An Roinn Talmhaíochta,
Bia agus Mara
Department of Agriculture,
Food and the Marine

16/RD/ERAGAS/3 Refining direct fed microbials (DFM) and silage inoculants for reduction of methane emissions from ruminants

Final Report

This project was funded under the Department of Agriculture, Food and the Marine Competitive Funding Programme.

SUMMARY

The MethLAB project focused on implementing the use of lactic acid bacteria (LAB) to reduce methane emissions from livestock such as cattle and sheep. The main goal of this project was to refine the methods that are currently used in onfarm technologies, such as silage inoculants or the use of direct-fed microbial supplements. This project began with the isolation of LAB strains from various agricultural environments, as well as LAB strains from various collections of bacteria and commercially available strains. These strains were screened for bacteriocin activity which inhibit the growth of methanogens within cattle and sheep. These bacteriocin producing LAB were tested against a range of methanogen species (Methanobrevibacter ruminantium, M. gottschalkii and M.boviskoreanii). Several of these LAB were found to reduce methanogen growth and methane production and were tested for their potential as silage inoculants. Large-scale silage was created which was administered to dairy cattle and sheep. LAB strains were tested for their ability to reduce methane in vivo. Parameters such as methane production, animal health, animal productivity and silage quality were tested. No effect was found when administering these MethLAB strains to sheep. In lactating dairy cattle, the MethLAB strains showed significant methane reduction, while simultaneously maintaining animal health and maintaining animal productivity. In vitro fermentations were also carried out to determine if these strains had any effect on gas production or silage quality. A collection of LAB antimicrobial strains were isolated with activity against methane producing bacteria (methanogens). These strains demonstrated methane reduction in Holstein dairy cattle, while maintaining animal health and productivity. This project will allow for further investigation of these methane reducing LAB strains, offering a safe, practical and natural way to influence the rumen microbial community for methane mitigation. This project will allow for a more sustainable, emission-efficient food production system.

KEYWORDS

Methane, Ruminant, Lactic acid bacteria

ACRONYM	METHLAB
PROJECT COORDINATOR, INSTITUTION	Dr Catherine Stanton. Teagasc, Food Research Centre, Moorepark.
EMAIL	catherine.stanton@teagasc.ie
COLLABORATORS, INSTITUTION	University College Cork, Ireland. Institute National de Recherche en Agronomie (INRA), France. Animal Nutrition Group, Wageningen University, The Netherlands. AgResearch Limited, New Zealand. SACCO S.R.L, Italy.
PUBLICATION DATE	April 2022

Section 1 - Research Approach & Results

Start Date

01 October 2017

End Date

30 September 2021

Select Research Programme

Food Institutional Research Measure

Please select the appropriate TRL Scale

TRL 4: Technology validated in lab

NRPE Priority area

Food for Health

Total DAFM Award

€218,920.00

Total Project Expenditure

€215,954.92

Rationale for undertaking the Research

Emissions from ruminants (e.g., cattle and sheep), is the single largest source of agricultural methane. These emissions are produced through enteric fermentation, the digestive process by which ruminants break down plant material into nutrients that can be used for energy. Currently, more than 100 countries have committed to reducing agricultural Greenhouse Gas (GHG) emissions in the 2015 Paris Agreement, including Ireland. Agricultural GHG emissions contribute to approximately 6-7% of all emissions. Agriculture mitigation efforts will help the EU meet its long-term target of reducing GHG emissions by 80-95% by 2050.

In the coming decades, livestock farmers will face numerous challenges and the development of technologies and practices which support efficient sustainable food production while moderating greenhouse gas emissions are urgently required. One possible approach is the use of lactic acid bacteria (LAB), bacteria that produce lactic acid as a major end product of carbohydrate fermentation. LAB can be readily isolated from ruminant animals and are currently used onfarm as direct-fed microbials (DFMs) and as silage inoculants. Little research has been carried out on the role of LAB for methane mitigation, and convincing animal data to support this concept are lacking. This project aims to bring together a global network of multidisciplinary researchers to enhance, impact and advance the knowledge transfer of LAB on-farm technologies to address the reduction of enteric methane emissions in ruminant production systems. This project should lead to environmental and societal benefits with the potential to deliver green jobs and increase competitiveness in the agri-food sector.

Methodology

1. The development of a 96-well plate assay to determine the effects of LAB on the growth of Methanobrevibacter JH1 (amethanogens species).
2. Gas chromatography methods to determine the effect of cell-free supernatants or lyophilised bacteriocins from LABon the reduction of methane in vitro. The cell free supernatant of each culture was prepared by centrifugation of the fully grown culture to pellet the bacterial cells and separate them from the cell-free supernatant. 3. Fermentation assays to determine the effect of LAB inoculants on in vitro gas production
3. Silage analysis to determine the effect of LAB on overall silage quality
4. In vivo investigations into animal health, productivity and methane emissions

Project Results

The main result from this experiment was the successful reduction of methane in cattle, through animal intervention studies. Significant methane reduction was seen in the ruminant livestock treatment group resulting in an average methane reduction of 5.7%. Methane reduction was observed while simultaneously maintaining animal health and productivity. It was also determined that LAB strains studied throughout the course of these trials, were effective in producing good quality silage. This project has resulted in a biobank of strains with proven methane reduction in vitro, as well as a wealth of data for their effect on methanogens and animal methane production. This project has also resulted in many peers reviewed publications.

