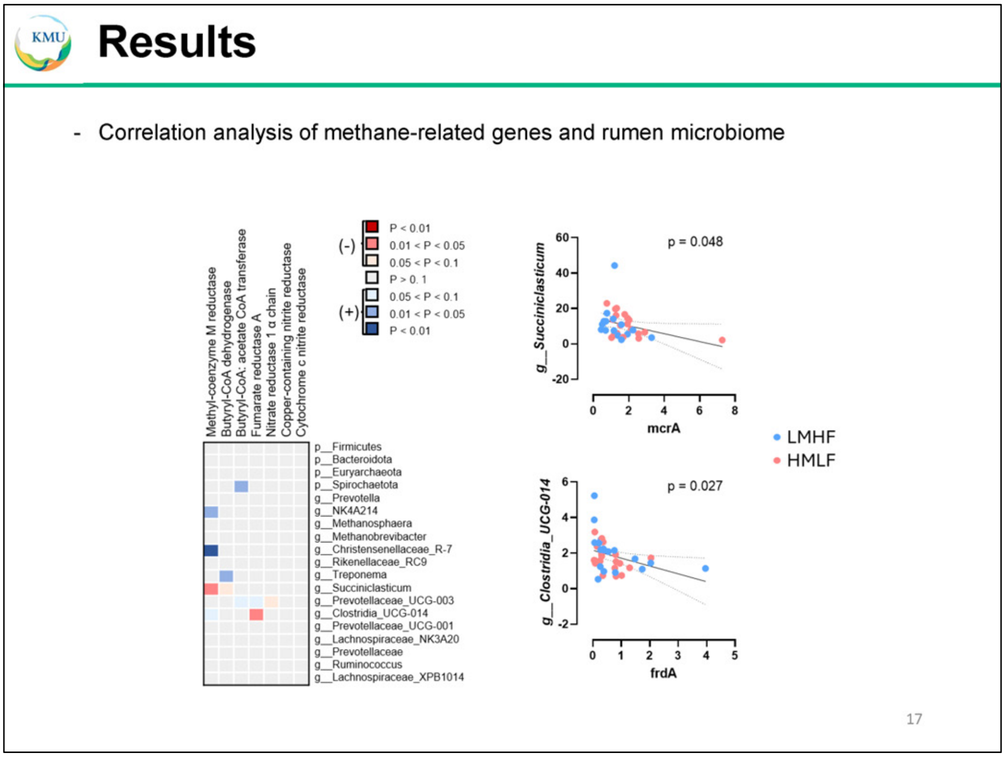
Results

- Correlation analysis of physiology results and rumen methanogen



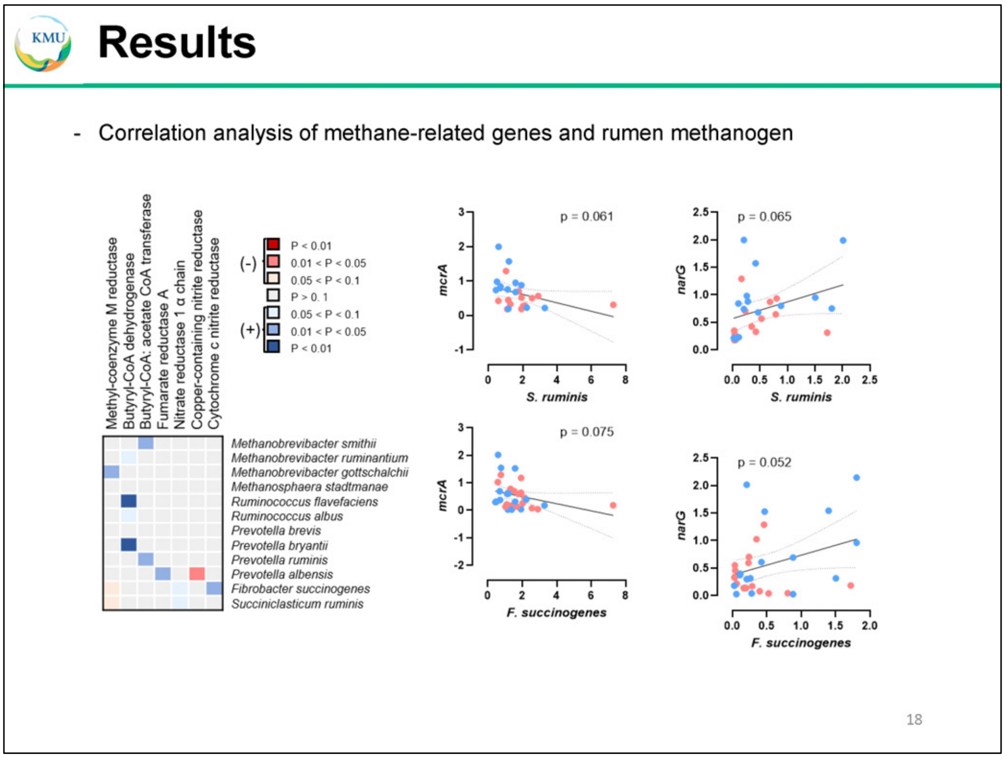
Results

- Correlation analysis of physiology results and methane-related genes



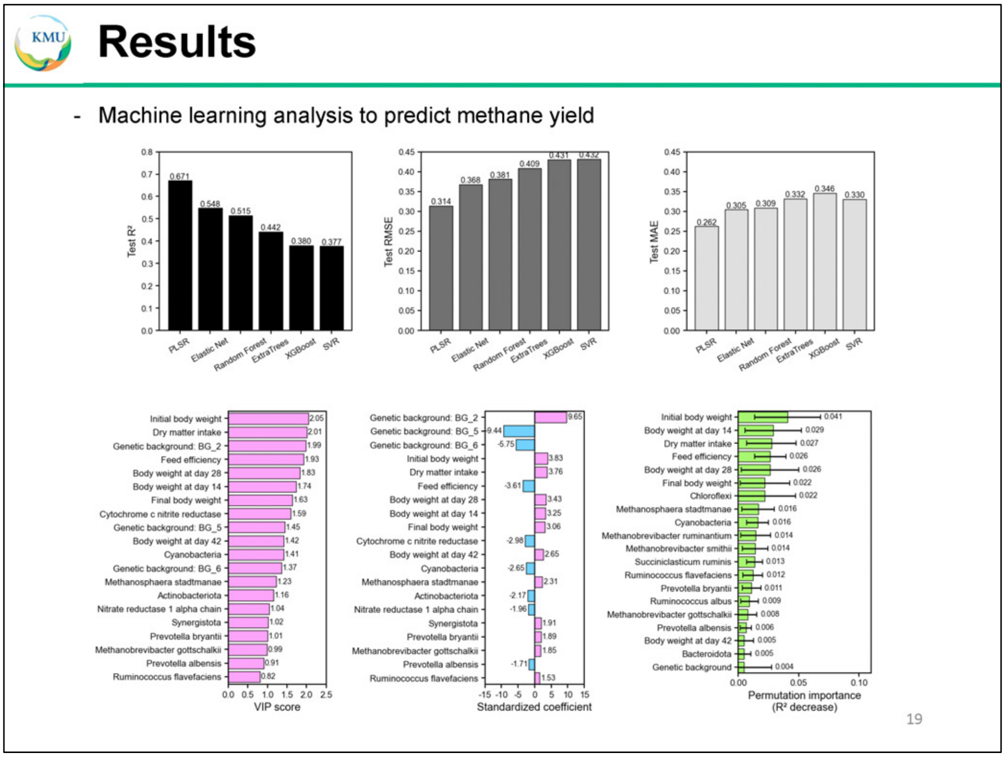
Results

- Correlation analysis of methane-related genes and rumen microbiome



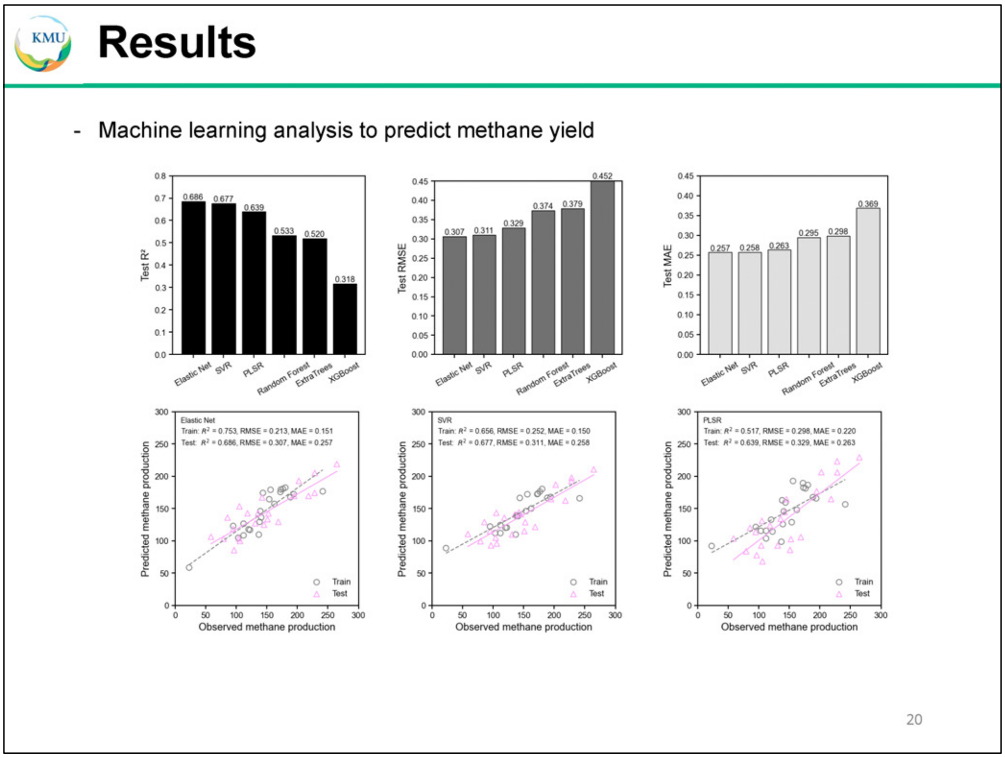
Results

- Correlation analysis of methane-related genes and rumen methanogen



Results

- Machine learning analysis to predict methane yield



Results

- Machine learning analysis to predict methane yield

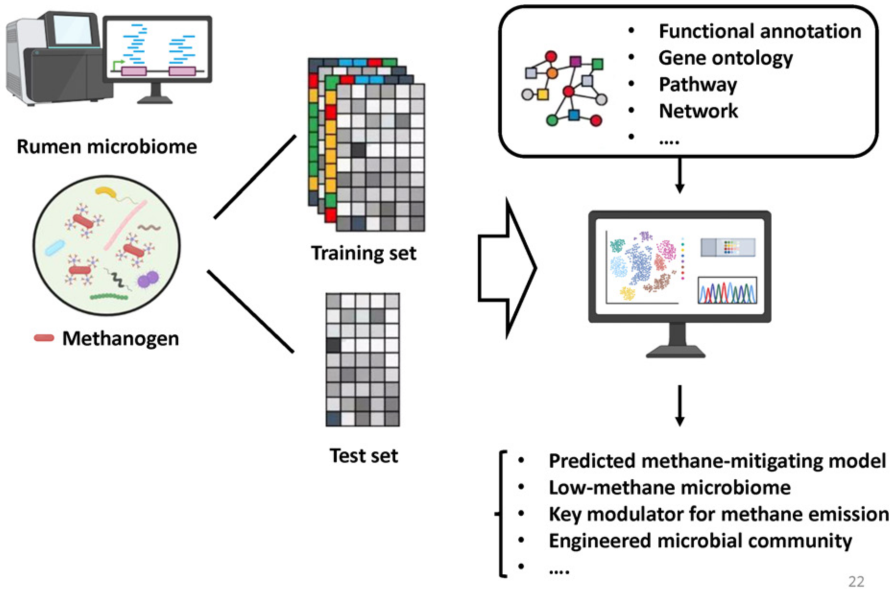
Elastic Net
Train: R^2 = 0.753, RMSE = 0.213, MAE = 0.151
Test: R^2 = 0.686, RMSE = 0.307, MAE = 0.257

