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

2017EN100–Harnessing the potato-microbiome interactions for development of sustainable breeding and production strategies

Final Report

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

SUMMARY

PotatoMETAbiome's overall objectives were to contribute to the development of more sustainable forms of potato cropping by making use of the power and functional traits provided by soil microorganisms and improved interactions between plants and microbes. The specific objectives of the Irish partner were to investigate how plant variety and plant bacteriome respond to fertilization events in general and towards sulfur fertilization in particular. Are certain potato varieties better adapted to limited nutrient supply and can these varieties make better use of their bacteriome when it comes to nutrient uptake? We identified that not only bacteria in the rhizosphere are key players in nutrient mobilization for the benefit of the plant but that the tuberosphere also carries a bacteriome that is different from the bulk soil, potentially contributing to nutrient supply that includes the macronutrient sulfur. Our fertilization experiments with 11 potato varieties in glasshouse settings using different soils revealed that responses towards fertilization greatly depended on the potato varieties as not all varieties produced higher biomass under fertilization in the early growth stage. Some indicators of sulfur cycling were enhanced upon fertilization, while other sulfur cycling indicators responded significantly higher under absence of fertilization. However, bacterial community structures responded to both, fertilization and variety. Sequencing results confirmed that some bacterial clades known to be important in mobilization of organosulfur in cereals and grasslands were under-represented in the potato rhizospheres, while potential new candidates of organosulfur mobilization were detected. Several plant genes were identified to be important for the interaction with microbes and sulfur cycling that responded differently towards lack of nutrient supply from fertilizers (with partner MPI Golm). Particular potato varieties were identified to be better adapted to limited nutrient supply and are supported by the bacteriome and are therefore potential candidates for future sustainable potato cropping.

KEYWORDS

Potato variety, microbial community structure, sulfur cycling

ACRONYM

PotatoMETAbiome

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PUBLICATION DATE

08/06/2023

Section 1 - Research Approach & Results

Start Date

01 April 2019

End Date

31 December 2022

Research Programme

Research Stimulus Fund

TRL Scale

TRL 3: Experimental Proof of Concept

NRPE Priority area

Sustainable Food Production and Processing

Total DAFM Award

€174,700.00

Total Project Expenditure

€139,931.23

Rationale for undertaking the Research

PotatoMETAbiome aimed at identifying potato genotypes that interact effectively with the soil microbiome, and therefore show reduced dependencies on external inputs under non-stress as well as (a)biotic stress conditions. The motivation of the project was to overcome current limitations in potato cropping that over-rely on intensive fertilizer and pesticide use and limited adaptation towards sustainable cropping with reduced fertilizer and pesticide input. In order to achieve this, potatoMETAbiome aimed to i) develop and exploit novel integrated pest and crop management methods and practices, ii) Improve resource-use efficiency of potato crops, iii) identify how potato crops can be an improved part of an ecosystem with interactions between plants and (micro-)organisms ("the plant as a meta-organism") than this is currently the case in conventional agriculture. This was to provide new knowledge for plant breeders to encourage enhanced predictive breeding technologies and development of new genotypes leading to new phenotypes and crop varieties for improvement of plant health, protection, production and resilience. As part of the potaotMETAbiome SusCrop consortium, UL has investigated the stress factor external input in the form of fertilizer in general and sulfur as a main plant nutrient in particular. The rationale was to identify potato varieties that were less dependent on fertilizer input and had higher levels of plantmicrobe interaction to maximise nutrient uptake and with-it improved nutrient use efficiencies.

.**Methodology**

As part of PotatoMETAbiome, UL has conducted two glasshouse trials with 11 potato varieties that were treated +/- fertilizer according to agricultural practice using two different soils. A third pot trial was conducted with only three potato varieties that differed only in the fertilization with sulfate. After approximately 50 days of cultivation, plant leaves were collected for metabolomics and metatranscriptomic analysis (at MPI Golm). The rhizosphere and bulk soil were collected for various types of analysis. Arylsulfatase activity was measured photometrically in the bulk soil after incubation with nitrophenylsulfate as a substrate. Available sulfate, nitrate and phosphate was measured in the bulk soils using ion chromatography. Cultivable bacteria that utilize arylsulfonates as sole S source were quantified from the rhizosphere soil via Most Probable Number and were subsequently isolated and sequenced. DNA was extracted from the rhizosphere soils and subjected to 16S amplicon sequencing and qPCR. A number of qPCR assays were tested alongside the quantification of 16S rRNA gene copies and eventually a number of group specific assays of asfA were established and a new universal asfA qPCR method was developed. While these qPCR methods were successfully utilized for the glasshouse trials with Irish soils, the assays did not produce reliable gene quantities in the field trial from the Netherlands. For the latter, UL was only able to establish the arylsulfatase measurement.

