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# Using experimental evolution to optimize probiotics for their application in wound care

*A Data Management Plan created using DMPonline.be*

**Creator:** Sybren Van Ginneken

**Affiliation:** KU Leuven (KUL)

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## **Project abstract:**

Against the backdrop of a global antimicrobial resistance crisis, wound infections remain a serious challenge for health care due to a lack of effective treatment options. To fill this gap, the topical application of probiotic Lactic Acid Bacteria (LAB) has been explored to combat wound infections. While both in vitro and in vivo data underpin the potential of this strategy, there is still considerable room for improvement. Therefore, this project aims to investigate whether experimental evolution can be used to tailor LAB for their application in wound care by enhancing their antimicrobial and anti-virulence properties against wound-associated pathogens. First, this project aims to deliver in vitro proof-of-concept by designing innovative setups for experimental evolution to improve various probiotic traits of LAB, including their competitive strength against wound-associated pathogens, their collagen-binding properties, their quorum quenching capacity and their co-aggregation with target pathogens. The most promising probiotics that emerge from these evolution experiments will be phenotypically and genotypically characterized and validated in an in vivo porcine wound infection model. If successful, this project will not only contribute to a significant advancement in the domain of wound care, but also provide a more general framework for enhancing the efficacy of probiotics as an antimicrobial strategy.

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## Application DMP

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### Questionnaire

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

Question not answered.

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)

Question not answered.

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)

Question not answered.

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)

Question not answered.

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

Question not answered.

## Using experimental evolution to optimize probiotics for their application in wound care

### DPIA

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#### DPIA

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

- Not applicable

## Using experimental evolution to optimize probiotics for their application in wound care

### GDPR

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#### GDPR

Have you registered personal data processing activities for this project?

- No

# Using experimental evolution to optimize probiotics for their application in wound care

## 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
		Please choose from the following options: <ul style="list-style-type: none"> <li>Generate new data</li> <li>Reuse existing data</li> </ul>	Please choose from the following options: <ul style="list-style-type: none"> <li>Digital</li> <li>Physical</li> </ul>	Please choose from the following options: <ul style="list-style-type: none"> <li>Observational</li> <li>Experimental</li> <li>Compiled/aggregated data</li> <li>Simulation data</li> <li>Software</li> <li>Other</li> <li>NA</li> </ul>	Please choose from the following options: <ul style="list-style-type: none"> <li>.por, .xml, .tab, .csv, .pdf, .txt, .rtf, .dwg, .gml, ...</li> <li>NA</li> </ul>	Please choose from the following options: <ul style="list-style-type: none"> <li>&lt;100MB</li> <li>&lt;1GB</li> <li>&lt;100GB</li> <li>&lt;1TB</li> <li>&lt;5TB</li> <li>&lt;10TB</li> <li>&lt;50TB</li> <li>&gt;50TB</li> <li>NA</li> </ul>	
<b>Evolved probiotics &amp; pathogens</b>	WP1-5: Experimentally evolved and genetically modified probiotic/pathogen strains will be generated starting from the wild-type strains available at the MiCA lab ( <i>L. plantarum</i> ATCC 10241, <i>L. reuteri</i> RC-14, <i>L. rhamnosus</i> GG, <i>P. aeruginosa</i> PAO1, <i>A. baumannii</i> NCTC 13423, <i>S. aureus</i> Newman). All new constructs will be stored in the bacterial collection of the Centre for Microbial and Plant Genetics (CMPG).	Generate new data	Physical (Glycerol stocks of bacterial cells frozen at -80°C)	Experimental	/	/	+/- 150 96-well plates
<b>Cell counts</b>	Pathogen and probiotic cell counts (plate counting and/or flow cytometry) following competition and adhesion assays under various conditions.	Generate new data	Digital	Experimental	.xlsx .xit	< 1 TB	/
<b>Inhibition zone diameters</b>	Inhibition zone diameters of probiotics and their supernatant versus different pathogens in various conditions.	Generate new data	Digital	Experimental	.xlsx	< 1 GB	/
<b>Optical density</b>	Aggregation assays with probiotics and pathogens will be carried out, resulting in optical density measurements to quantify the sedimentation rate.	Generate new data	Digital	Experimental	.xlsx	< 1 GB	/
<b>Microscopy images</b>	Aggregation assays with probiotics and pathogens will be carried out, resulting in microscopy images to visualise aggregation.	Generate new data	Digital	Experimental	.czi	< 100 GB	/
<b>Fluorescence</b>	The ability of probiotics to interfere with quorum sensing in <i>S. aureus</i> will be quantified using a reporter assay based on fluorescence measurements (population level and single-cell level with flow cytometry).	Generate new data	Digital	Experimental	.xlsx .xit	< 1 TB	/
<b>In vivo data</b>	The experimentally evolved probiotics will be tested in an <i>in vivo</i> porcine wound infection model. Biopsies of infected wounds treated with different probiotic groups will be analyzed. Pathogen and probiotic load will be evaluated by determining cell counts (plate counting). Wound healing will be evaluated using standard histopathological analysis on microscopy images of tissue sections. Inflammation will be evaluated using RT-qPCR on inflammatory markers.	Generate new data	Digital	Experimental	.xlsx .czi .eds	< 100 GB	/
<b>Genome sequences</b>	Whole Genome Sequencing data of ancestral and evolved pathogens and probiotics	Generate new data	Digital	Experimental	.fastq .fasta	< 100 GB	/

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:

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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.

