Identification and characterization of biocontrol organisms and unraveling their mode of action to tackle Phytophthora cactorum in strawberry

A Data Management Plan created using DMPonline.be

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Project abstract:

In Flanders and worldwide, strawberry growers are facing increasing problems caused by fungal and oomycete pathogens. One of them, Phytophthora cactorum, triggers crown, leather, and root rot diseases, resulting in considerable strawberry yield losses yearly. Due to European legislation, chemical fungicides currently used in strawberry disease management are progressively phased out. Combined with the apparition of tolerant strains, there is an urgency for a sustainable solution. Biocontrol organisms (BCOs) with antagonistic activity towards phytopathogens are a promising sustainable alternative to chemical fungicides. The few microbial BCO-based products allowed in Europe are not dedicated to and/or less effective for management of strawberry diseases. Our aim is to identify and characterize new BCOs to manage P. cactorum in strawberry cultivation. A new screening platform for mycelium growth and/or zoospore germination inhibition will be optimized. The potential of identified BCO candidates is further evaluated in a bioassay and greenhouse trial. The mode of action of the best performing strain is examined with complementary in silico genome analysis and identification of the nature of the compound(s) with LC or GC-MS. Ultimately, this project contributes to a tangible solution for management of P. cactorum in strawberry cultivation, the development of an innovative screening method for BCOs, and more insight into the mode of action of the best performing BCO(s).

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Application DMP

Questionnaire

Describe the datatypes (surveys, sequences, manuscripts, objects …) the research will collect and/or generate and /or (re)use. (use up to 700 characters)

For this project, bacterial strains isolated from previous experiment will be used. New data will be generated after testing these strains ability to inhibit *Phytophthora cactorum* in vitro and in strawberry. No personal data will be included.
The data will comprise the following types:

New data generated:

- in vitro screenings for antagonistic activity:
  - raw images: jpeg and tif
  - data table summary: .xlsx
  - statistic analysis and graph: .prism, .R, .tif, .xlsx
  - microscopy images: .jpeg and .zvi
- in vivo screenings for antagonistic activity:
  - raw images: jpeg and tif
  - data table summary: .xlsx
  - daily monitoring of plant growth: .xlsx
  - statistic analysis and graph: .prism, .R, .tif, .xlsx
- bioinformatic analysis
  - DNA quality check: .xlsx, .sql
  - qPCR results: .xlsx
  - sequencing results: .fasta, .ab1, .pdf, .fastq
  - gel pictures: .tiff
  - phylogenetic trees: .tiff, .mega
  - alignment: .mega, .tiff
  - identification of genes of interest: .fasta, xlsx, .gbk, .mol
  - statistic analysis and graph: .prism, .R, .tif, .xlsx
- chemical analysis:
  - LC-MS or GC-mS: .andi, .mzML, .xlsx
  - HPLC: .tiff, .xlsx
- lab process:
  - storage, daily monitoring, results summary, protocols: .xlsx, .one, .docx, .db
  - presentation and illustration: .ppt, .pdf, .tiff, .ai, .psd
-

Existing data re-used:

- information regarding the lab bacterial collection (.fasta, .xls)
- R scripts (.R)

The estimated data storage size will be in the order of 500GB.

Specify in which way the following provisions are in place in order to preserve the data during and at least 5 years after the end of the research? Motivate your answer. (use up to 700 characters)

The supervisor of this project Hans Rediers will be responsible for the long-term preservation of data. KUL's RDR (Research Data Repository) provides enough storage space for long term preservation. The data will remain accessible for the supervisor of the project. The publication will be in open access journals as much as possible. As the project presents an economic interest, we will consider patenting the results depending on the evolution. In this case, the data access will be restricted to KUL R&D and the main actors of the project, i.e. the supervisor Hans Rediers and the PhD student Juliane Ferreira.

What's the reason why you wish to deviate from the principle of preservation of data and of the minimum preservation term of 5 years? (max. 700 characters)

NA

Are there issues concerning research data indicated in the ethics questionnaire of this application form? Which specific security measures do those data require? (use up to 700 characters)

NA

Which other issues related to the data management are relevant to mention? (use up to 700 characters)

NA

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DPIA

DPIA

Have you performed a DPIA for the personal data processing activities for this project?

- Not applicable

Identification and characterization of biocontrol organisms and unraveling their mode of action to tackle Phytophthora cactorum in strawberry
GDPR

GDPR

Have you registered personal data processing activities for this project?

