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

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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


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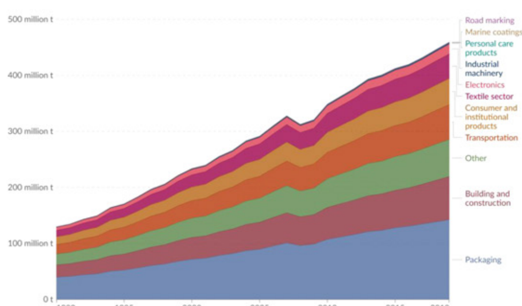
Seoul National University
Department of Agricultural Biotechnology
Younghoon Kim
Source: Tanaka et al. (2022) Polymers, 14(15):3063

01

Background

Current problems of petroleum-based plastics

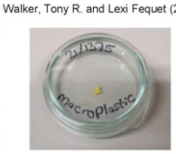
Global primary plastic production By industrial sector, 1990 to 2019



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

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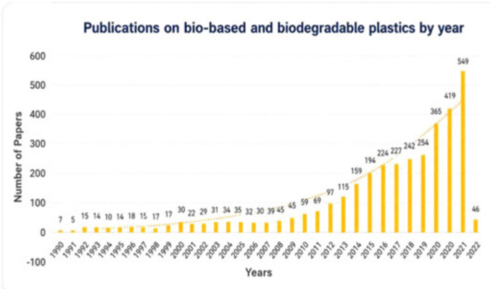
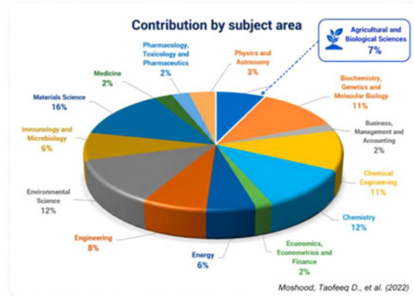
Bioplastics: Plastics that are bio-based, biodegradable, or both





Three types of bioplastics

Bio-based
- bio-PE
- bio-PET
- bio-PP

Biodegradable
- PCL
- PVA
- PBAT

Bio-based & Biodegradable

PHA
- PLA
- bio-PBS

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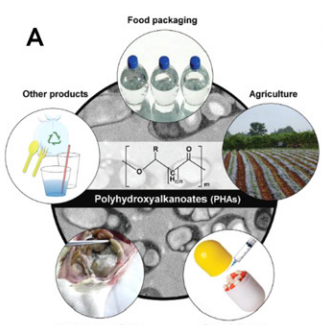
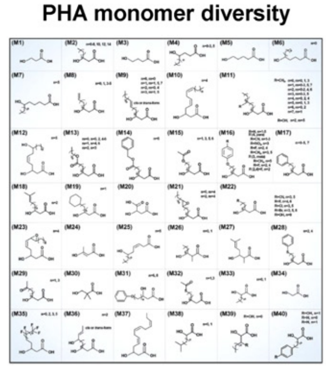
PHAs for sustainable bioplastics

A



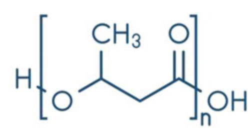
B

PHA monomer diversity



Choi et.al. (2020)

C



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

6

Discovery of PHA producing bacteria

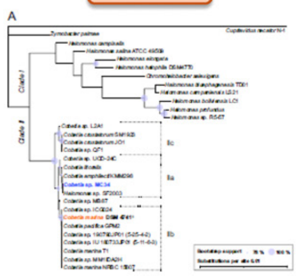
RESEARCH Open Access

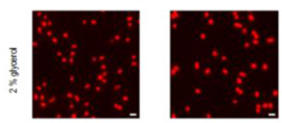
High natural PHA production from acetate in *Cobetia* sp. MC34 and *Cobetia marina* DSM 4741^T and in silico analyses of the genus specific $PhaC_2$ polymerase variant

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

Microbial Cell Factories (2021)

Isolation

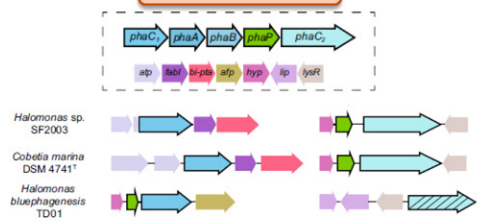




Carbon source screening

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

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Large-scale production

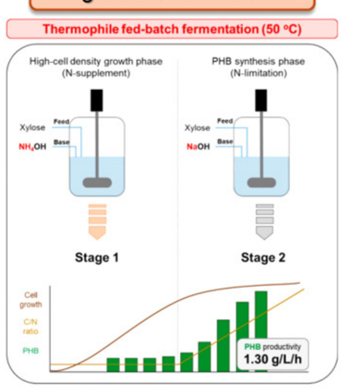
Production of polyhydroxybutyrate with high cell density cultivation using thermophile *Caldimonas thermodepolymerans*