SVR
Train: R^2 = 0.656, RMSE = 0.252, MAE = 0.150
Test: R^2 = 0.677, RMSE = 0.311, MAE = 0.258

PLSR
Train: R^2 = 0.517, RMSE = 0.298, MAE = 0.220
Test: R^2 = 0.639, RMSE = 0.329, MAE = 0.263

Conclusion & Perspectives

ML-based mitigating methane emission



Rumen microbiome

Methanogen

Training set

Test set

- Functional annotation
- Gene ontology
- Pathway
- Network
- ….

- Predicted methane-mitigating model
- Low-methane microbiome
- Key modulator for methane emission
- Engineered microbial community
- ….

THANK YOU

FOR YOUR ATTENTION!!!

ANY QUESTION ?

Rumen-Derived Next Generation Probiotics for Sustainable Bioplastic Production and Microplastic Toxicity Mitigation

김 영 훈
Kim, Younghoon
(서울대학교)
(Seoul National University)

Curriculum Vitae

2019~현재	서울대학교 농생명공학부 동물생명공학전공 교수
2025~2026	University of Florida Visiting Professor
2024~현재	식약처 축산물위생심의위원회 전문위원
2022~현재	한국축산학회 영문지 JAST 편집위원장
2021~2024	한국연구재단 생명과학단 기반생명 동물자원학분야 전문위원

Rumen-Derived Next Generation Probiotics for Sustainable Bioplastic Production and Microplastic Toxicity Mitigation

Seoul National University
Department of Agricultural Biotechnology
Younghoon Kim

Source: Tanaka et al. (2022) Polymers, 14(15):3063

1 | Background

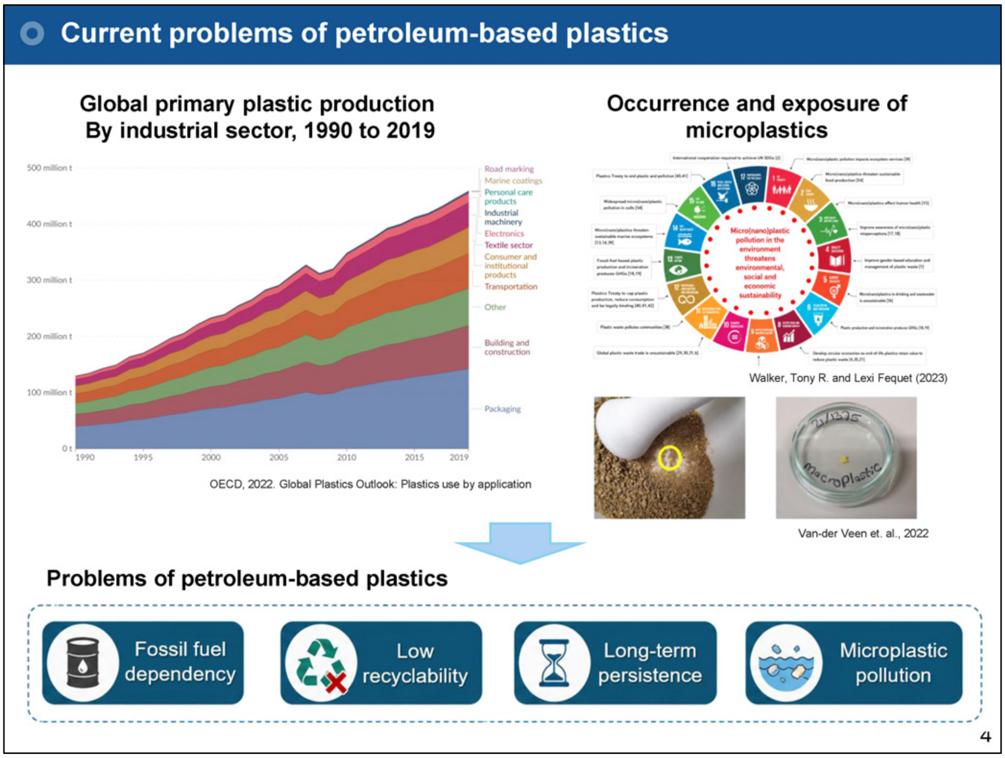
2 | Results (1)
- PHA producing bacteria

3 | Results (2)
- Microplastic toxicity

4 | Further study

01

Background



Current problems of petroleum-based plastics

Global primary plastic production By industrial sector, 1990 to 2019

OECD, 2022. Global Plastics Outlook: Plastics use by application

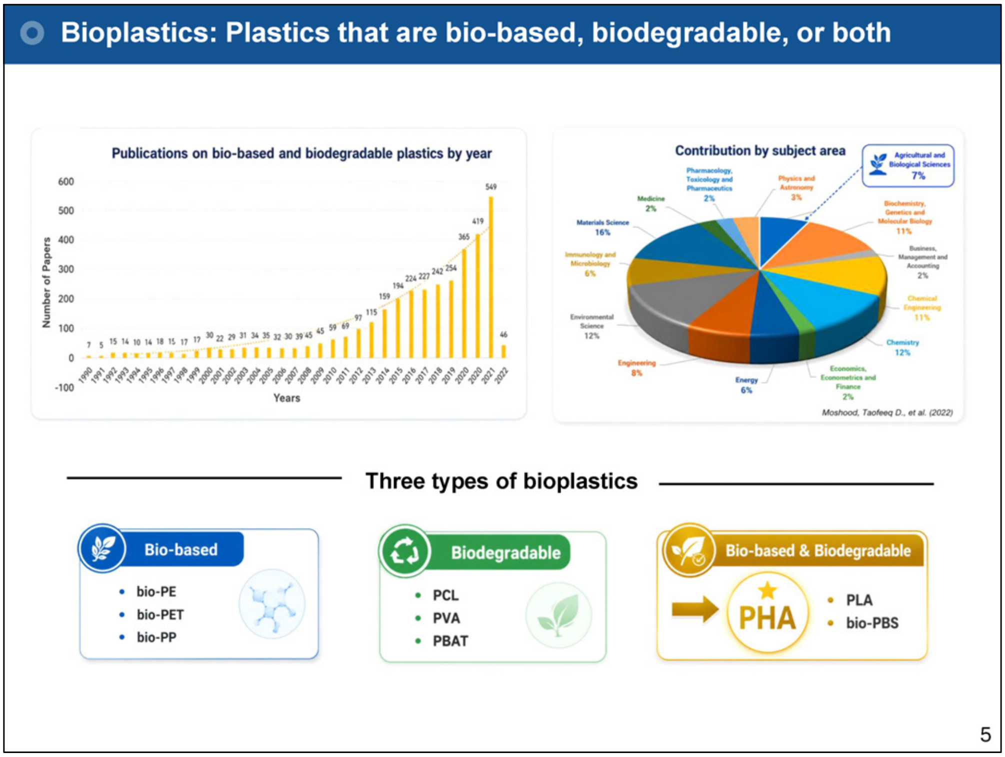
Occurrence and exposure of microplastics

Walker, Tony R. and Lexi Fequet (2023)

Van-der Veen et. al., 2022

Problems of petroleum-based plastics

- Fossil fuel dependency
- Low recyclability
- Long-term persistence
- Microplastic pollution



Bioplastics: Plastics that are bio-based, biodegradable, or both

Moshood, Taofeeq D., et al. (2022)