- No

Identification and characterization of biocontrol organisms and unraveling their mode of action to tackle Phytophthora cactorum in strawberry
FWO DMP (Flemish Standard DMP)

1. Research Data Summary

List and describe all datasets or research materials that you plan to generate/collect or reuse during your research project. For each dataset or data type (observational, experimental etc.), provide a short name & description (sufficient for yourself to know what data it is about), indicate whether the data are newly generated/collected or reused, digital or physical, also indicate the type of the data (the kind of content), its technical format (file extension), and an estimate of the upper limit of the volume of the data.

				Only for digital data	Only for digital data	Only for digital data	Only for physical data			
Dataset Name	Description	New or reused	Digital or Physical	Digital Data Type	Digital Data format	Digital data volume (MB/GB/TB)	Physical volume			
Development of a new screening platfrom		*Please choose from the following options:* • Generate new data • Reuse existing data	*Please choose from the following options:* • Digital • Physical	*Please choose from the following options:* • Observational • Experimental • Compiled/aggregated data • Simulation data • Software • Other • NA	*Please choose from the following options:* • .por, .xml, .tab, .cvs,.pdf, .txt, .rtf, .dwg, .gml, … • NA	*Please choose from the following options:* • <100MB • <1GB • <100GB • <1TB • <5TB • <10TB • <50TB • >50TB • NA				
Development of a new screening platform	to validate the new screening platform, mycelium growth inhibition and zoospore germination tests will be executed on a selection of ~50 bacterial strains, and compared with the new platform. For MGI, picture of agar plates and corresponding quantitative data will be stored (mycelium radius, percentage ofinhibition compared to the control inoculation). For ZGI, microscopy pictures and corresponding quantitative data will be saved (number of zoospores in a picture frame and percentage of germination). For the platform, picture of the petri dish will be saved and corresponding quantitative data (size of the inhibition zone). All quantitative data will be saved on a xls file, all picture will be saved on a dedicated folder. One folder is created per replicate.	generate new data	digital	experimental data, compiled/aggregated data	.xls and .jpg	<1GB				
screening for new BCOs in vitro	~1000 bacterial strains will be tested using the platform newly developed. Picture of the petri dish will be saved and corresponding quantitative data (size of the inhibition zone). All quantitative data will be saved on a xls file, all picture will be saved on a dedicated folder. One folder is created per replicate.	generate new data	digital	experimental data, compiled/aggregated data	.xls and .jpg	<1GB				
identification of the bacteria species by 16S sequencing	for unknown bacteria, DNA will be extracted and 16S sequence will be sequenced using sanger sequencing (Marogen). Resulting DNA will be stored at -20°C and fasta sequencing results will be kept.	generate new data	digital and physical	experimental data, compiled/aggregated data	.fasta	<100MB	up to 5 1.5ml tube storage boxes			
identification of a phylegenetic signal for antagonistic bacteria	16S sequence will be aligned in order to build a phylogenetic tree using MEGA	generate new data	digital	experimental data	.aln	<100MB				
in vitro screening for antagonistic metabolites	use medium-specific assay to identify the production of antagonistic compounds: cellulase, protease, biosurfactant, siderophores. Plates pictures will be saved and quantitative data (size of the inhibition zone) will be kept.	generate new data	digital	experimental data	.jpg .xls	<1GB				
in vitro screening for production of volatile organic compounds	production of volatile by the bacteria will be assessed by physically separating the bacteria from Phytophthora cactorum, and assess its growth after 7 days. Plates pictures will be saved and quantitative data (diameter of the mycelium) will be kept.	generate new data	digital	experimental data	.jpg .xls	<1GB				