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

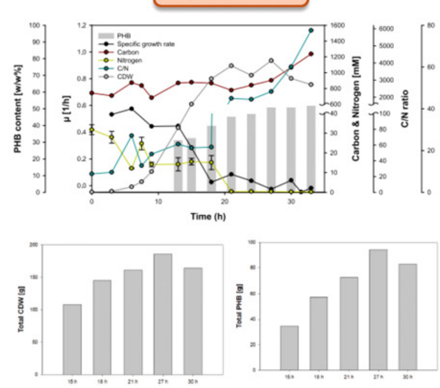
[a] Department of Chemical Engineering (BK21 FOUR Integrated Engineering Program) College of Engineering Kyung Hee University Republic of Korea

Bioresource Technology (2024)

Large-scale fermentation



Optimization



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

8

Metabolic engineering

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

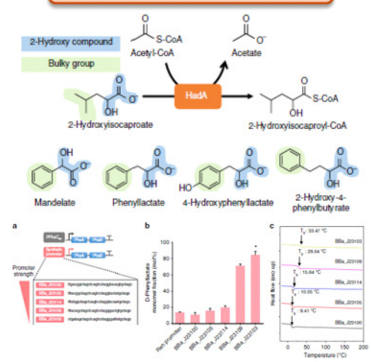
One-step fermentative production of aromatic polyesters from glucose by metabolically engineered *Escherichia coli* strains

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.

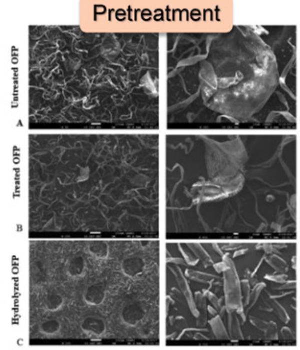
Industrial by-product upcycling for PHB production

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

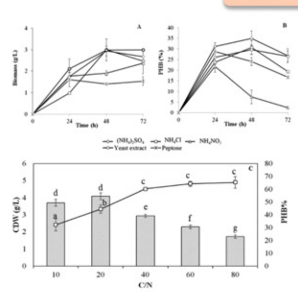
Journal of Cleaner Production **(2018)**

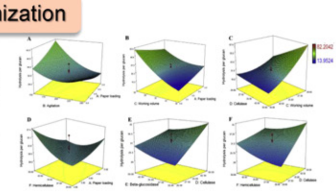
Huda Al-Battashi [a], Neelamegam Annamalai [a], Shatha Al-Kindi [a], Anu Sadasivan Nair [a], Saif Al-Bahry [a], Jay Prakash Verma [b], Nallusamy Sivakumar [a,*]
[a] Department of Biology, College of Science, Sultan Qaboos University, PO Box 36, PC 123, Oman
[b] Hawkesbury Institute for Environment, Western 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

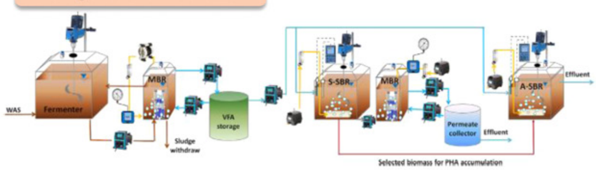
From waste activated sludge to polyhydroxyalkanoate: Insights from a membrane-based enrichment process

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

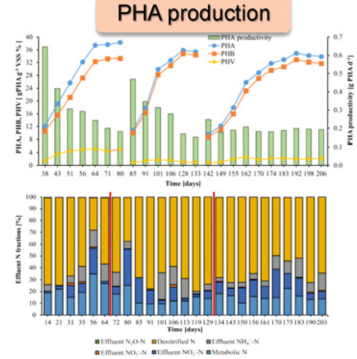
[a] Engineering Department, Palermo University, Viale delle Scienze ed. 8 90128 Palermo, Italy
[b] Department of Biotechnology, Delft University of Technology, Van der Maasweg 9 2629 HZ Delft, The Netherlands