Three types of bioplastics

Bio-based

- bio-PE
- bio-PET
- bio-PP

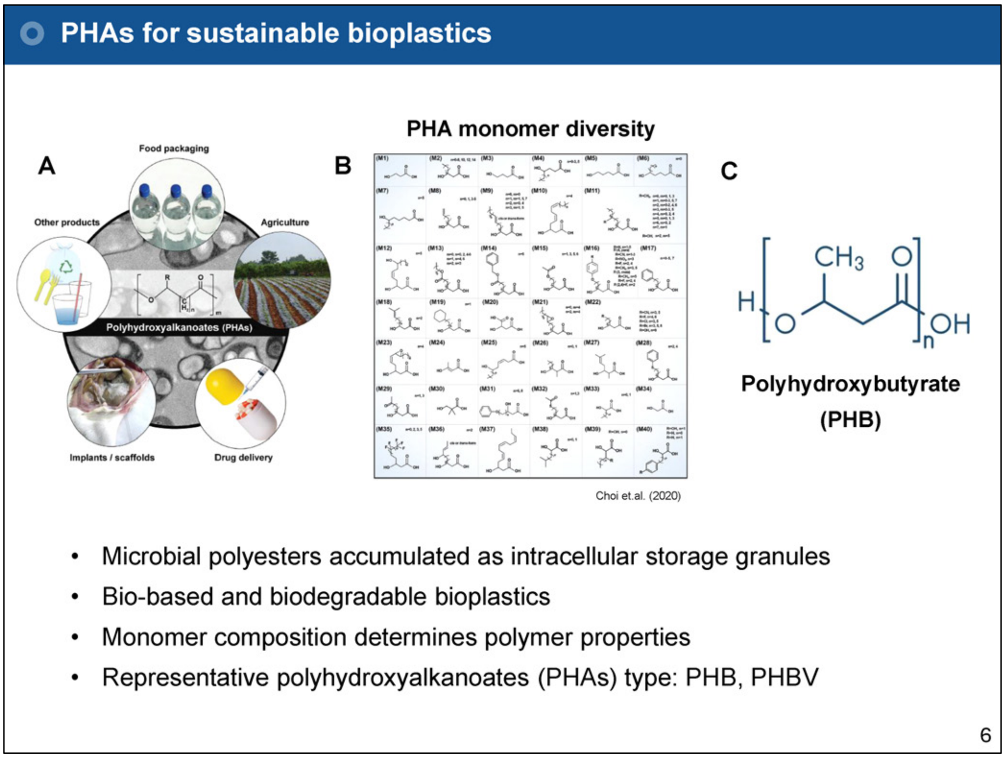
Biodegradable

- PCL
- PVA
- PBAT

Bio-based & Biodegradable

PHA

- PLA
- bio-PBS



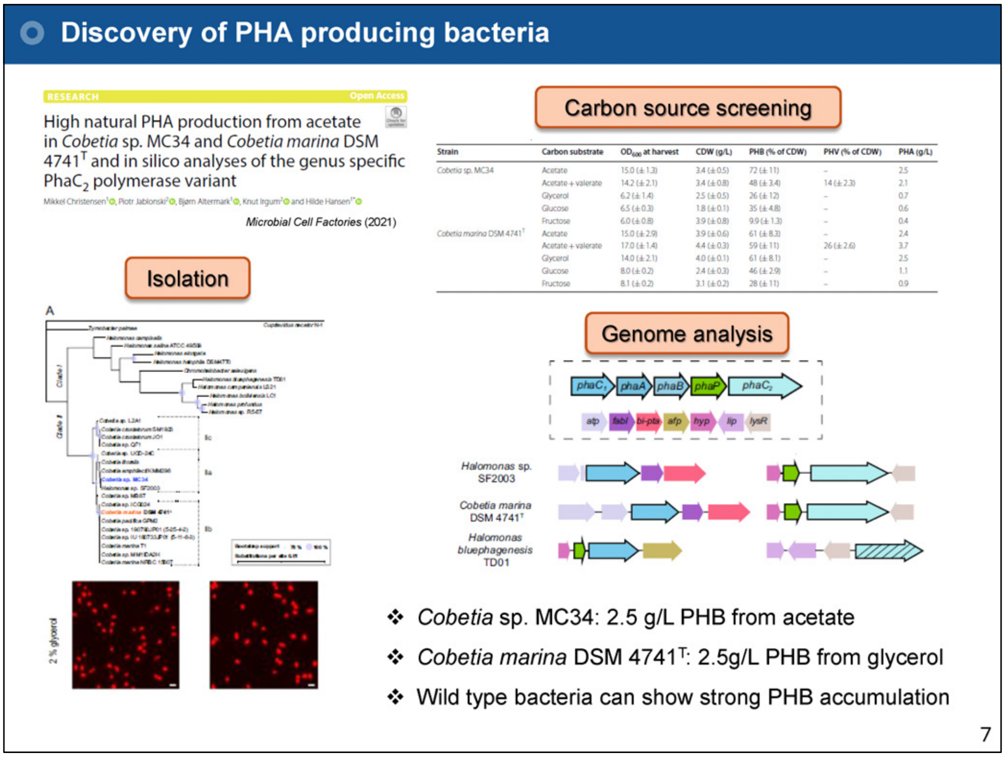
PHAs for sustainable bioplastics

PHA monomer diversity

Choi et.al. (2020)

Polyhydroxybutyrate (PHB)

- Microbial polyesters accumulated as intracellular storage granules
- Bio-based and biodegradable bioplastics
- Monomer composition determines polymer properties
- Representative polyhydroxyalkanoates (PHAs) type: PHB, PHBV



Discovery of PHA producing bacteria

RESEARCH Open Access

High natural PHA production from acetate in *Cobetia* sp. MC34 and *Cobetia marina* DSM 4741^T and in silico analyses of the genus specific $PhaC_2$ polymerase variant

Mikkel Christensen[1], Piotr Jablonski[2], Bjørn Altermark[1], Knut Irgum[2] and Hilde Hansen[1*]

Microbial Cell Factories (2021)

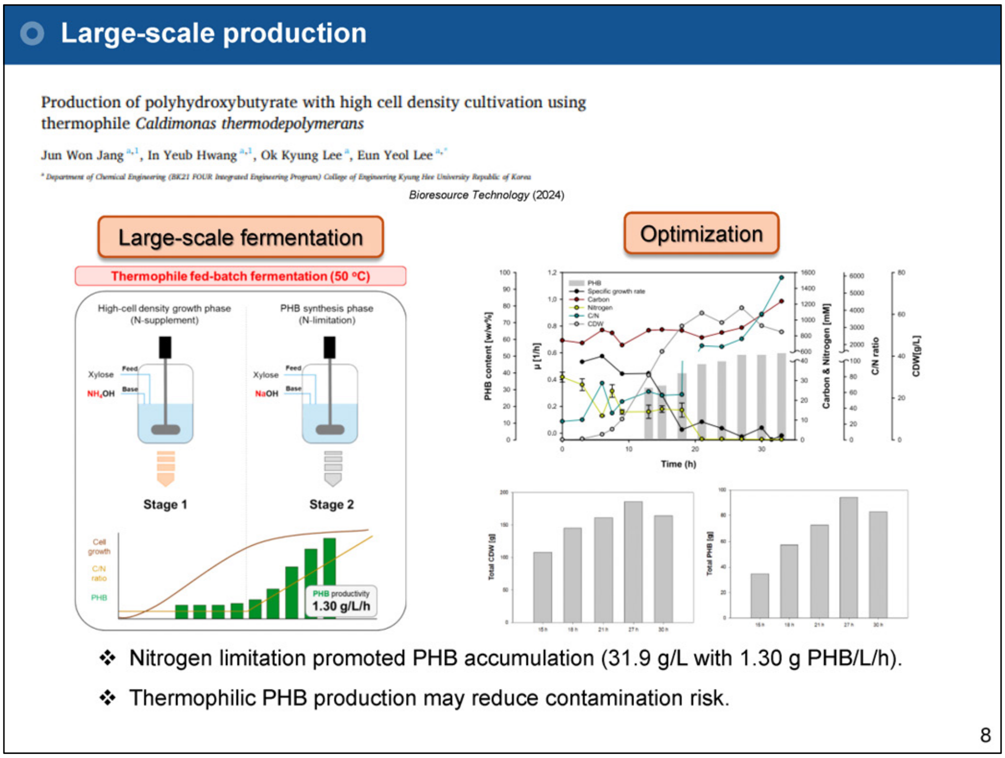
Isolation

Carbon source screening

Strain	Carbon substrate	OD_{600} at harvest	CDW (g/L)	PHB (% of CDW)	PHV (% of CDW)	PHA (g/L)
Cobetia sp. MC34	Acetate	15.0 (± 1.3)	3.4 (± 0.5)	72 (± 11)	–	2.5
	Acetate + valerate	14.2 (± 2.1)	3.4 (± 0.8)	48 (± 3.4)	14 (± 2.3)	2.1
	Glycerol	6.2 (± 1.4)	2.5 (± 0.5)	26 (± 12)	–	0.7
	Glucose	6.5 (± 0.3)	1.8 (± 0.1)	35 (± 4.8)	–	0.6
	Fructose	6.0 (± 0.8)	3.9 (± 0.8)	9.9 (± 1.3)	–	0.4
Cobetia marina DSM 4741^T	Acetate	15.0 (± 2.9)	3.9 (± 0.6)	61 (± 8.3)	–	2.4
	Acetate + valerate	17.0 (± 1.4)	4.4 (± 0.3)	59 (± 11)	26 (± 2.6)	3.7
	Glycerol	14.0 (± 2.1)	4.0 (± 0.1)	61 (± 8.1)	–	2.5
	Glucose	8.0 (± 0.2)	2.4 (± 0.3)	46 (± 2.9)	–	1.1
	Fructose	8.1 (± 0.2)	3.1 (± 0.2)	28 (± 11)	–	0.9

Genome analysis

- ❖ *Cobetia* sp. MC34: 2.5 g/L PHB from acetate
- ❖ *Cobetia marina* DSM 4741^T: 2.5g/L PHB from glycerol
- ❖ Wild type bacteria can show strong PHB accumulation



Large-scale production

Production of polyhydroxybutyrate with high cell density cultivation using thermophile *Caldimonas thermodepolymerans*

Jun Won Jang[a,1], In Yeub Hwang[a,1], Ok Kyung Lee[a], Eun Yeol Lee[a,*]

[a] Department of Chemical Engineering (BK21 FOUR Integrated Engineering Program) College of Engineering Kyung Hee University Republic of Korea

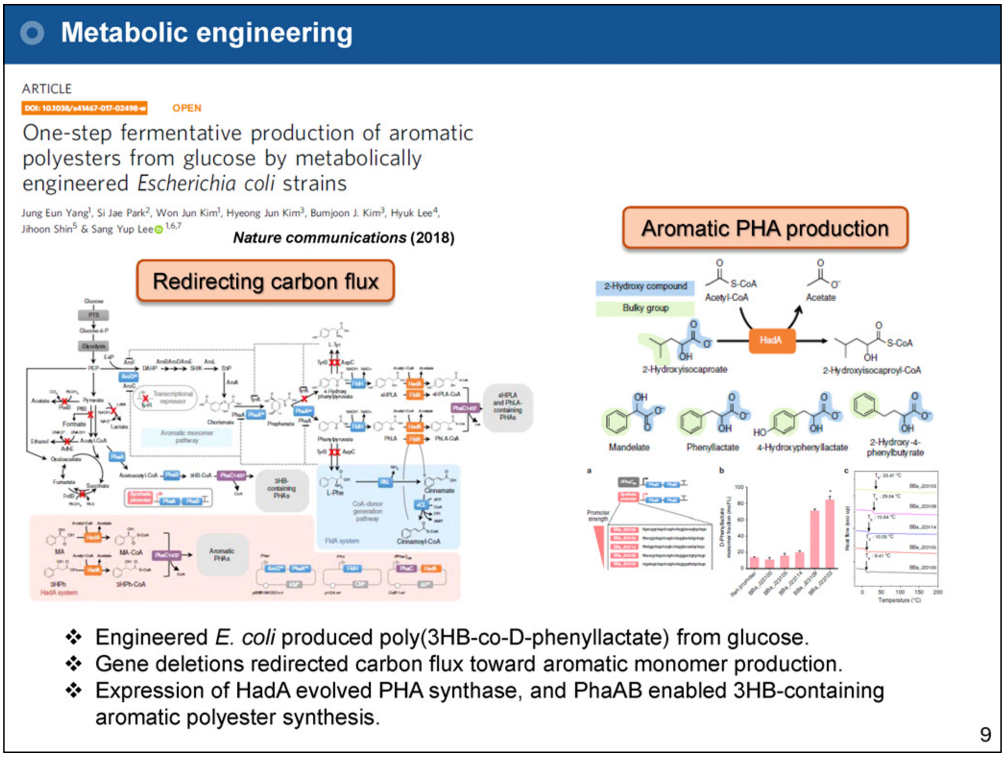
Bioresource Technology (2024)

Large-scale fermentation

Thermophile fed-batch fermentation (50 °C)

Optimization

- ❖ Nitrogen limitation promoted PHB accumulation (31.9 g/L with 1.30 g PHB/L/h).
- ❖ Thermophilic PHB production may reduce contamination risk.