in vitro screening for production of water soluble antagonistic compounds	production of antagonistic compound by the bacteria will be assessed by repeating the in vitro screening for mycelium growth and zoospore germination inhibition with cell-free supernatant of bacteria instead of cell culture. Plates pictures will be saved and quantitative data (diameter of the mycelium) will be kept.	generate new data	digital	experimental data	.jpg .xls	<1GB				
assessment of the BCO activity spectrum in vitro	BCO will be tested for their activity in vitro against other phyto-pathogens' mycelium growth. Plates pictures will be saved and quantitative data (diameter of the mycelium) will be kept.	generate new data	digital	experimental data	.jpg .xls	<1GB				
Detached leaf assay to evaluate the potential of BCO in planta	Strawberry leaves will be inoculated with BCO and/or pathogen and lesion size will be monitored. qPCR will be done to quantify *Phytophthora cactorum*. Leaves pictures will be saved and quantitative data (area of the lesion) will be kept. DNA extract will be stored at -20°C and processed. qPCR results will also be saved.	generate new data	digital and physical	experimental data, compiled/aggregated data	.jpg .xls	<1GB	3-4 1.5ml tube storage boxes at -20°C			
Evaluation of the biocontrol potential of bacteria in planta	Whole plants will be inoculated with Phytophthora and BCOs to check for their protective ability. Plants will be monitored weekly for above ground-size, number of stems, number of flowers, number of buds, number of fruits, collapsing status. After 2 months, plants will be harvested and the crown will be inspected for development of Phytophthora cactorum crown rot. Pictures will be taken and qPCR will be done to quantify PC in the crown. Whole plants will be stored at -20°C, DNA extracts will be stored at -20°C, crown picture and lesion size quantitative data will be kept	generate new data	digital and physical	experimental data, compiled/aggregated data	.jpg .xls	<1GB	3 whole shelves at -20°C for plant storage (temporary) and 3-4 1.5ml tube storage boxes at -20°C			
In silico screening for biosynthetic gene clusters involved in the antagonistic activity	A few promising strains will be sequenced using illumina sequencing (by Genomics Core in Leuven).DNA will be kept at -20°C. Output will be saved as fastq files and further processed: fastqc report (pdf files), genome assembly using spades (fasta files). Genome mining will be done using spades and/or prism3 (online tools) and output information will be saved on a tabler.	generate new data	digital and physical	experimental data, compiled/aggregated data	.fasta .fastq .pdf .xls	<1GB	3 whole shelves at -20°C for plant storage (temporary) and 3-4 1.5ml tube storage boxes at -20°C			
identification of bio-active metabolites	Metabolites will be extracted with different solvents and their nature will be determined by LC-MS. Activity will be tested by repeating the screening assay for mycelium activity with crude extract only. Plates pictures will be saved and corresponding quantitative data will be summarized in a tabler (inhibition zone size). LC-MS output will also be saved as andi files. Crude extract will be kept at -20°C for further investigation.	generate new data	digital and physical	experimental data, compiled/aggregated data	.jpg .xls .ANDI	<100GB	shelve at -20°C for crude extract storage (temporary)			
validation of the mechanism by KO	Candidate genes will be knocked out using CRISPR technology. New plasmid coding for the target sequence complementary RNA will be generated and mutant bacterial lines will be generated. They will be added to the lab microbial collection at -80°C. Correct mutation will be tested by sequencing the target region. Sequencing results will be managed as described previously. Screening for activity in vitro and in planta of the mutant bacterial strains will be assessed similarly to the previous experiment. Picture of the plates/leaves/plant will be saved and corresponding quantitative data will be compiled in a tabler.	generate new data	digital and physical	experimental data, compiled/aggregated data	.jpg .xls .fasta	<100GB	1.5ml tube storage box at -80°C.			

°C

If you reuse existing data, please specify the source, preferably by using a persistent identifier (e.g. DOI, Handle, URL etc.) per dataset or data type:

NA

Are there any ethical issues concerning the creation and/or use of the data (e.g. experiments on humans or animals, dual use)? Describe these issues in the comment section. Please refer to specific datasets or data types when appropriate.

- No

Will you process personal data? If so, briefly describe the kind of personal data you will use in the comment section. Please refer to specific datasets or data types when appropriate.

- No

Does your work have potential for commercial valorization (e.g. tech transfer, for example spin-offs, commercial exploitation, …)? If so, please comment per dataset or data type where appropriate.

- Yes

In the expectation we identify a viable biological control agent to manage Phytophthora cactorum in strawberry cultivation (and potentially other pathosystems), we will consider patenting the discoveries. Biological control technology is generating increasing interest from agrochemical producing companies in order to comply with the European policy aiming at reducing chemical usage for food production. Considering this, the project could be commercially valorized on later stages of the research. All the data set generated will be encompassed, as they are legally required for the commercialization of BCO formulation.

Do existing 3rd party agreements restrict exploitation or dissemination of the data you (re)use (e.g. Material/Data transfer agreements/ research collaboration agreements)? If so, please explain in the comment section to what data they relate and what restrictions are in place.

- No

Are there any other legal issues, such as intellectual property rights and ownership, to be managed related to the data you (re)use? If so, please explain in the comment section to what data they relate and which restrictions will be asserted.

- No

2. Documentation and Metadata

Clearly describe what approach will be followed to capture the accompanying information necessary to keep data understandable and usable, for yourself and others, now and in the future (e.g., in terms of documentation levels and types required, procedures used, Electronic Lab Notebooks, README.txt files, Codebook.tsv etc. where this information is recorded).

All protocols used in this project will be explicitly described according to the format we use at our lab. This format comprises the following information:

- purpose and application
- principle
- equipment and reagents
- quality control
- safety precaution
- procedure
- results
- recording data
- method limitation
- reporting results
- reference procedure
- references
- appendices

Daily work will be monitored in an electronic lab notebook on OneNote, with the location of the data resulting from the experiment on the computer. Data will be sorted according to the work package of the project, and each folder will comprise:

- raw data (example: pictures)
- compiled data (example: excel file)
- trace of data analysis (using excel, R or GraphPad Prism)
- progress report with the "story line" around the data on powerpoint
- when needed for the comprehension, a .txt file with explanation of the data/protocol

Will a metadata standard be used to make it easier to find and reuse the data? If so, please specify (where appropriate per dataset or data type) which metadata standard will be used. If not, please specify (where appropriate per dataset or data type) which metadata will be created to make the data easier to find and reuse.