- Yes, animal data

Only the final work package of the project requires ethical approval to validate the previously obtained *in vitro* results in an *in vivo* porcine wound infection model. When more *in vitro* data is available to better support an ECD application, such an application will be prepared and submitted. Evidently, no animal experiments will be carried out until ethical approval is acquired.

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

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**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

If the results of my project are promising, the evolution setups that will be designed and the experimentally evolved probiotics resulting from these evolution setups could potentially result into novel antimicrobial wound care products. Therefore, opportunities to protect intellectual property will be regularly discussed with Leuven Research and Development (LRD), and at least prior to publicly releasing any information.

**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

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**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

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## 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).**

The MiCA lab works with a SharePoint-based electronic lab notebook (ELN) supported by KU Leuven. This digital notebook organises all data acquired within a project in a standardized manner and attaches a fixed set of metadata (see below). In addition, separate folders are created for the raw, processed, and final data.

**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

The ELN incorporates a fixed set of metadata, including the user coordinating the experiment, the user performing the experiment, the date, and the experimental protocol used.

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

**Where will the data be stored?**

The data will be stored on the hard drive of my laptop, as well as on the SharePoint-based electronic lab notebook (ELN). The ELN is an application hosted on-site within KU Leuven (meaning it is not a cloud storage). Large data files such as microscopy images and whole genome sequencing files will be stored on the internal server of KU Leuven and linked to the ELN.

**How will the data be backed up?**

The ELN and the internal KU Leuven server contain an automatic back-up for the drive capacity that is in use:

- A back-up every few hours (at 9h, 12h30, and 17h), of which the latest 7 versions are saved
- A daily back-up (at 21h), of which the latest 10 version are saved

A weekly back-up (Sunday at 11h), of which the latest 6 version are saved

In addition to these daily and weekly backups on the internal KU Leuven servers, a regular backup on an external drive will be made to protect against the unlikely scenario in which the KU Leuven servers malfunction.

**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

KU Leuven provides easily expandable storage space based on their internal server maintained by the IT service of KU Leuven. Our lab uses two drives on the KU Leuven internal server: the J-drive, and the K-drive. The J-driver is intended for daily use, while the K-drive mainly serves as a long-term storage space.

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

- Access to the ELN only works via KU Leuven Single Sign On
- Each user should have a valid KU Leuven intranet user ID and password
- Permission for access can be defined in detail by the local admin of the SharePoint site.

Biological samples will be stored in a secured -80°C freezer at the facility.

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

As the use of ELN is relatively new at KU Leuven, no costs are accounted yet. If costs were to be accounted, they will likely resemble these of other SharePoint sites with similar storage capacity, which is

- €344.80 for the first year
- €274.80 for the next years

Additional storage space on the KU Leuven internal server can be acquired. Our lab uses two drives on the KU Leuven internal server: the J-drive, and the K-drive:

- The costs for the J-drive are €519/TB per year
- The costs for the K-drive are €100/TB/year

The costs will be covered by the allocated project budget.

#### 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 data will be retained for at least 5 years after the end of the project.

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

Using the ELN which is connected to the KU Leuven internal server.

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

As the use of ELN is relatively new at KU Leuven, no costs are accounted yet. If costs were to be accounted, they will likely resemble these of other SharePoint sited with similar storage capacity, which is

- €344.80 for the first year
- €274.80 for the next years

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- The costs for the J-drive are €519/TB per year
- The costs for the K-drive are €100/TB/year

The costs will be covered by the allocated project budget.

#### 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.**

- Yes, in an Open Access repository

We plan to make all final datasets publicly available.

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

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**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.**

- No

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

We aim to make all datasets publicly available through publication in Open Access, peer-reviewed scientific journals. At the end of the project, final datasets that have not been published in Open Access scientific journals will be made publicly available using the Research Data Repository (RaDaR), managed by the KU Leuven.

**When will the data be made available?**

Upon publication of research results or upon granting of a patent.

At the end of the project, final datasets that have not been published in Open Access scientific journals will be made publicly available using the Research Data Repository (RaDaR), managed by the KU Leuven.

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

Open Access publications in scientific, peer-reviewed journals are typically covered by a Creative Commons Attribution License (CC-BY). Also the RaDaR repository allows to share data using the CC-BY license.

**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

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

Open Access publication costs in scientific, peer-reviewed journals will be covered by the allocated project budget.

KU Leuven staff has free access to the Research Data Repository (RaDaR). In case the data size exceeds the freely available size limit, additional costs will be covered by the allocated project budget.

## 6. Responsibilities

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

Sybren Van Ginneken

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

Sybren Van Ginneken

**Who will manage data preservation and sharing?**

Sybren Van Ginneken, Hans Steenackers

**Who will update and implement this DMP?**

Sybren Van Ginneken

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