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

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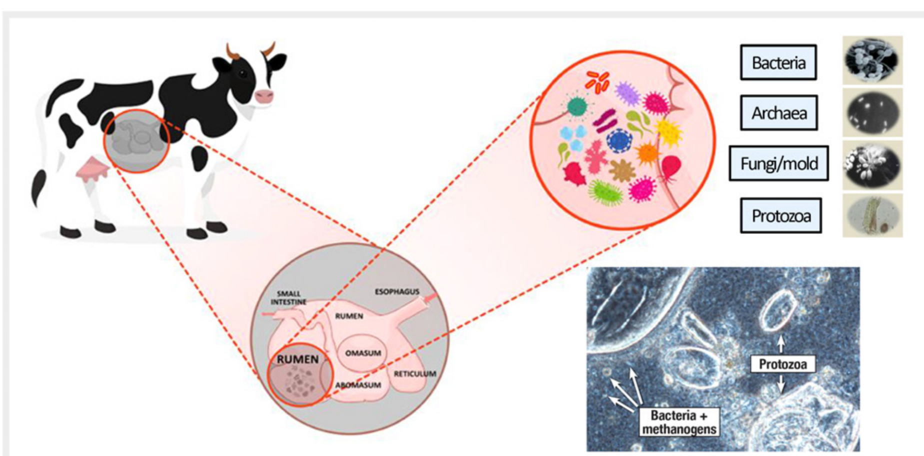
Rumen microbiome

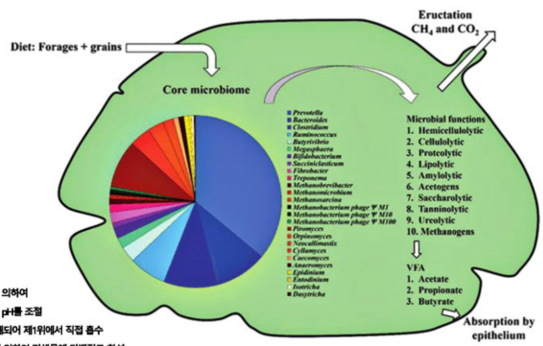
Biodiversity of Rumen Microbiome

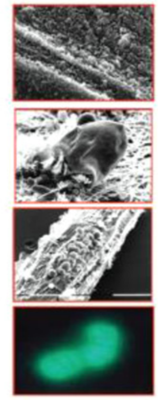


Rumen Microbiome: Reservoir of uncultivable microorganisms and new functional biological enzyme resources

Rumen microbiome

Biodiversity of Rumen Microbiome



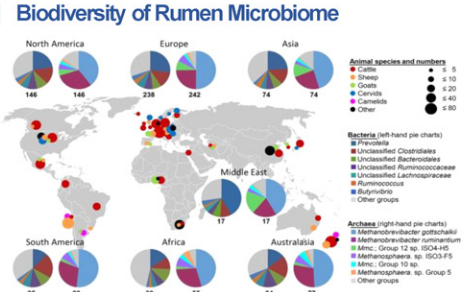


① 제1위의 소화는 미생물 발효에 의하여
② 되새김시 타액에 의해 제1위내 pH를 조절
③ 탄수화물은 미생물에 의해 분해되어 제1위에서 직접 흡수
④ 조단백질은 암모니아 분해균에 의하여 미생물체 단백질로 합성
⑤ 미생물은 제1위에서 염산에 의해 분해되어 장에서 소화 흡수
⑥ 腸은 탄수화물, 지방, 단백질을 소화효소로 소화, 흡수
⑦ 소화기에서 흡수된 영양분은 간에서 에너지원, 단백질, 지방으로 합성이용

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


nature biotechnology

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Open Access | Published: 19 March 2018

Cultivation and sequencing of rumen microbiome members from the Hungate1000 Collection

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

20k Accesses | 147 Citations | 157 Altmetric | Metrics

Carbon neutrality and rumen microbiome


Science Advances

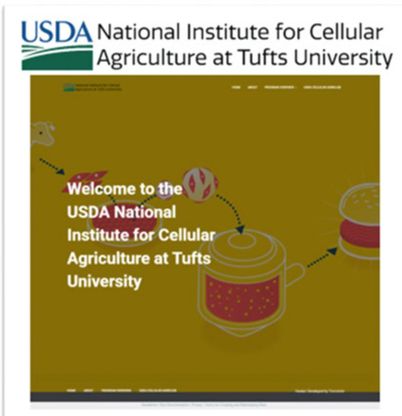
CURRENT ISSUE

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

방귀 적게 뀌는 젖소, 장내미생물로 골라 메탄가스 줄인다

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 **$10 million** investment (2021)