Project Results

Irish field trial with 2 local varieties to identify differences between bulk soil, rhizosphere and tuberosphere: Different mechanisms are implicated in shaping the structure of the tuberosphere microbiome that is dependent on the variety and the soil type. Specifically, the tuberosphere acquires its most of its microbiome directly from the bulk soil but it is also influenced by the root exudates produced by the root system.

Glasshouse trial 11 varieties with cropland soil +/- fertilizer: Potato genotype and fertilizer as well as their interaction constitute significant determinants of microbial community structure in the rhizosphere. Comparison of organic sulfur cycling between the two fertilization conditions revealed that fertilizer had a positive or neutral effect on microbial sulfur cycling, as well as on plant biomass of different varieties. In addition, varieties presented greater differences amongst them in their overall and sulfur cycling communities when fertilizer was applied. Interestingly, bacterial genera with major role in sulfur cycling such as Variovorax and Polaromonas weren't enriched in the rhizosphere soil (extremely low relative abundances) suggesting that other bacterial genera may play this role in rhizosphere of potatoes growing in Irish soils. For instance, Sphingomonadaceae which possess the ssuD gene were identified as one of the 10 most abundant families. Alternatively, dominant taxa together with rare taxa may participate in bacterial sulfur cycling communities. Analysis of the core microbiome revealed a large number of bacterial genera were associated with the rhizosphere of the potato genotypes. Among them, Shpyngopixis was recovered from the bacterial cultures of arylsulfonate utilizers while other core genera belonging to the family Sphingomonadaceae and Rhizobiacea as well as the genus Devosia have been correlated with potato yield in previews studies.

Plant growth chamber trial with 3 varieties +/- sulfate: The potato varieties had similar arylsulfatase activity while one variety had higher populations of arylsulfonate utilizing bacteria under control conditions. Application of sulfate didn't have an effect on arylsulfatase activity and plant biomass but significantly decreased the population of arylsulfonate utilizing bacteria in two varieties. Structure of bacterial communities was also significantly influenced by sulfate application and the genotype. Metabolomic analysis showed that the three potato varieties had completely different metabolomic profiles under control conditions but application of sulfate resulted in a shift of the metabolomic profile of one variety.

Glasshouse trial 11 varieties with grassland soil +/- fertilizer: Results on organic sulfur cycling in the rhizosphere showed that fertilizer didn't have an affect on arylsulfatase activity as all varieties presented the same levels of activity. Contrary to that, application of fertilizer resulted in inhibition or had no effect on arylsulfonate utilizing bacteria growth. Plant biomass increased in all 11 varieties upon fertilization. Variety and fertilizer and their interaction had significant effect on the structure of bacterial communities, but only five varieties presented significant differences among the fertilizer treatments revealing the significance of the soil factor when selecting varieties for improved bacterial associations and sulfur cycling properties.

Section 2 - Research Outputs

Summary of Project Findings

1) Industry and scientific community: PotatoMETAbiome has successfully tested a range of biopesticide formulations that were able to replace synthetic pesticides in potato cropping (TU Graz). These formulations may be commercialised for implementation in the industry. Among the 11 potato varieties tested by UL, the variety Atol was particularly effective in providing yields at limited nutrient input. For sulfur uptake, this variety also showed a high level of bacterial activity. Furthermore, Atol accumulated the highest amount of nitrogen in the shoot biomass upon limited nutrient supply. Current findings will encourage further research into optimization of plant nutrients via (micro-)biological processes in order to ensure future potato cropping. The comparison of the compartments soil, rhizosphere-soil and potatosphere-soil (tuberosphere) by UL identified that the tuberosphere has a microbial community that can be distinct from the rhizosphere and the bulk soil and thus has the potential to support the plant with essential nutrients or to protect the tubers from plant pathogens. Future research should take the tuberosphere as a place of microbial activity into consideration when it comes to improve sustainable forms of agriculture.
2) Consumers: The application of to date underutilized potato varieties may change the consumer landscape with varieties such as Atol may become more prominent for consumers to purchase in the future and with it supporting more sustainable forms of agriculture.
3) Regulatory authorities and policymakers: Further development and optimization of biopesticides may affect the regulation of what pesticides can be utilized commercially in the mid- to long-term future (TU Graz). Approval of new potato varieties may be influenced by the selection of more sustainable varieties (higher nutrient use efficiencies) in the future rather than just focusing on yield and resistance markers alone in order to achieve several of the EU's 2030 targets related to agriculture.