- No

3. Data storage & back-up during the research project

Where will the data be stored?

The data will be stored in three environment:

- locally on the computer (:C environment) for daily work
- on the KU Leuven network drive associated with the staff number (:I environment)
- on the cloud storage used at KU (One drive)
- Long-term storage is provided on the KULeuven network (K-)drive managed by the supervisor (Prof. H. Rediers)
- The KU Leuven network drives are automatically backed up every day

For material data (DNA extracts, plants samples...) each box will be labelled and will contain an information sheet summarizing the experiment and the dates, in order to be able to link it with the lab notebook on OneNote.

How will the data be backed up?

Every month, the data stored on the local environment of the computer (:C environment) will be backed-up on the network environment of KUL (:I environment) and on OneDrive.
Every 6 months, the data will be manually exported to a hard drive for the supervisor of the project to upload on KUL servers (:K environment)

Is there currently sufficient storage & backup capacity during the project? If yes, specify concisely.
If no or insufficient storage or backup capacities are available, then explain how this will be taken care of.

- Yes

How will you ensure that the data are securely stored and not accessed or modified by unauthorized persons?

The local environment as well as the network and one drive environment are only accessible with personal credential. The physical storage of experiments results are only accessible for colleagues and other staff members.

What are the expected costs for data storage and backup during the research project? How will these costs be covered?

The cost for storage are the following:

- external hard drive: covered by PME&BIM lab
- OneDrive subscription: covered by KU Leuven
- K-drive: covered by KU Leuven
- physical storage: covered by PME&BIM lab (-80°C freezer and -20°C freezer)

4. Data preservation after the end of the research project

Which data will be retained for at least five years (or longer, in agreement with other retention policies that are applicable) after the end of the project? In case some data cannot be preserved, clearly state the reasons for this (e.g. legal or contractual restrictions, storage/budget issues, institutional policies...).

All the digital data will be saved for at least 10 years according to KU Leuven policy. Plant material will be stored the time needed for the samples to be processed, during the project timeframe (and will be discarded afterward). DNA extracts will be saved for the time of the project. All the strains being used are already stored in the PME&BIM microbial culture collection and will be stored here indefinitely.

Where will these data be archived (stored and curated for the long-term)?

Data will be stored on the KUL RDR (Research Data Repositories).

What are the expected costs for data preservation during the expected retention period? How will these costs be covered?

Preservation of data will be free of charge, on the KUL servers.

5. Data sharing and reuse

Will the data (or part of the data) be made available for reuse after/during the project? In the comment section please explain per dataset or data type which data will be made available.

- Other, please specify:

The data will be shared on Open Access Repository if possible. For example, genome sequences will be shared on NCBI. In case some project results are patented, the data regarding the biological control agent will not be disclosed for the public until the patent is submitted.

If access is restricted, please specify who will be able to access the data and under what conditions.

In the case the project or part of the project results are submitted to patenting, the R&D team, supervisor and the PhD student working on the project will have access to the data.

Are there any factors that restrict or prevent the sharing of (some of) the data (e.g. as defined in an agreement with a 3rd party, legal restrictions)? Please explain in the comment section per dataset or data type where appropriate.

- Yes, Intellectual Property Rights

If the project is patented, the genomic and chemical information of the strain will not be disclosed to the public.

Where will the data be made available? If already known, please provide a repository per dataset or data type.

If there is no patenting:

- The genomic information will be available for public usage on NCBI
- Publication will be done in Open Access journal as much as possible, following KUL policy

When will the data be made available?

upon publication of research results

Which data usage licenses are you going to provide? If none, please explain why.

If no patent: Creative Commons Attribution

Do you intend to add a PID/DOI/accession number to your dataset(s)? If already available, you have the option to provide it in the comment section.

- Yes

Not available yet.

What are the expected costs for data sharing? How will these costs be covered?

None, the RDR is free of charge. Data sharing will also happen through publication in scientific and professional oriented journals, and the costs associated will be covered by the PME&BIM lab.

6. Responsibilities

Who will manage data documentation and metadata during the research project?

Juliane Ferreira

Who will manage data storage and backup during the research project?

Hans Rediers

Who will manage data preservation and sharing?

Juliane Ferreira and Hans Rediers

Who will update and implement this DMP?

Juliane Ferreira