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

1 ton of cultivated-meat

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

Generation of the spent media

Lactate, proteins, metabolites, ammonium

PHB production

Remediation | Carbon valorization | Bioplastic | Circular economy

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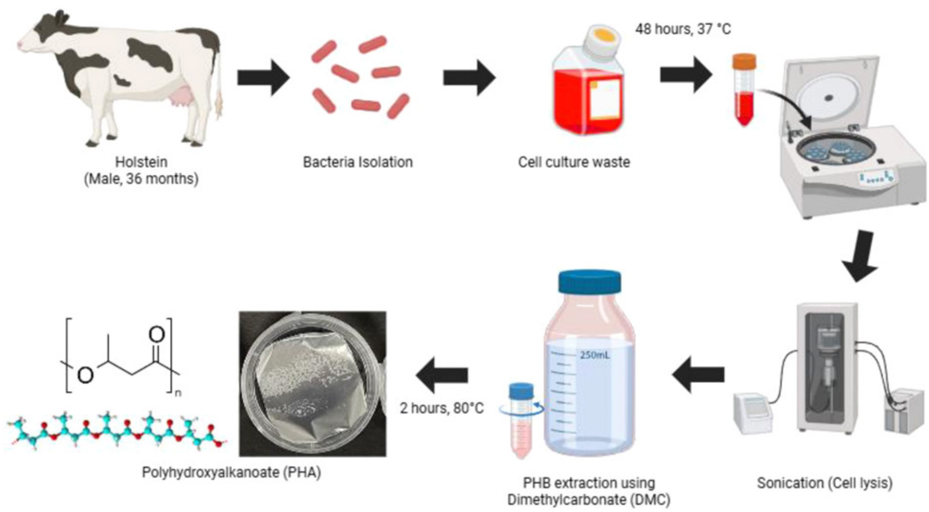
Bioconversion of cell culture spent medium

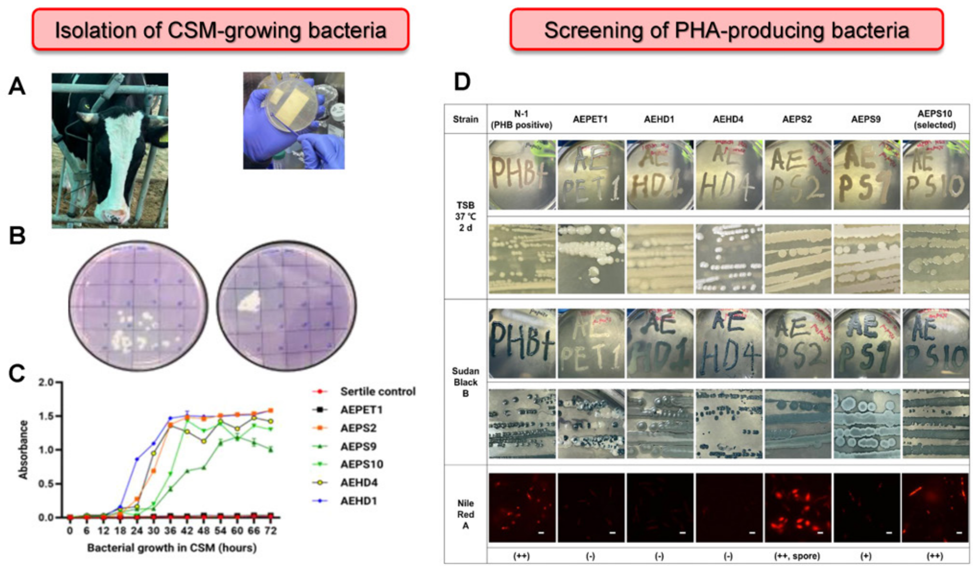


02

Results (1)