Metabolic engineering

ARTICLE

DOI: 10.1038/s41467-017-02498-w OPEN

One-step fermentative production of aromatic polyesters from glucose by metabolically engineered *Escherichia coli* strains

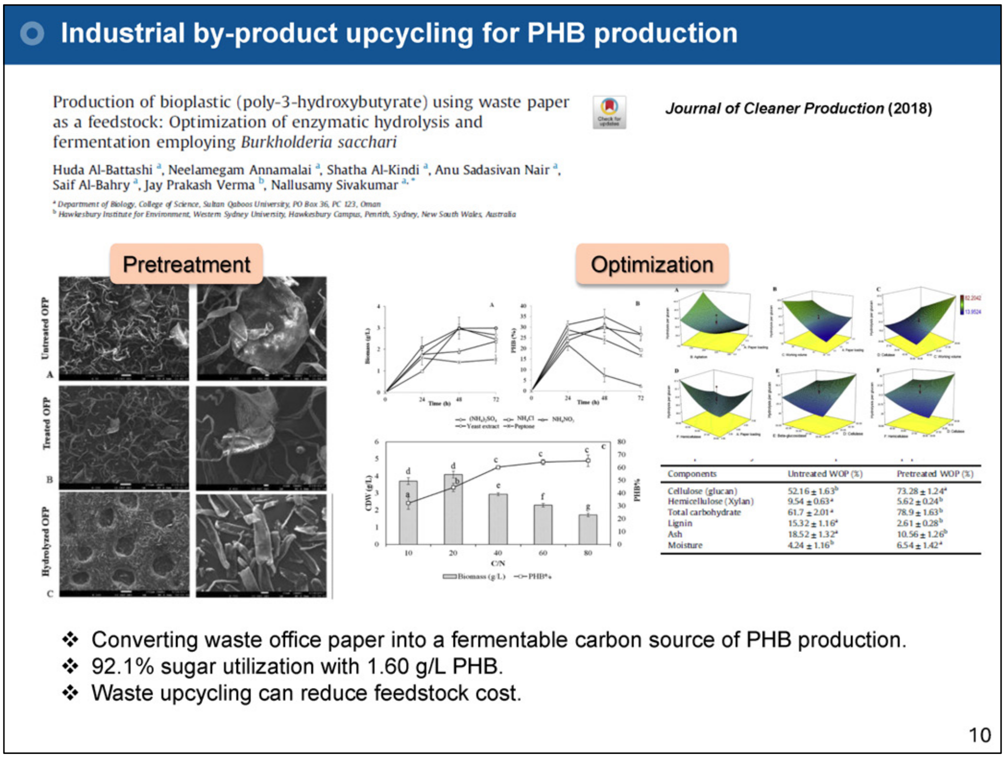
Jung Eun Yang[1], Si Jae Park[2], Won Jun Kim[1], Hyeong Jun Kim[3], Bumjoon J. Kim[3], Hyuk Lee[4], Jihoon Shin[5] & Sang Yup Lee[1,6,7]

Nature communications **(2018)**

Redirecting carbon flux

Aromatic PHA production

- ❖ Engineered *E. coli* produced poly(3HB-co-D-phenyllactate) from glucose.
- ❖ Gene deletions redirected carbon flux toward aromatic monomer production.
- ❖ Expression of HadA evolved PHA synthase, and PhaAB enabled 3HB-containing aromatic polyester synthesis.



Industrial by-product upcycling for PHB production

Production of bioplastic (poly-3-hydroxybutyrate) using waste paper as a feedstock: Optimization of enzymatic hydrolysis and fermentation employing *Burkholderia sacchari*

Journal of Cleaner Production **(2018)**

Huda Al-Battashi [a], Neelamegam Annamalai [a], Shatha Al-Kindi [a], Anu Sadasivan Nair [a], Saif Al-Bahry [a], Jay Prakash Verma [b], Nallusamy Sivakumar [a,*]

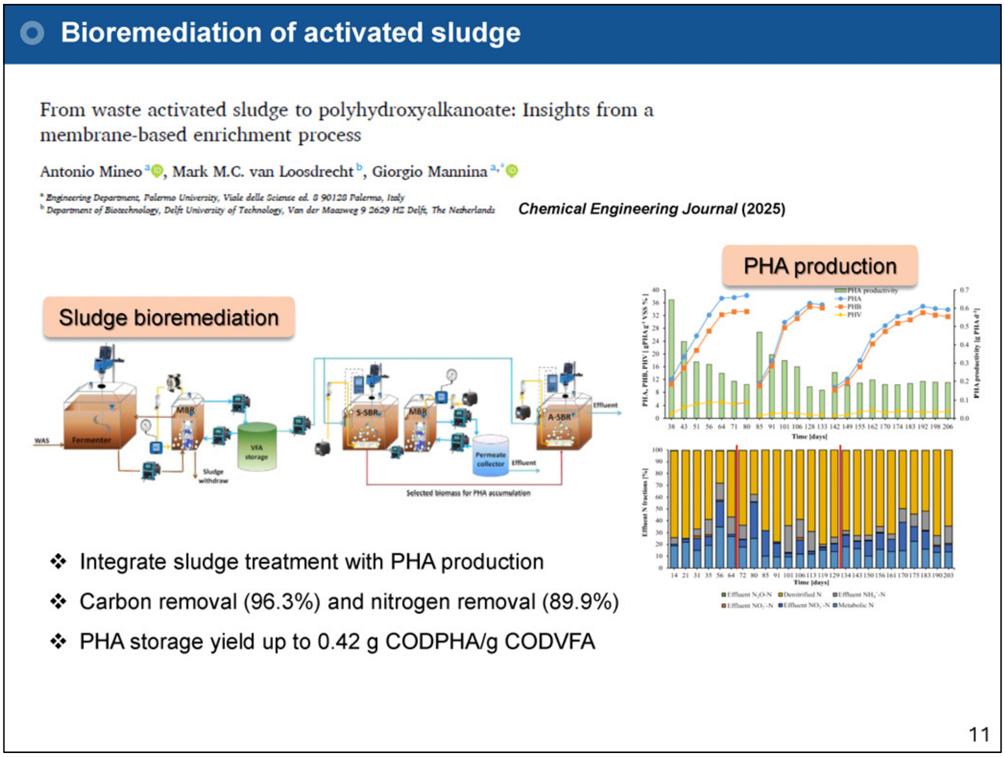
[a] *Department of Biology, College of Science, Sultan Qaboos University, PO Box 36, PC 123, Oman*
[b] *Hawkesbury Institute for Environment, Western Sydney University, Hawkesbury Campus, Penrith, Sydney, New South Wales, Australia*

Pretreatment

Optimization

Components	Untreated WOP (%)	Pretreated WOP (%)
Cellulose (glucan)	52.16 ± 1.63^b	73.28 ± 1.24^a
Hemicellulose (Xylan)	9.54 ± 0.63^a	5.62 ± 0.24^b
Total carbohydrate	61.7 ± 2.01^a	78.9 ± 1.63^b
Lignin	15.32 ± 1.16^a	2.61 ± 0.28^b
Ash	18.52 ± 1.32^a	10.56 ± 1.26^b
Moisture	4.24 ± 1.16^b	6.54 ± 1.42^a

- ❖ Converting waste office paper into a fermentable carbon source of PHB production.
- ❖ 92.1% sugar utilization with 1.60 g/L PHB.
- ❖ Waste upcycling can reduce feedstock cost.



Bioremediation of activated sludge

From waste activated sludge to polyhydroxyalkanoate: Insights from a membrane-based enrichment process

Antonio Mineo[a], Mark M.C. van Loosdrecht[b], Giorgio Mannina[a,*]

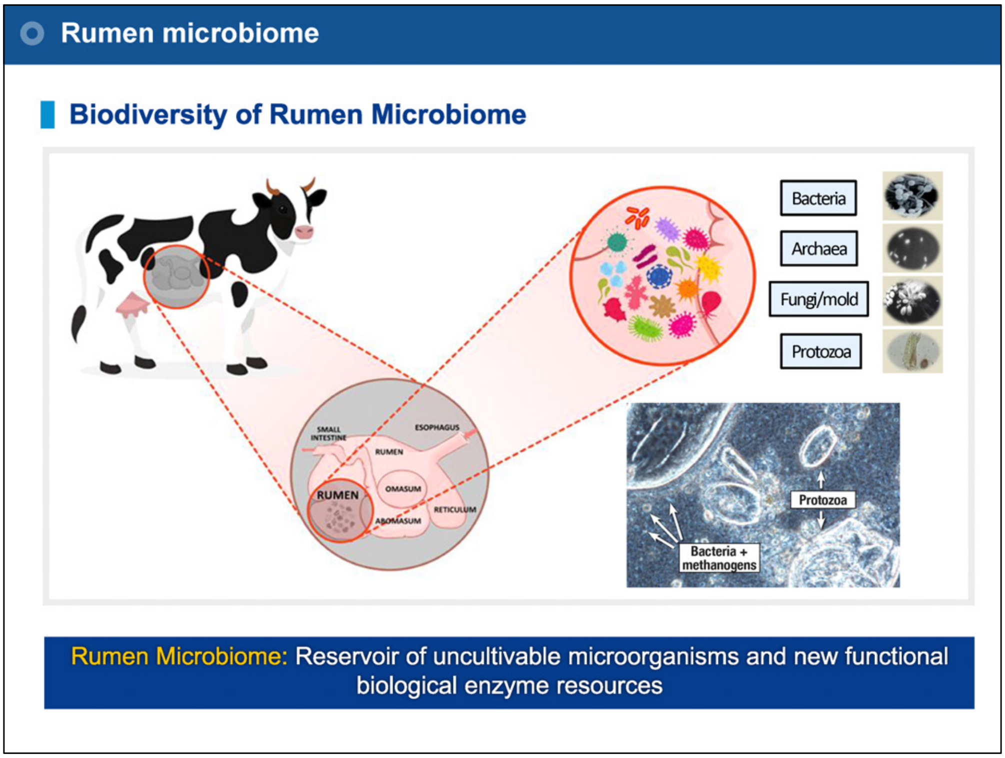
[a] *Engineering Department, Palermo University, Viale delle Scienze ed. 8 90128 Palermo, Italy*
[b] *Department of Biotechnology, Delft University of Technology, Van der Maasweg 9 2629 HZ Delft, The Netherlands*

Chemical Engineering Journal (2025)

Sludge bioremediation

PHA production

- ❖ Integrate sludge treatment with PHA production
- ❖ Carbon removal (96.3%) and nitrogen removal (89.9%)
- ❖ PHA storage yield up to 0.42 g CODPHA/g CODVFA