Summary of Staff Outputs

Research Output	Male	Female	Total Number
PhD student	0	1	1
Other/ Temporary	0	1	1

Summary of Academic Outputs

Research Output	Total Number	Details
Publications in Peer Reviewed Scientific Journals	1	The manuscript "Bacterial communities in the potato tuberosphere share similarities with bulk soil and rhizosphere communities, yet possess distinct features" by authors Georgia Voulgari and Achim Schmalenberger is currently under peer review at Plant and Soil and is available as a pre-print under https://doi.org/10.21203/rs.3.rs-2894746/v1
Peer Reviewed Conference Papers	4	MikrobioKosmos 2021 16-18 December 2021, 13th International Conference on Agrophysics, 15-16 November 2021, Lublin, Poland, as well as Microbiology Society Conference, 4-7 April 2022, Belfast. Contributions described the bacterial community structures as identified in the rhizosphere-soil, tuberopshere-soil and bulk soil from two Irish varieties. The ISME Symposium on the 14-19 August 2022 in Lausanne (Switzerland) described how potato genotypes influence soil sulfur cycling and rhizosphere community structures under different fertilization conditions.
PhD Theses	1	Thesis preparation is in an advanced stage for submission in Q3 of 2023 for consideration of the Q4 2023 exam board at UL.

Intellectual Property

There are currently no plans to protect intellectual property from the conducted research. Instead, we aim to make all our findings publicly available for the benefit of all stakeholders.

Summary of other Project Outputs

Project Outputs	Details	Total No.
New Processes	We developed a new qPCR assay to detect/quantify arylsulfonate utilizing bacteria. Up to now, qPCR assays were only able to quantify asfA of phylogentic clades of bacteria, i.e. Variovorax, Polaromonas and Burkholderia. Due to the challenges encountered in potatoMETAbiome in detecting arylsulfonate utilizing bacteria using existing assays, this new assay was developed that is based on new universal primers for asfA that can be utilized for qPCR as well as next generation amplicon sequencing.	1

Potential Impact related to Policy, Practice and Other Impacts

Impact	Details
Environmental Sustainability	UL's research has identified beneficial traits of a range of potato varieties that are beneficial for potato cropping under limited fertilizer use which may result into more extensive practices of potato cropping in the future in order to reach EU 2030 targets of reduced fertilizer applications, reduced environmental pollution and improved nutrient use efficiencies by 50%.

Dissemination Activities

Activity	Details
Media Events	An animated video outlining the potatoMETAbiome principles was created by the coordinator, which is unfortunately not currently available on youtube. The video is currently revised and it is planned to release a shortened version of the video to the potatoMETAbiome website.
Seminars at which results were presented	Findings from the PotatoMETAbiome project from the Irish partner were disseminated at the Departmental Research and Innovation Day in December 2019 (introducing the project).
Workshops at which results were presented	Progress reports were presented on PotatoMETAbiome consortium meetings in person in February 2020 in Pau as well as online in July 2020, November 2020, and March 2023.

Knowledge Transfer Activities

Identify knowledge outputs generated during this project.	The identification of the tuberopshere as a site of potential microbial activity needs to be better exploited in the form of knowledge transfer activities with potato growers as well as breeders. The lockdowns and restricted access to the laboratories has delayed the knowledge generation into late 2021 and 2022 and thus knowledge transfer activities are only now becoming of interest while the project has already ended. However, UL is hosting a successor project called PotatoBIO that will utilize the knowledge gained in the SusCrop project for dissemination and knowledge transfer. PotatoBIO will be running alongside the new ReNu2Cycle Interreg project that is utilizing the Living Lab concept. Thus, UL will aim to create knowledge transfer from potatoMETAbiome into the new projects from 2023 to 2026 and pass it on to stakeholders. For instance, as part of ReNu2Cycle, UL wants to recruit a participating farmer to demonstrate the increments in nutrient use efficiencies in potato cropping, of which microbial driven nutrient mobilization will be one key aspect.
Identify any knowledge transfer activities executed within the project.	There has been too little time to engage in knowledge transfer to date. This will be corrected for the duration of the follow-up project.
List any impacts resulting from the knowledge transferred during the project.	N/A