- **Isolation and Characterization of PHA producing bacteria from rumen fluids**

Bacterial isolation for PHA producing bacteria



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

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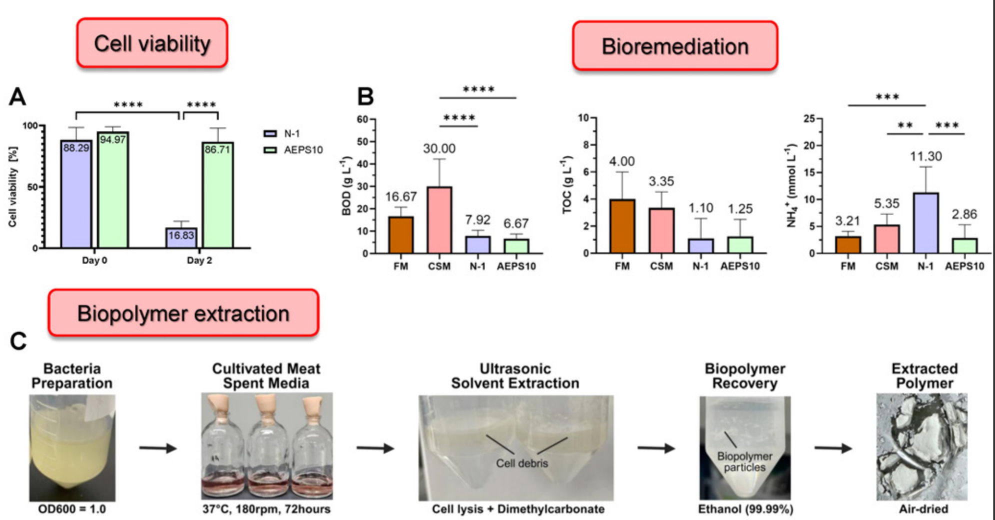
Bioremediation and polymer production



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

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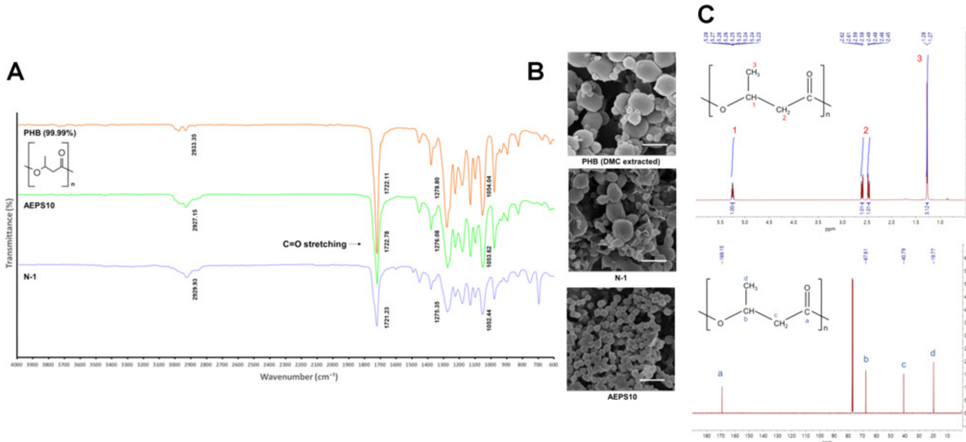
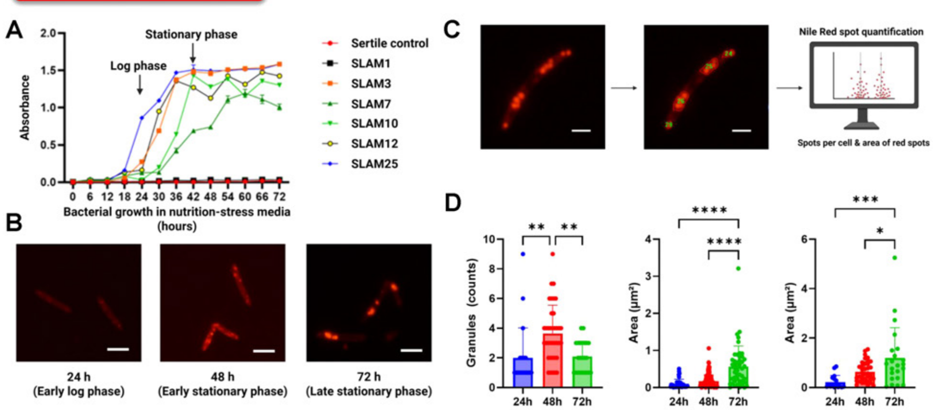
Polymer characterization



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

Dynamics of PHB production

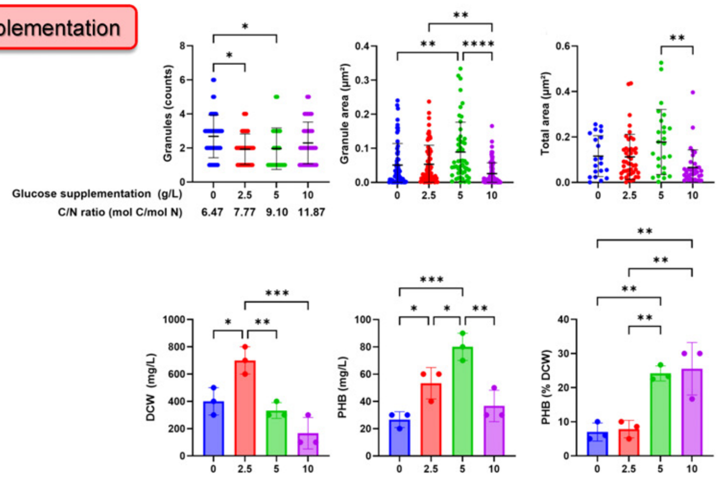
Single-cell analysis



- ❖ 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.