The take home message from this project is that LAB are a viable method to reduce methane emissions from cattle through ingestion via inoculated silage, while maintaining animal health and productivity.

Section 2 - Research Outputs

Summary of Project Findings

This project has identified that lactic acid bacteria are a viable method to reduce enteric methane emissions produced from ruminant livestock. Methodology has been developed to identify LAB which can inhibit methanogen growth and methane inhibition in vitro. These methodologies will prove invaluable in accessing the possibility of microbial interventions for methane reduction. The methods used provide a direct route to market, as silage inoculants are already used in most on-farm technologies. Adaptation of these methods would provide a simple yet effective solution to reduce livestock GHG emissions.

Summary of Staff Outputs

Research Output	Male	Female	Total Number
PhD Students	0	2	2

Summary of Academic Outputs

Research Outputs	Total Number	Details
Training Courses	**22**	**Philiswa Mbandlwa:** 1. An introduction into the National and global food sector module - UCC 2. Leadership for Agrifood researchers: UCC 3. Future learn: Introduction to bioinformatics 4. Current Topics in food and Nutrition Research module - UCC 5. Statistics module - UCC 6. Law and Technology University wide module: UCC 7. Introduction to GC: Teagasc 8. PhD and beyond Masterclass - AFGDP 9. Early Career Researcher Summer School on GHG monitoring andmitigation in agriculture and forestry: Facce- Eragas Amsterdam. 10. STEPS, Scientific Training for Enhanced PostGrad Students - UCC 11. Research Integrity and Ethics module- UCC **Natasha Doyle:** 1. 1.An introduction into the National and global food sector module - UCC 2. Bioinformatics Workshop - Teagasc 3. C.V Writing Workshop - UCC and Reference Management and writing skills workshop - Teagasc Moorepark 4. Current Topics in food and Nutrition Research module - UCC

		5. Statistics module - UCC 6. Grant and proposal writing skills - NoProblems, Naples, Italy 7. ILM Certificate in Leadership and Management 8. PhD and beyond Masterclass - AFGDP 9. Programming in R/Teagasc coding club 10. STEPS, Scientific Training for Enhanced PostGrad Students - UCC 11. Research Integrity and Ethics - UCC
Publications in Peer Reviewed Scientific Journals	**4**	1. Mbandlwa P, Doyle N, Hill C, Stanton C, Ross RP. Bacteriocins: Novel Applications in Food, and Human and Animal Health. 2. Doyle N, Mbandlwa P, Kelly WJ, Attwood G, Li Y, Ross RP, Stanton C,Leahy S. Use of lactic acid bacteria to reduce methane production in ruminants, a critical review. Frontiers in Microbiology. 2019:2207. 3. Stanton C, Leahy S, Kelly B, Ross RP, Attwood G. Manipulating therumen microbiome to address challenges facing Australasian dairy farming. Animal Production Science. 2019 Dec 13;60(1):36-45. 4. Huyen NT, Martinez I, Pellikaan W. Using lactic acid bacteria assilage inoculants or direct-fed microbials to improve in vitro degradability and reduce methane emissions in dairy cows. Agronomy. 2020 Oct;10(10):1482.
PhD Theses	**2**	1. Philiswa Mbandlwa:Lactic acid bacteria (LAB) as animal probiotics for the modification of the Gastrointestinal tract (GIT) 2022 2. Natasha Doyle: The use of lactic acid bacteria as methane mitigation strategies in ruminants 2022

Other	**1**	Book chapter: Doyle, Natasha & Mbandlwa, Philiswa & Leahy, Sinead & Attwood, Graeme & Kelly, Bill & Hill, Collin & Ross, R. & Stanton, Catherine. (2021). The use of feed supplements to reduce livestock greenhouse gas emissions: direct-fed microbials. 10.19103/AS.2020.0077.14.

Intellectual Property

IDF submitted on Lactic acid bacteria (LAB) as silage inoculants for inhibition of methane production in ruminants

Summary of other Project Outputs

Project Outputs	Details	Total No.
New Processes	Novel methodology for the investigations of LAB for methane reduction	1

Potential Impact related to Policy, Practice and Other Impacts

Impact	Details
Environmental Sustainability	Results of this study have demonstrated that selected strain of LAB demonstrating antimicrobial activity again rumen methanogens of are a viable method to reduce methane emissions from cattle through ingestion via inoculated silage, while maintaining animal health and productivity.