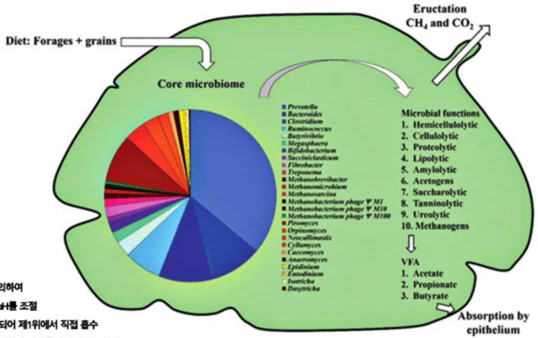
Rumen microbiome

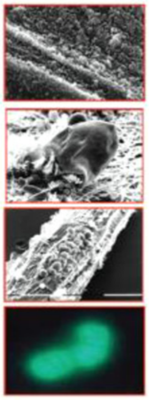
Biodiversity of Rumen Microbiome

Rumen Microbiome: Reservoir of uncultivable microorganisms and new functional biological enzyme resources

Rumen microbiome

Biodiversity of Rumen Microbiome



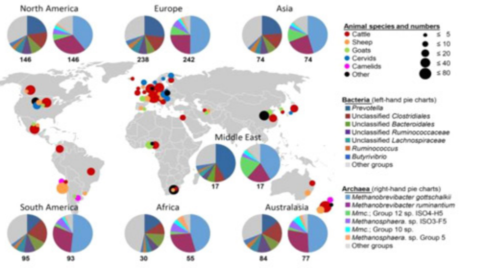


① 제1위의 소화는 미생물 발효에 의하여
② 되새김시 타액에 의해 제1위내 pH를 조절
③ 탄수화물은 미생물에 의해 분해되어 제1위에서 직접 흡수
④ 조단백질은 암모니아 분해균에 의하여 미생물체 단백질로 합성
⑤ 미생물은 제1위에서 염산에 의해 분해되어 장에서 소화 흡수
⑥ 腸은 탄수화물, 지방, 단백질을 소화효소로 소화, 흡수
⑦ 소화기에서 흡수된 영양분은 간에서 에너지원, 단백질, 지방으로 합성이용

Diet composition directly influences the rumen microbiome structure, which affects microbial functions and consequently biomass degradation, resulting in the release of methane (CH_4) and carbon dioxide (CO_2) via eructation and volatile fatty acids that are absorbed by the epithelium abomasum.

Rumen microbiome

Biodiversity of Rumen Microbiome



Henderson et al. 2015

Cultivation and sequencing of rumen microbiome members from the Hungate1000 Collection

Rekha Seshadri, Sinead C Leahy, Graeme T Attwood, Koon Hoong Teh, Suzanne C Lambie, Adrian L Cookson, Emiley A Eloe-Fadrosh, Georgios A Pavlopoulos, Michalis Hadjithomas, Neha J Varghese, David Paez-Espino, Hungate1000 project collaborators, Rechelle Perry, Gemma Henderson, Christopher J Creevey, Nicolas Terrapon, Pascal Lapebie, Elodie Drula, Vincent Lombard, Edward Rubin, Nikos C Kyrpides, Bernard Henrissat, Tanja Woyke, Natalia N Ivanova & William J Kelly

Nature Biotechnology 36, 359–367 (2018) | Cite this article

20k Accesses | 147 Citations | 157 Altmetric | Metrics

Global Rumen Census

Carbon neutrality and rumen microbiome

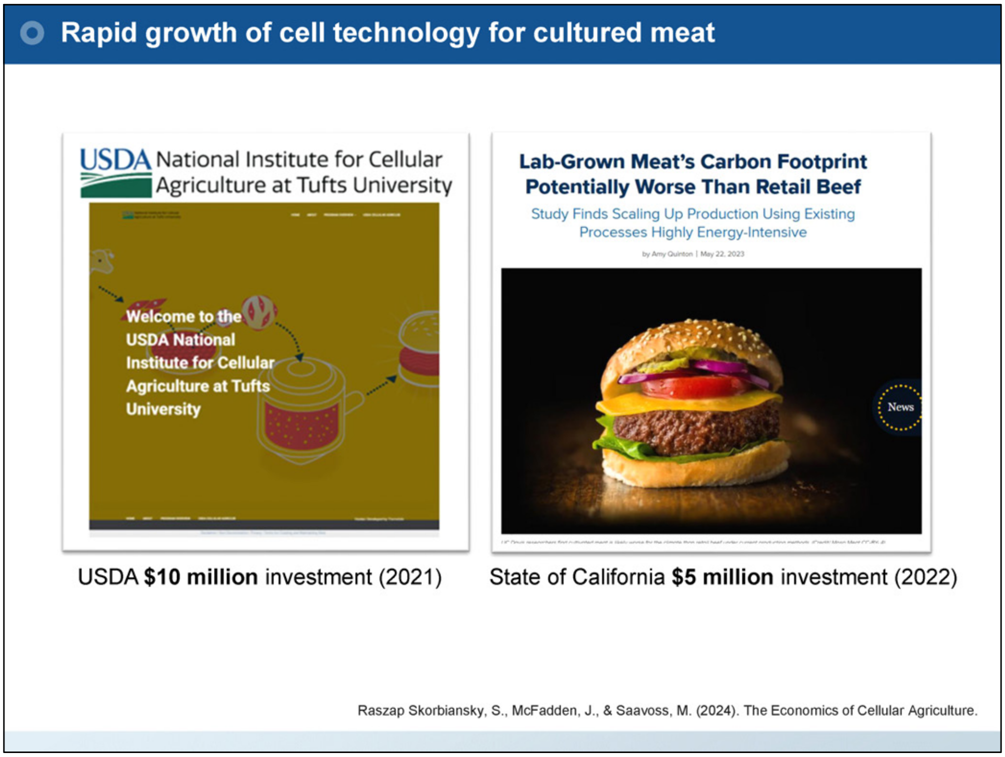
A heritable subset of the core rumen microbiome dictates dairy cow productivity and emissions

방귀 적게 뀌는 젖소, 장내미생물로 골라 메탄가스 줄인다

[이근영의 기상천외한 기후이야기]
국제연구팀 젖소 1000마리 장내세균 분석해
메탄가스 생산에 관여하는 미생물들 찾아내
방귀 조금 만드는 세균 지닌 유전자 소 선택
“우유 양과 질 유지되면 온실가스 감축 효과”

국제 연구팀이 메탄가스를 덜 생산하는 장내 미생물을 지닌 소를 선택해 육종하는 방법으로 온실가스를 감축할 수 있는 방안을 제시했다.

Future strategy for the animal science and industry that improves productivity and achieves carbon neutrality through the discovery of highly functional microbiome derived from rumen fluids



Rapid growth of cell technology for cultured meat

USDA National Institute for Cellular Agriculture at Tufts University

Welcome to the USDA National Institute for Cellular Agriculture at Tufts University

USDA **$10 million** investment (2021)

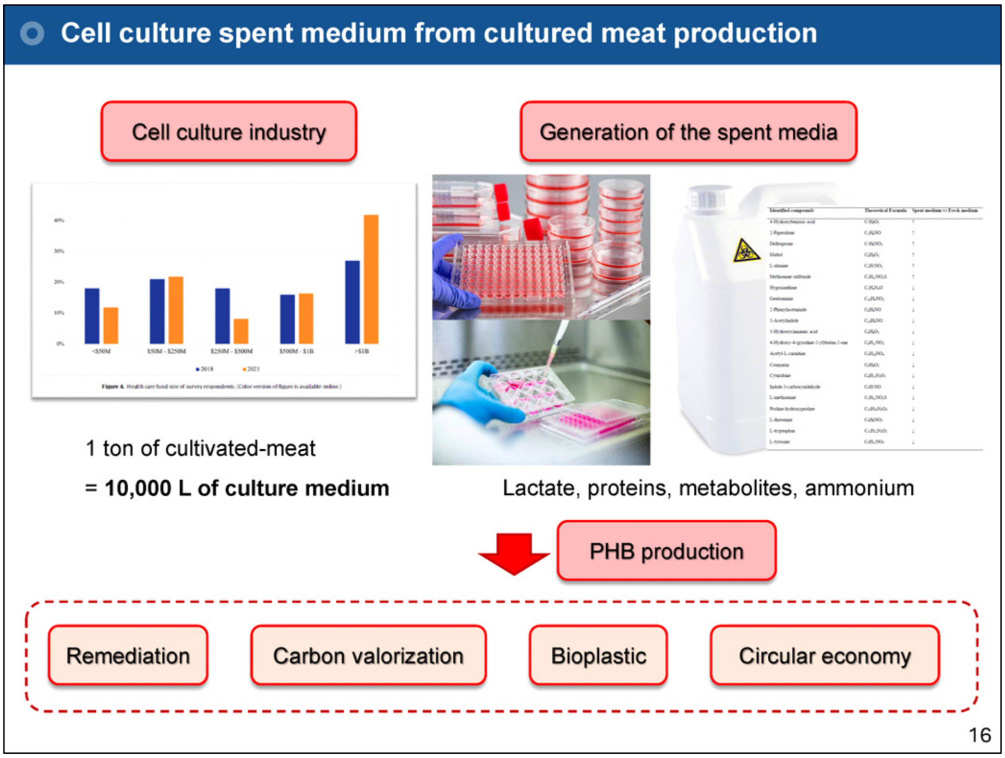
Lab-Grown Meat's Carbon Footprint Potentially Worse Than Retail Beef

Study Finds Scaling Up Production Using Existing Processes Highly Energy-Intensive

by Amy Quinton | May 22, 2023

State of California **$5 million** investment (2022)

Raszap Skorbiansky, S., McFadden, J., & Saavoss, M. (2024). The Economics of Cellular Agriculture.



Cell culture spent medium from cultured meat production

Cell culture industry

Figure 4. Health care fund size of survey respondents. (Color version of figure is available online.)