Section 3 - Leveraging, Future Strategies & Reference

Leveraging Metrics

Type of Funding Resource	Funding €	Summary
Non Exchequer National Funding	€95,000.00	A structured PhD scholarship has been awarded following an internal competition from the Faculty of Science & Engineering Sustainability Scholars Scheme (S3). The scholarship will provide funding for 4 years and will cover a stipend and fees for the partaking student. Furthermore, financial support for consumables has been pledged for the project "PotatoBIO" since commencement of the project. PotatoBIO: Current agricultural management practices over-depend on synthetic fertiliser to directly supply plants with nutrients instead of utilizing the soil's multi-functionality in the form of nutrient storage/release through its beneficial soil microbiota. This is especially the case for potato cropping where current conventional farming practices are not sustainable and are at risk of becoming economically non-viable. PotatoBIO will establish sustainable fertilization practices and variety selection to maximise nutrient use efficiencies and soil microbiota function. Soil column experiments will test the use of innovative recycling derived fertilizer (RDF) blends to replace synthetic fertilizers and minimise nutrient losses. Effects of RDF on the microbiota diversity and function will be assessed to identify beneficial correlations between variety selection and RDF use. Potato tubers will be analysed for resistant starch (RS) content as prebiotic property to improve production of healthy food. PotatoBIO will support several SDGs including 2-zero hunger, 3-food health, 12-responsible production, 13-climate action, 15-life on land as well as EU’s proposed Integrated Nutrient Management Action Plan (INMAP).

Future Strategies

Immediate publication plans are underway. 1) UL's 10+1 pot trial data will be combined with the metabolomic and transcriptomic data generated in MPI Golm. 2) on the sulfur fertilizer effect on three potato varieties with MPI Golm metabolomic data. 3) stand alone research manuscript is developed on the first UL10+1 pot trial (no metabolomic or transcriptomic data). The follow-up project PotatoBIO is due to start in June 2023 and will combine findings from PotatoMETAbiome and an Interreg project to study the effect of recycling derived fertilizers on the growth and the microbiome of potato varieties, some of which will be utilized from potatoMETAbiome. For that reason, a collaboration has been established with one of the potatoMETAbiome partners from IHAR, Poland.

At the time of the establishement of potatoMETAbiome, the UL partner was also leading a GreenDeal 2020 proposal called Solanuverse which ultimately was not successful. However, there will be an emphasis on utilizing the gained knowledge during potatoMETAbiome and the contact from the same project as well as Solanuverse to apply for an EU wide project again, likely as part of the Horizon Europe's Soil Missions or another ERA-NET initiative.

Dissemination and knowledge transfer activities as part of PotatoBIO.

Project Publications

1) Voulgari, G. and A. Schmalenberger (2021) INVESTIGATING THE BACTERIAL COMMUNITIES IN THE BELOWGROUND COMPARTMENTS OF POTATO PLANTS, Mikrobiokosmos Conference, Athens, December 16-18

2) Voulgari, G. and A. Schmalenberger (2021) Comparison of bacterial communities and their organosulfur utilization in the potato rhizopshere, tuberospahere and bulk soil. Internation Conference on Agrophysics, November 15-16.

3) Voulgari G., Schmalenberger A. (2022) BACTERIAL COMMUNITIES IN THE BELOWGROUND COMPARTMENTS OF POTATO PLANTS. Microbiology Society Symposium, Belfast, April 4-7

4) Voulgari G., Ikoyi, I., and Schmalenberger A. (2023) Potato genotype influence on soil sulfur cycling and rhizosphere community structure under different fertilization conditions. ISME Symposium, Lausanne, August 14-19

5) Voulgari, G. and A. Schmalenberger (2023) Bacterial communities in the potato tuberosphere share similarities with bulk soil and rhizosphere communities, yet possess distinct features. ResearchSquare doi.org/10.21203/rs.3.rs-2894746/v1 under review @ Plant and Soil