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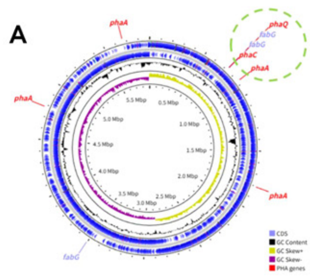
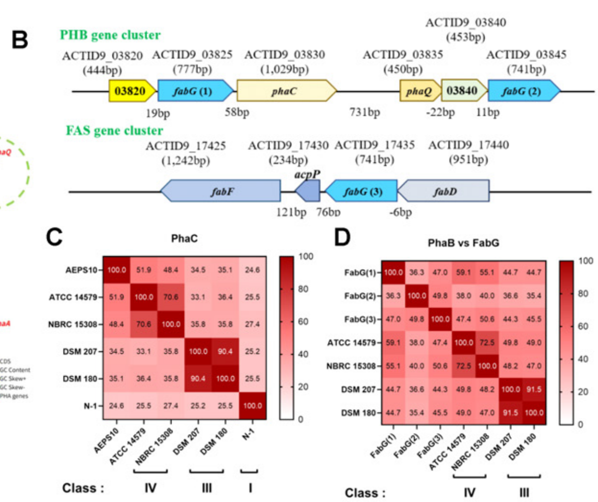
Molecular characterization of PHB producing bacteria using WGS

Genomic analysis



Whole genome of AEPS10



- 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.

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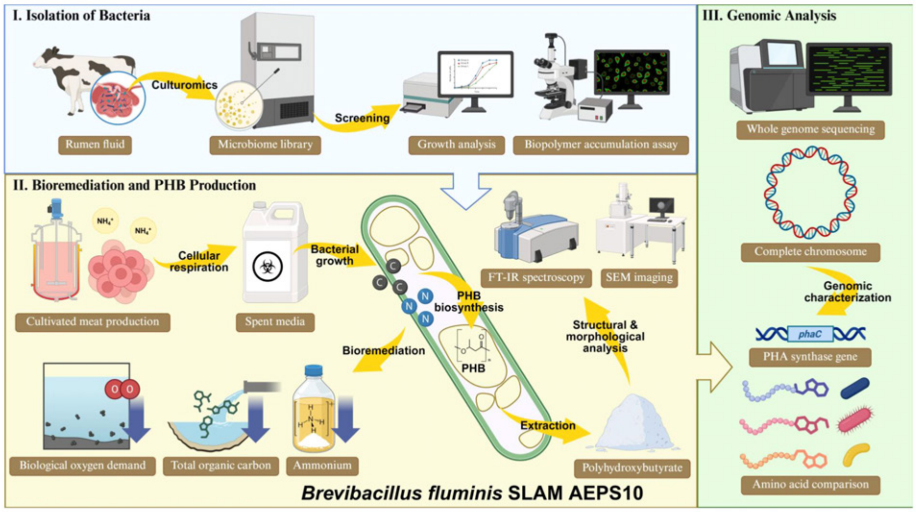
Molecular characterization of PHB producing bacteria



- 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.