1 ton of cultivated-meat

= **10,000 L of culture medium**

Generation of the spent media

Identified compounds	Theoretical Formula	Spent medium vs Fresh medium
4-Hydroxybenzoic acid	$C_7H_6O_3$	↑
2-Piperidone	C_5H_9NO	↑
Deferiprone	$C_7H_9NO_2$	↑
Maltol	$C_6H_6O_3$	↑
L-alanine	$C_3H_7NO_2$	↑
Methionine sulfoxide	$C_5H_{11}NO_3S$	↑
Hypoxanthine	$C_5H_4N_4O$	↓
Gentianine	$C_{10}H_9NO_2$	↓
2-Phenylacetamide	C_8H_9NO	↓
3-Acetylindole	$C_{10}H_9NO$	↓
3-Hydroxycinnamic acid	$C_9H_8O_3$	↓
4-Hydroxy-4-(pyridine-2-yl)butan-2-one	$C_9H_{11}NO_2$	↓
Acetyl-L-carnitine	$C_9H_{17}NO_4$	↓
Coumarin	$C_9H_6O_2$	↓
Cytarabine	$C_9H_{13}N_3O_5$	↓
Indole-3-carboxyaldehyde	C_9H_7NO	↓
L-methionine	$C_5H_{11}NO_2S$	↓
Proline-hydroxyproline	$C_{10}H_{16}N_2O_4$	↓
L-threonine	$C_4H_9NO_3$	↓
L-tryptophan	$C_{11}H_{12}N_2O_2$	↓
L-tyrosine	$C_9H_{11}NO_3$	↓

Lactate, proteins, metabolites, ammonium

PHB production

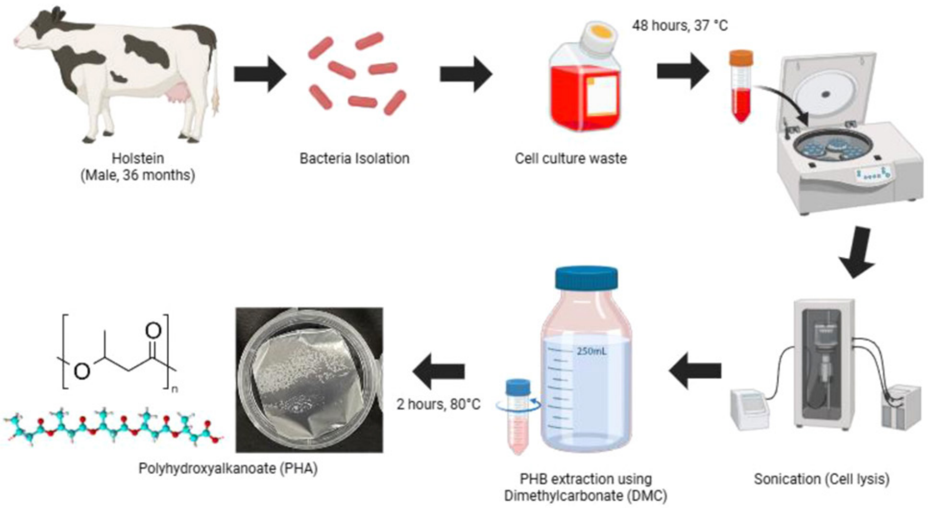
Remediation

Carbon valorization

Bioplastic

Circular economy

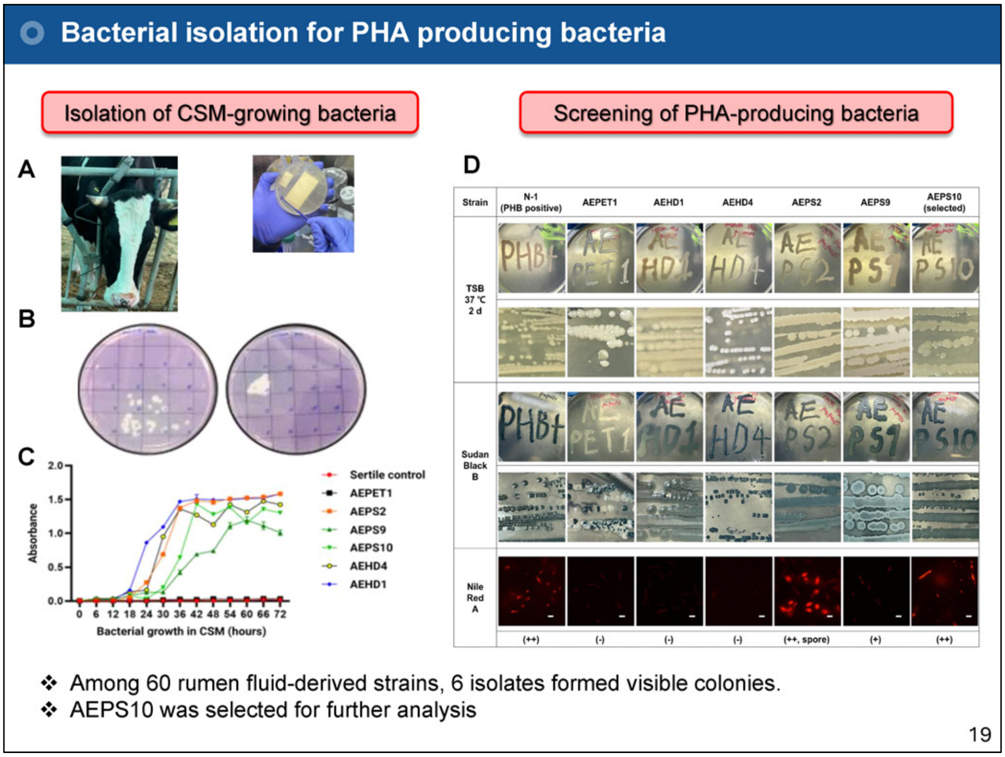
Bioconversion of cell culture spent medium



02

Results (1)

- Isolation and Characterization of PHA producing bacteria from rumen fluids

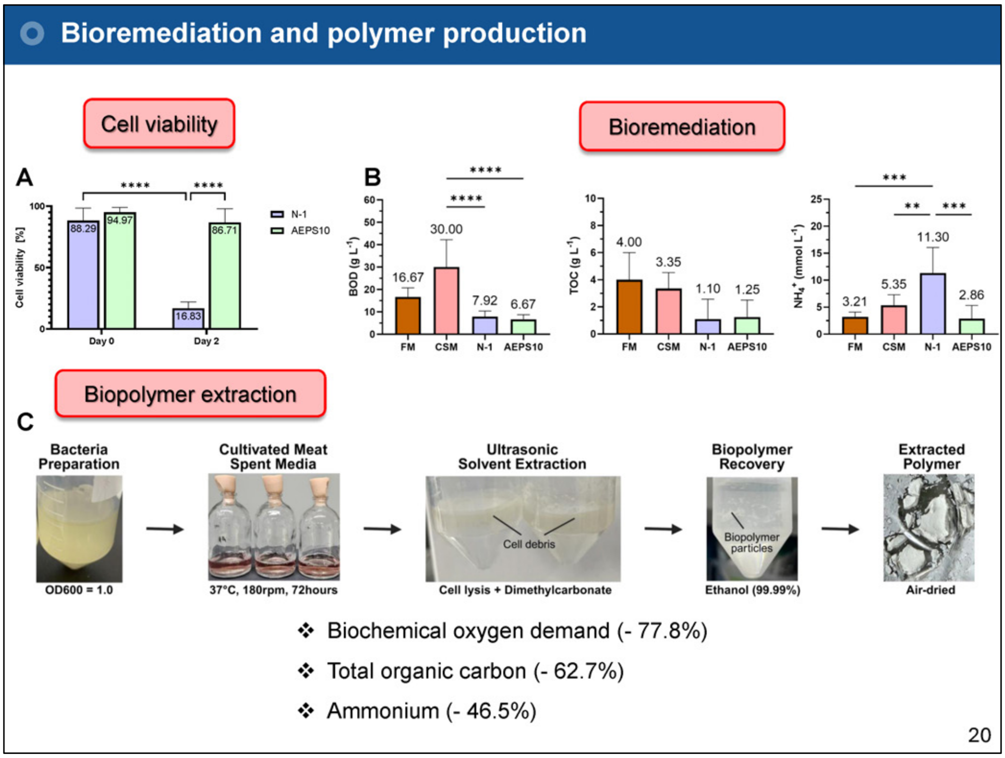


Bacterial isolation for PHA producing bacteria

Isolation of CSM-growing bacteria

Screening of PHA-producing bacteria

- ❖ Among 60 rumen fluid-derived strains, 6 isolates formed visible colonies.
- ❖ AEPS10 was selected for further analysis



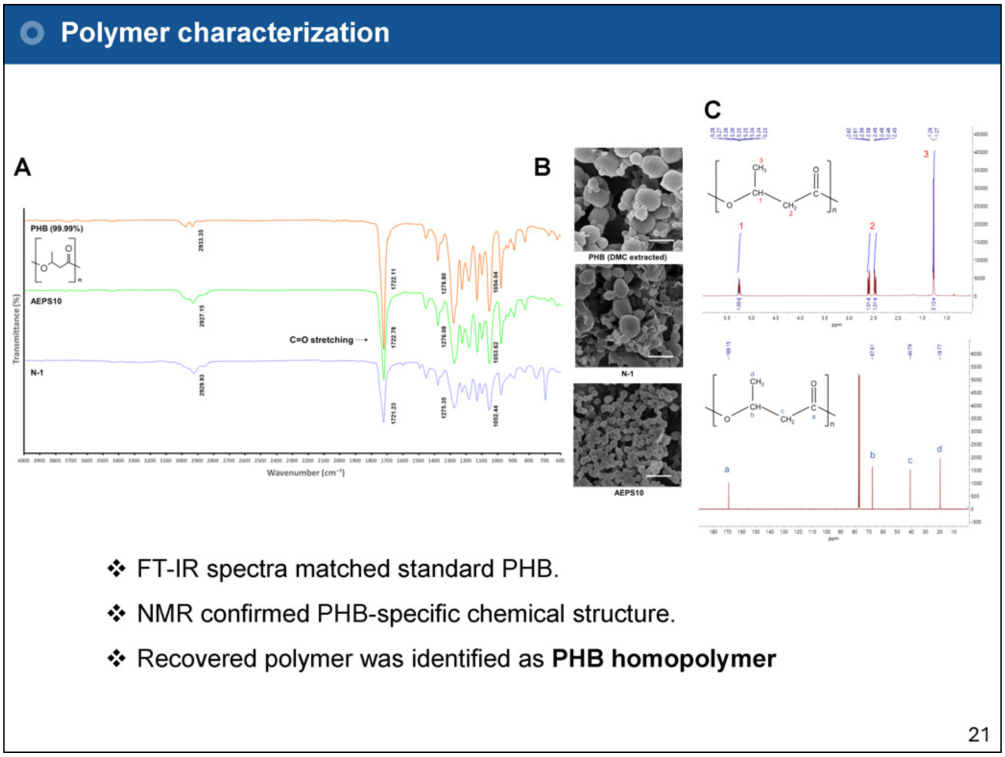
Bioremediation and polymer production

Cell viability

Bioremediation

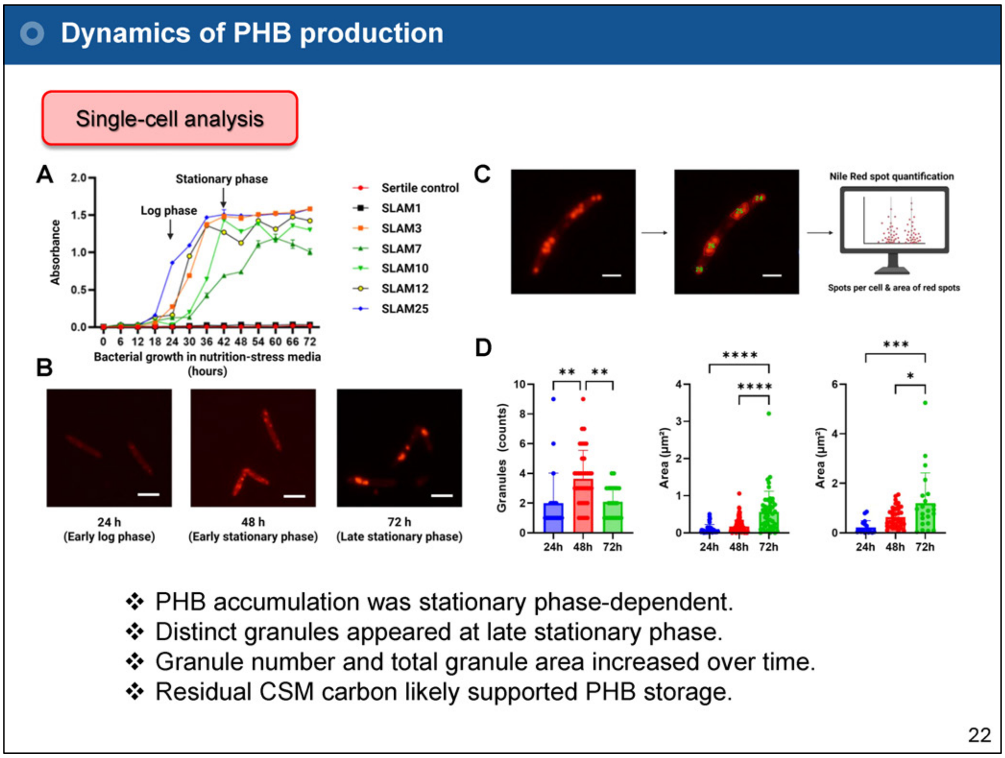
Biopolymer extraction

- ❖ Biochemical oxygen demand (- 77.8%)
- ❖ Total organic carbon (- 62.7%)
- ❖ Ammonium (- 46.5%)