Dissemination Activities

Activity	Details

Seminars at which results were presented	Organization of the MethLAB annual meeting for all members. Hosted in Chicago, USA while attending the congress on gastrointestinal function.
Workshops at which results were presented	FACCE ERA-GAS Summer School – attended by Natasha Doyle and Philiswa Mbandlwa
Other	School visit to demonstrate microbiological skills, Cork, Ireland – completed by Natasha Doyle and Philiswa Mbandlwa on two occasions BT Young Scientist Mentors – completed by Natasha Doyle and Philiswa Mbandlwa
Media Events	TResearch magazine publication on reduction of enteric methane using Lab.

Knowledge Transfer Activities	
Identify knowledge outputs generated during this project.	Throughout the course of this project key knowledge outputs have been identified which include a broad range of peer-reviewed literature from all partners. Knowledge has been gained on the role of LAB bacteriocin production against methanogen growth and methane inhibition. A biobank of cultures is available for future initiatives and research. Publications in TResearch magazine allowed for information regarding this project to reach a wider audience. The main knowledge outputs generated by project: 1. Identification of Lactic acid bacteria and bacteriocins with methaneinhibition potential. 2. Development of vitro assays for methane quantification from puremethanogen cultures using GC; and methanogen inhibition using microtitre plate format based on the ability of this M.boviskoreani to grow on ethanol in the absence of H2, allowing high throughput of samples using a small sample volume. This assay allows high throughput identification of

	promising LABs and is an advancement on tools previously available. 3. Methane reduction in ruminants using LAB strains, which are already welldocumented silage inoculants with probiotic characteristics.
Identify any knowledge transfer activities executed within the project.	Knowledge transfer activities included many poster presentations held at conferences globally. Two BT young scientist students were mentored and learned the importance of enteric GHG emissions, as well as LAB as silage inoculants for methane reduction. Several press publications highlighting the importance of this research took place during this project duration, including news articles in the Irish Times, the Irish examiner and through SFI publications. A consortium meeting was held in Chicago which allowed for expertise and knowledge transfer between project members. List of outreach activities include 1. Teagasc Newsletter, published in all Teagasc facilities – produced by Teagasc https://www.irishexaminer.com/business/arid-30955641.html https://www.sfi.ie/researchnews/stories/climate/cows-greenhouse-gases/ https://thetakeout.com/ireland-methlab-climate-change-cows-dairy-beefmethane-1841020902 2. Centre for Animal Nutrition (CAN) Newsletter, published online July 2017 – produced by CAN (https://www.wur.nl/Animal-Nutrition.htm) 2. Facce era-gas 10 year event: Methlab presentation and video 3. Sharing on UCC social Media page (Microbes for Starters) about the MethLAB project and importance of ruminants and climate change.

This project has resulted in the training of many new scientists, including PhD researchers who are now working on developing their skills further with the knowledge gained from this project. Practical knowledge on the cultivation of

strict anaerobes gained by PHD student from MethLAB is now being used in

a different project where they hold post-doc position. As of yet, there is no

change in policies or technologies as a result of this project, however it would be anticipated that in the coming months, withpublication of these results, that this knowledge would result in such.

Section 3: Leveraging, Future Strategies & Reference

Future Strategies

Outline plans for future strategies

Continue to investigate possible LAB strains which may have proven methane reduction capabilities in vivo, that may not have been fully investigated throughout the course of this project duration.

Investigate mechanism(s) by which LAB are able to reduce methane in vivo. (including shotgun sequencing of rumen microbiome for Teagasc Methlab Trial) Dissemination/ publication of results achieved during project duration, i.e. in vitro essays for methane reduction and use of LAB to reduce methane in vivo.

Project Publications

List publications numerically.

1. Mbandlwa P, Doyle N, Hill C, Stanton C, Ross RP. Bacteriocins: Novel Applications in Food, and Human and AnimalHealth.
2. Doyle, N., et al. (2021). The use of feed supplements to reduce livestock greenhouse gas emissions: direct-fedmicrobials. Reducing greenhouse gas emissions from livestock production: 261-286.
3. Doyle N, Mbandlwa P, Kelly WJ, Attwood G, Li Y, Ross RP, Stanton C, Leahy S. Use of lactic acid bacteria to reducemethane production in ruminants, a critical review. Frontiers in Microbiology. 2019:2207.
4. Stanton C, Leahy S, Kelly B, Ross RP, Attwood G. Manipulating the rumen microbiome to address challenges facingAustralasian dairy farming. Animal Production Science. 2019 Dec 13;60(1):36-45.

Huyen NT, Martinez I, Pellikaan W. Using lactic acid bacteria as silage inoculants or direct-fed microbials to improve invitro degradability and reduce methane emissions in dairy cows. Agronomy. 2020 Oct;10(10):1482.