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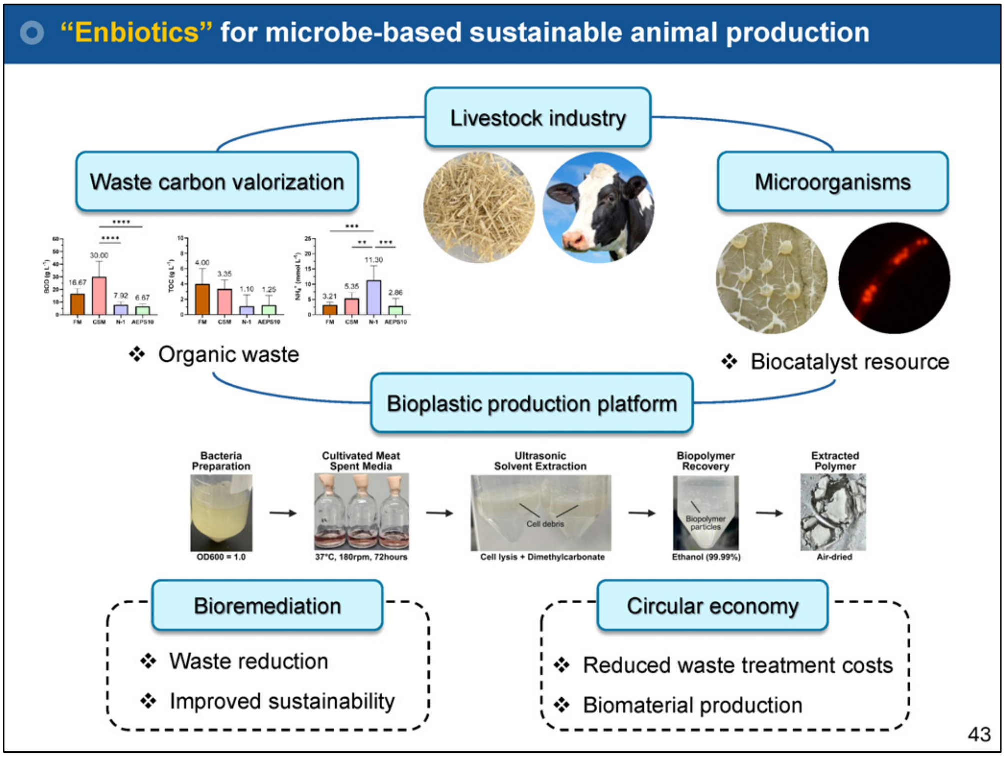
04

Further study


"Enbiotics" for microbe-based sustainable animal production
Livestock industry
Waste carbon valorization
Microorganisms
❖ Organic waste
❖ Biocatalyst resource
Bioplastic production platform
Bacteria Preparation
OD600 = 1.0
Cultivated Meat Spent Media
37°C, 180rpm, 72hours
Ultrasonic Solvent Extraction
Cell debris
Cell lysis + Dimethylcarbonate
Biopolymer Recovery
Biopolymer particles
Ethanol (99.99%)
Extracted Polymer
Air-dried
Bioremediation
❖ Waste reduction
❖ Improved sustainability
Circular economy
❖ Reduced waste treatment costs
❖ Biomaterial production
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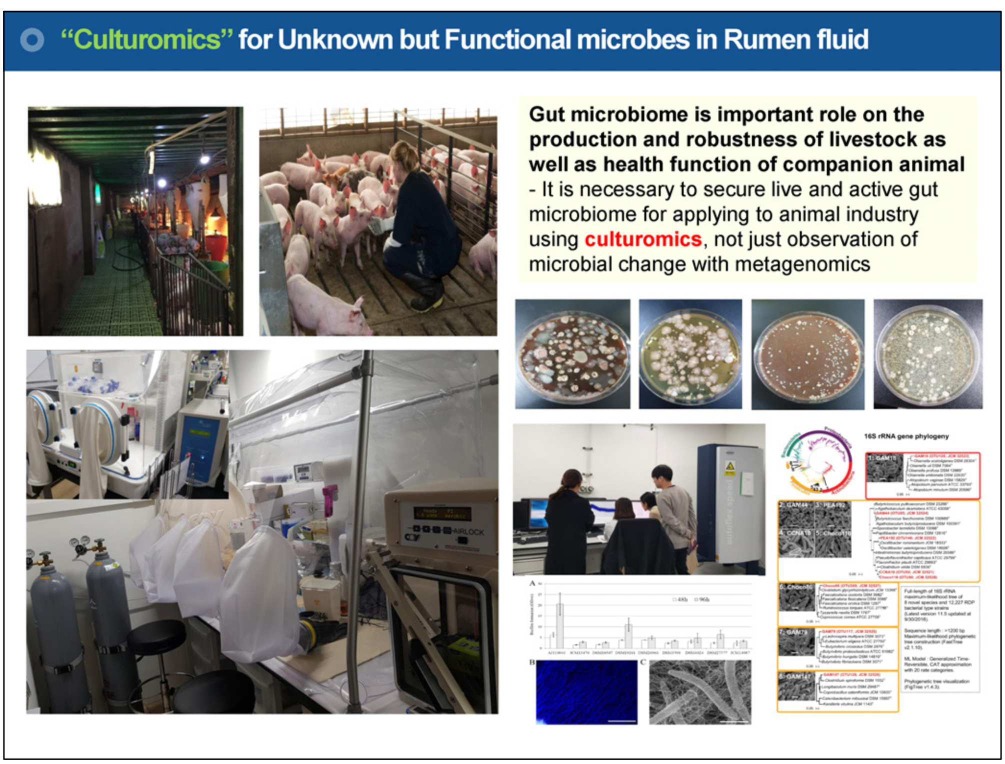
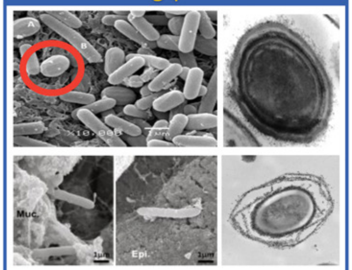