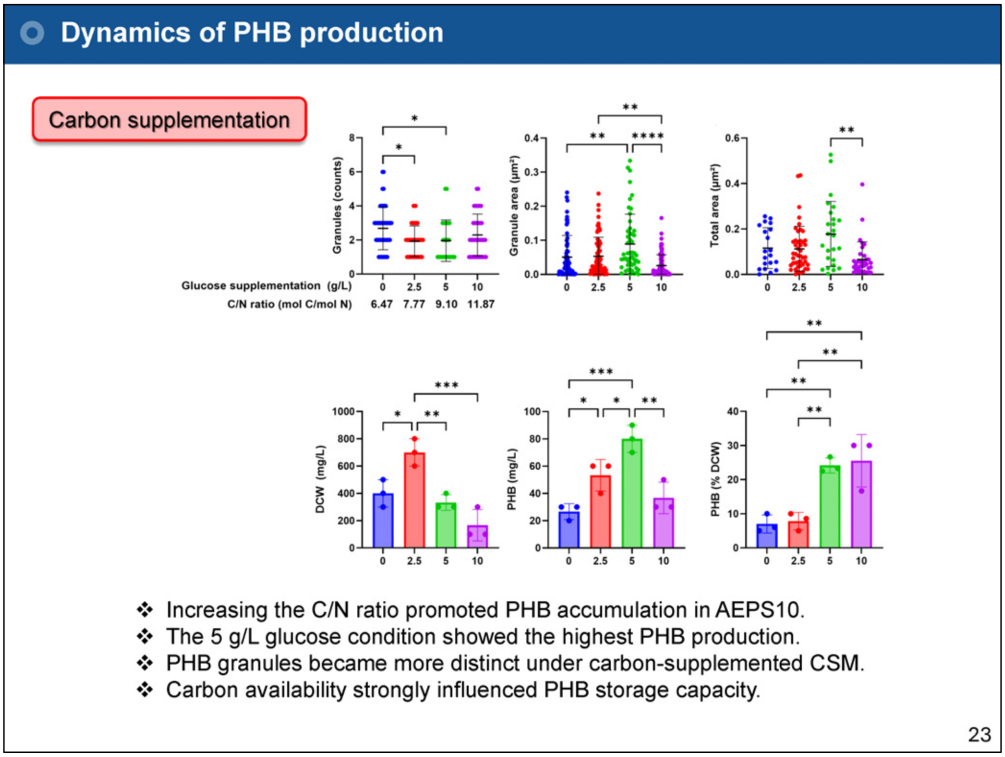
Polymer characterization

- ❖ FT-IR spectra matched standard PHB.
- ❖ NMR confirmed PHB-specific chemical structure.
- ❖ Recovered polymer was identified as **PHB homopolymer**



Dynamics of PHB production

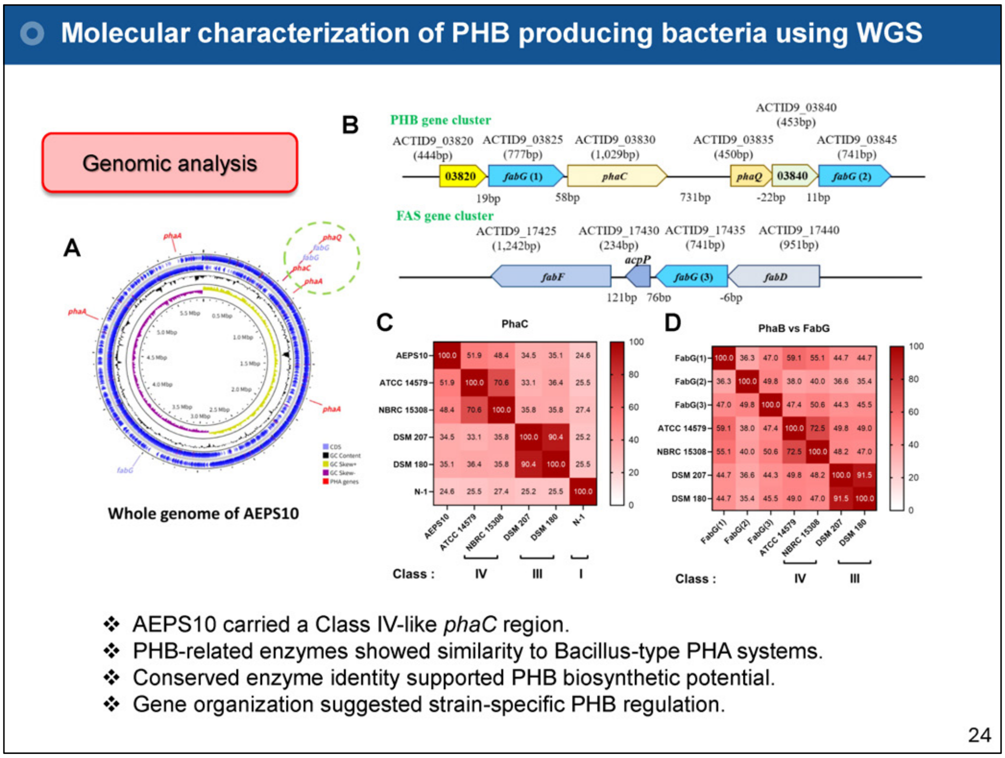
- ❖ PHB accumulation was stationary phase-dependent.
- ❖ Distinct granules appeared at late stationary phase.
- ❖ Granule number and total granule area increased over time.
- ❖ Residual CSM carbon likely supported PHB storage.



Dynamics of PHB production

Carbon supplementation

- ❖ Increasing the C/N ratio promoted PHB accumulation in AEPS10.
- ❖ The 5 g/L glucose condition showed the highest PHB production.
- ❖ PHB granules became more distinct under carbon-supplemented CSM.
- ❖ Carbon availability strongly influenced PHB storage capacity.



Molecular characterization of PHB producing bacteria using WGS

Genomic analysis

Whole genome of AEPS10

C PhaC

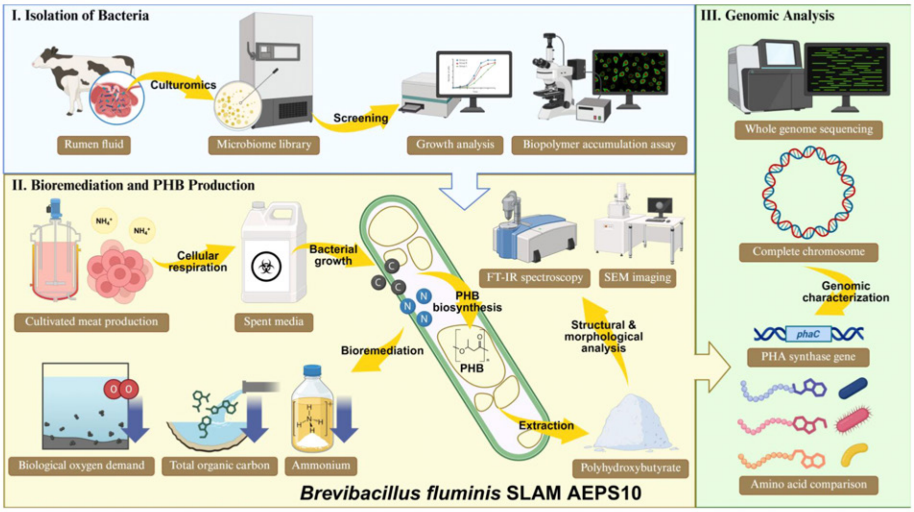
	AEPS10	ATCC 14579	NBRC 15308	DSM 207	DSM 180	N-1
AEPS10	100.0	51.9	48.4	34.5	35.1	24.6
ATCC 14579	51.9	100.0	70.6	33.1	36.4	25.5
NBRC 15308	48.4	70.6	100.0	35.8	35.8	27.4
DSM 207	34.5	33.1	35.8	100.0	90.4	25.2
DSM 180	35.1	36.4	35.8	90.4	100.0	25.5
N-1	24.6	25.5	27.4	25.2	25.5	100.0

D PhaB vs FabG

	FabG(1)	FabG(2)	FabG(3)	ATCC 14579	NBRC 15308	DSM 207	DSM 180
FabG(1)	100.0	36.3	47.0	59.1	55.1	44.7	44.7
FabG(2)	36.3	100.0	49.8	38.0	40.0	36.6	35.4
FabG(3)	47.0	49.8	100.0	47.4	50.6	44.3	45.5
ATCC 14579	59.1	38.0	47.4	100.0	72.5	49.8	49.0
NBRC 15308	55.1	40.0	50.6	72.5	100.0	48.2	47.0
DSM 207	44.7	36.6	44.3	49.8	48.2	100.0	91.5
DSM 180	44.7	35.4	45.5	49.0	47.0	91.5	100.0

- ❖ AEPS10 carried a Class IV-like *phaC* region.
- ❖ PHB-related enzymes showed similarity to Bacillus-type PHA systems.
- ❖ Conserved enzyme identity supported PHB biosynthetic potential.
- ❖ Gene organization suggested strain-specific PHB regulation.