"Culturomics" for Unknown but Functional microbes in Rumen fluid
Gut microbiome is important role on the production and robustness of livestock as well as health function of companion animal
- It is necessary to secure live and active gut microbiome for applying to animal industry using culturomics, not just observation of microbial change with metagenomics
16S rRNA gene phylogeny

"Next Generation Probiotics" in Animal Science

Multiomics analysis

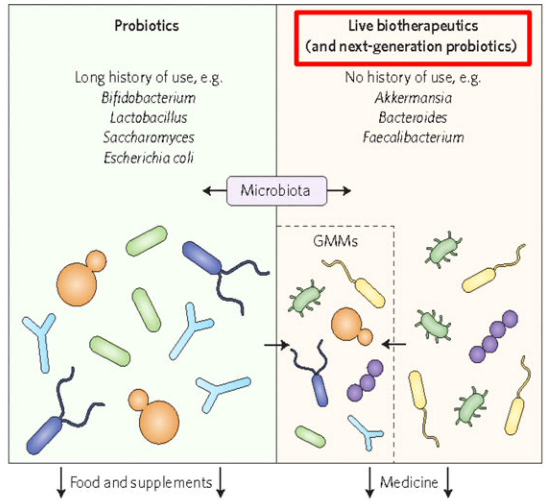
+

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

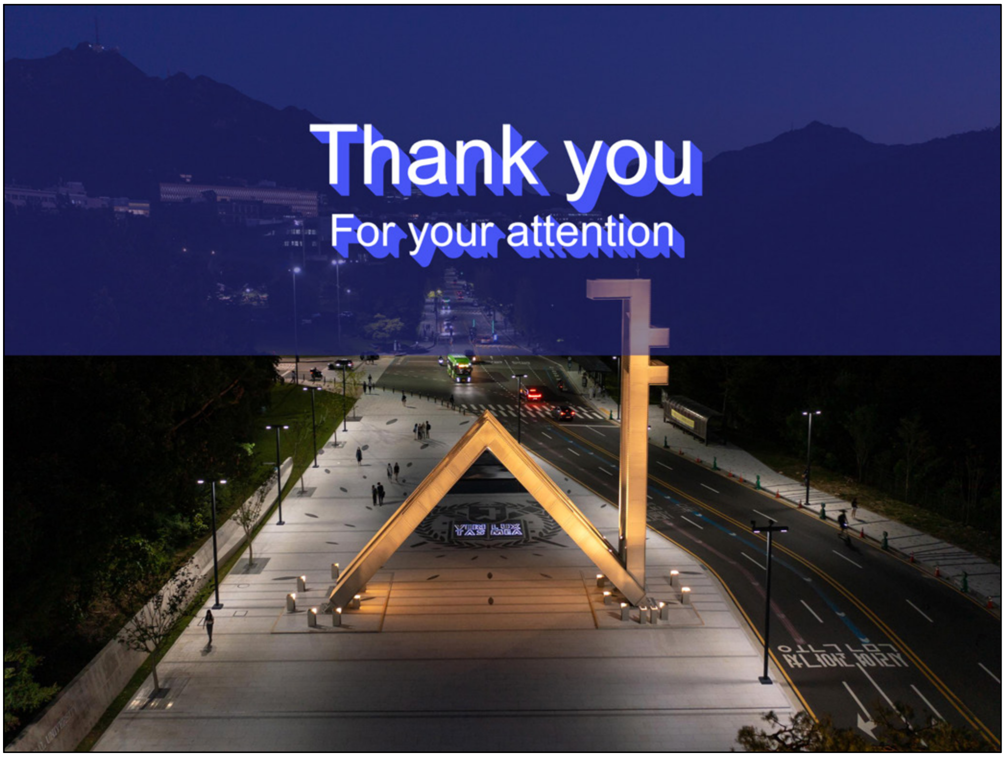


Combination of multiomics analysis and DFM application with spore-forming probiotics will be open new era for next generation probiotics with concept of LBP in the animal industry

Acknowledgement

SNU Lab. of Animal Microbiology (SLAM)

NRF 한국연구재단 농촌진흥청 산업통상부 iPET 농림식품기술기획평가원


Thank you
For your attention