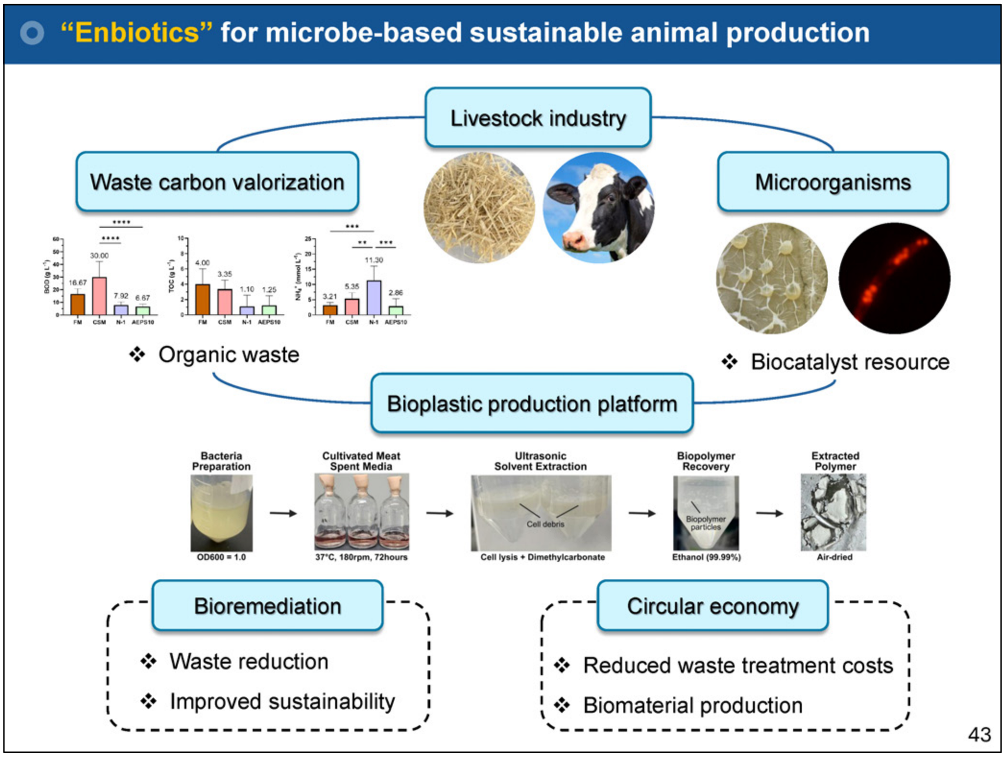
Molecular characterization of PHB producing bacteria



- This study connects waste treatment and bioplastic production in one bacterial process.
- AEPS10 reduced BOD, TOC, and ammonium in CSM and accumulated recoverable PHB.
- This strain provides a starting point for applying animal-associated microbial resources to waste-carbon conversion.

04

Further study



"Enbiotics" for microbe-based sustainable animal production

Livestock industry

Waste carbon valorization

Microorganisms

❖ Organic waste

❖ Biocatalyst resource

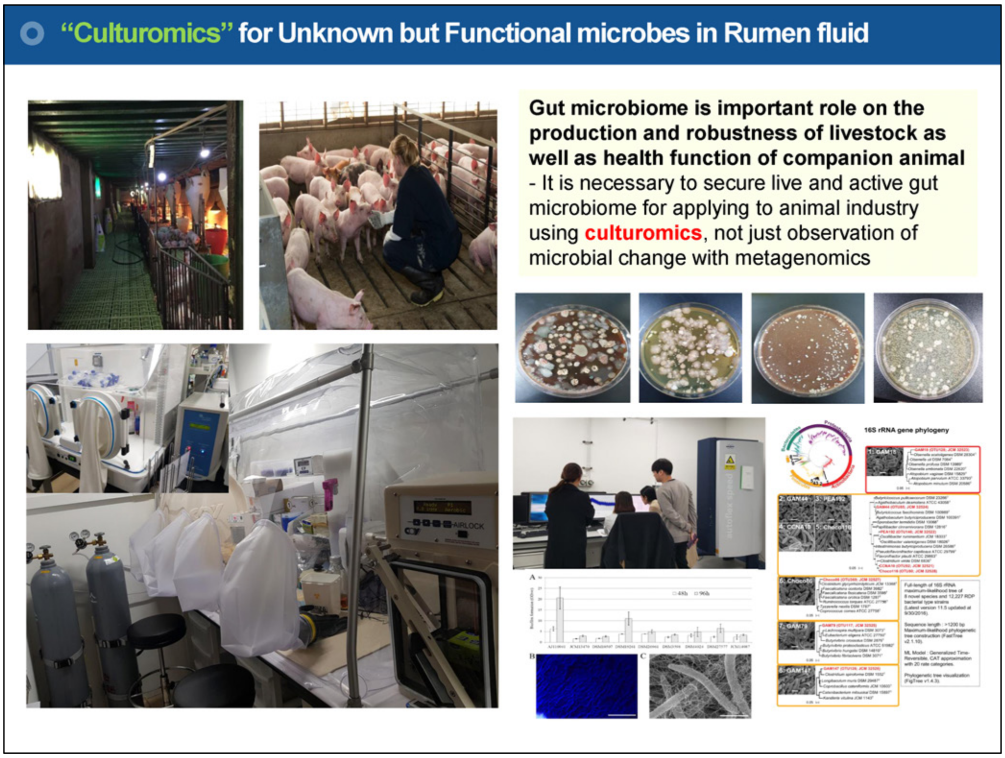
Bioplastic production platform

Bioremediation

- ❖ Waste reduction
- ❖ Improved sustainability

Circular economy

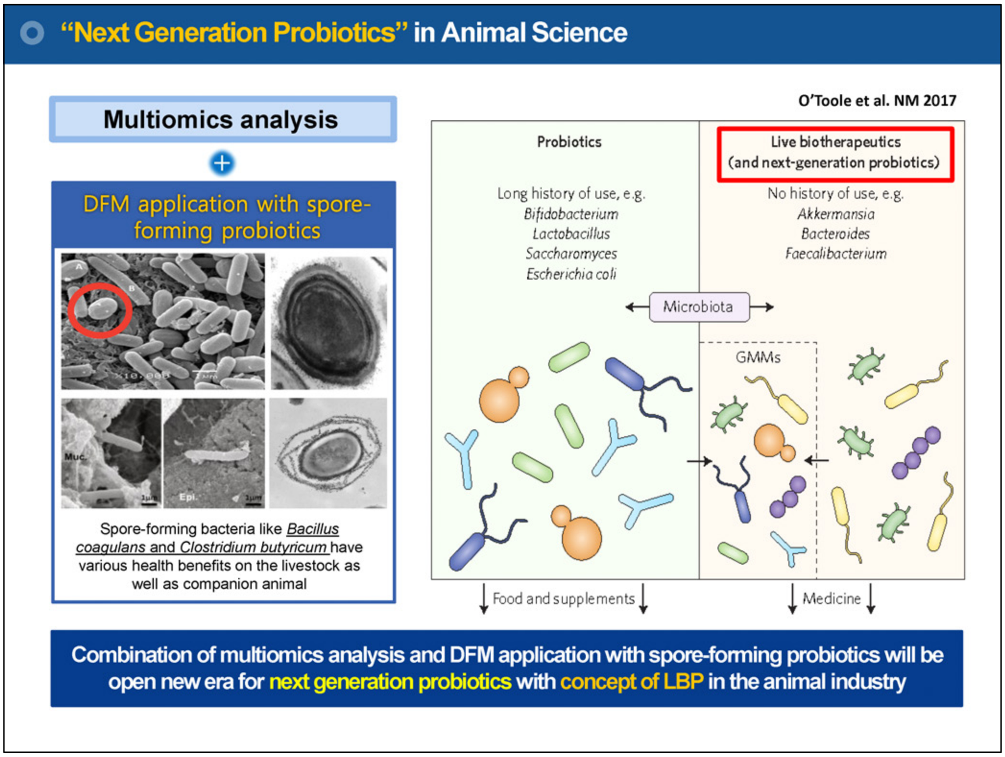
- ❖ Reduced waste treatment costs
- ❖ Biomaterial production



"Culturomics" for Unknown but Functional microbes in Rumen fluid

Gut microbiome is important role on the production and robustness of livestock as well as health function of companion animal

- It is necessary to secure live and active gut microbiome for applying to animal industry using **culturomics**, not just observation of microbial change with metagenomics



"Next Generation Probiotics" in Animal Science

Multiomics analysis

+

DFM application with spore-forming probiotics

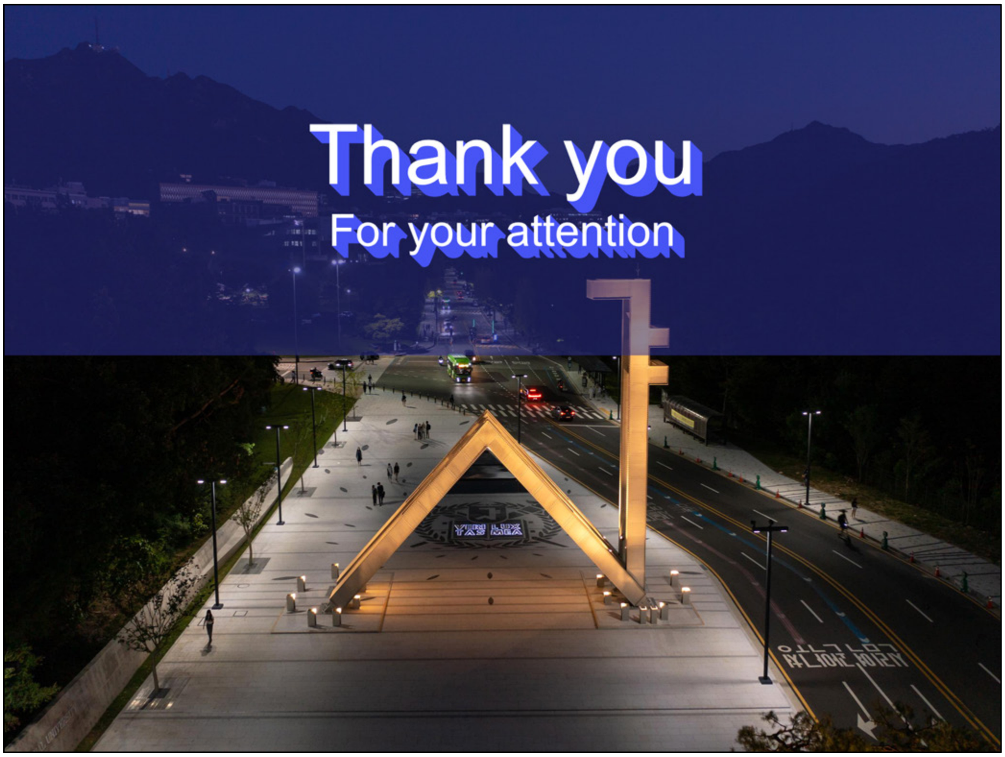
Spore-forming bacteria like *Bacillus coagulans* and *Clostridium butyricum* have various health benefits on the livestock as well as companion animal

O'Toole et al. NM 2017

Combination of multiomics analysis and DFM application with spore-forming probiotics will be open new era for next generation probiotics with concept of LBP in the animal industry

Acknowledgement

SNU Lab. of Animal Microbiology (SLAM)



Thank you

For your attention