

DR HAB. TOMASZ JAGIELSKI

# Environmental reservoir and infectious cycle of pathogenic algae of the genus *Prototheca*. The occurrence of *Prototheca* spp. in soil environments.



# Prototheca Algae

☼ **Unicellular, colourless (achlorophyllous), saprophytic microalgae, phylogenetically related to *Chlorella* spp.**

**Superkingdom:** Eukaryota

**Kingdom:** Viridiplantae

**Phylum:** Chlorophyta

**Class:** Trebouxiophyceae

**Order:** Chlorellales

**Family:** Chlorellaceae

**Genus:** *Prototheca*

*P. zopfii* (Krüger, 1894)

*P. wickerhamii* (Tubaki & Soneda, 1959)

*P. stagnora* (Cooke, 1968)

*P. blaschkeae* (Rösler et al., 2006)

*P. cutis* (Satoh, 2010)

*P. miyajii* (Masuda et al., 2016)

*P. tumulicola* (Nagatsuka et al., 2016)

*P. bovis* (Jagielski et al., 2019)

*P. cerasi* (Jagielski et al., 2019)

*P. ciferrii* (Jagielski et al., 2019)

*P. cookei* (Jagielski et al., 2019)

*P. moriformis* (Jagielski et al., 2019)

*P. pringsheimii* (Jagielski et al., 2019)

*P. xanthoriae* (Jagielski et al., 2019)

*P. paracutis* (Kunthiphun et al., 2019)

☼ **Ubiquitous in nature (soil, natural and artificial aquatic bodies, sewage, plant debris, animal faeces etc.).**

☼ **Opportunistic pathogens of animals and humans → protothecosis.**

☼ **Protothecosis:**

- results from infection by the only known plants pathogenic to vertebrates!
- occurs predominantly as clinical and subclinical mastitis in dary cattle.
- has been described also in dogs, cats, goats, horses, and fishes.



# Human Protothecosis

At least 335 cases have been reported (Jagielski T., unpublished data).

Most cases are due to: *Prototheca wickerhamii*.

Major clinical forms are:

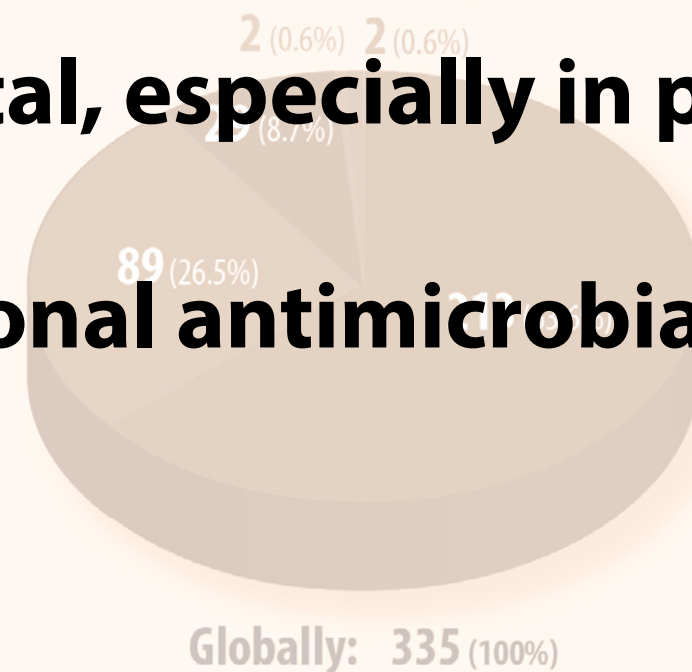
 cutaneous,

 articular,


 disseminated.

Disease is rare, but can be fatal, especially in patients with immunosuppression.

Resistance to most conventional antimicrobial agents → treatment failure, chronicity, recurrence.



 *P. wickerhamii*

 *P. zopfii\**

 *P. cutis*

 *P. miyajii*

 *Prototheca sp.*



# Habitat

 **J. Dairy Sci.** 102:619–628  
<https://doi.org/10.3168/jds.2018-15495>  
 © American Dairy Science Association®, 2019.

**A survey on the incidence of *Prototheca* mastitis in dairy herds in Lublin province, Poland**

Tomasz Jagielski,<sup>1\*</sup> Katarzyna Roeske,<sup>1</sup> Zofia Bakula,<sup>1</sup> Tomasz Piech,<sup>2</sup> Łukasz Wlazło,<sup>3</sup> Mariola Bochniarz,<sup>2</sup> Piotr Woch,<sup>4</sup> and Henryk Krukowski<sup>3</sup>

<sup>1</sup>Department of Applied Microbiology, Institute of Microbiology, Faculty of Biology, University of Warsaw, I. Miecznikowa 1, 02-096 Warsaw, Poland  
<sup>2</sup>Department and Clinic of Animal Reproduction, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, Głęboka 30, 20-612 Lublin, Poland  
<sup>3</sup>Department of Animal and Environmental Hygiene, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, Poland  
<sup>4</sup>Regional Agrochemical Station in Lublin, Sławinkowska 5, 20-810 Lublin, Poland

Mycopathologia (2006) 161: 141–145  
 DOI: 10.1007/s11046-005-0145-8 © Springer 2006

**Epidemiological and clinical aspects of the first outbreak of bovine mastitis caused by *Prototheca zopfii* in Goiás State, Brazil**

Válter Ferreira Félix Bueno<sup>1</sup>, Albenones José de Mesquita<sup>1</sup>, Rodrigo Balduino Soares Neves<sup>1</sup>, Marcos Almeida de Souza<sup>1</sup>, Andréa Rentz Ribeiro<sup>2</sup>, Edmar Soares Nicolau<sup>1</sup> & Antônio Nonato de Oliveira<sup>1</sup>

<sup>1</sup>Centro de Pesquisa em Alimentos, Escola de Veterinária, Universidade Federal de Goiás, Brazil; <sup>2</sup>Faculdade de Medicina Veterinária, PUC Minas Gerais – Poços de Caldas, Brazil


Received 29 August 2005; accepted in revised form 25 October 2005

J. Dairy Sci. 91:3474–3477  
 doi:10.3168/jds.2008-1115  
 © American Dairy Science Association, 2008.

**Short Communication: Isolation of *Prototheca* Species Strains from Environmental Sources in Dairy Herds**

L. Scaccabarozzi,\* B. Turchetti,† P. Buzzini,† G. Pisoni,\* L. Bertocchi,‡ N. Arrigoni,‡ P. Boettcher,§ V. Bronzo,\* and P. Moroni\*<sup>¶</sup>

\*Department of Veterinary Pathology, Hygiene and Public Health, University of Milan, via Celoria 10, 20133 Milan, Italy  
 †Department of Applied Biology–Microbiology, University of Perugia, 06100 Perugia, Italy  
 ‡Istituto Zooprofilattico Sperimentale di Lombardia and Emilia Romagna, 25100 Brescia, Italy  
 §Institute of Agricultural Biology and Biotechnology (IBBA), Consiglio Nazionale delle Ricerche (CNR), 20133 Milan, Italy

**microbial biotechnology** 

Research Article | Open Access | CC BY

**Prevalence of *Prototheca* spp. on dairy farms in Poland – a cross-country study**

Tomasz Jagielski ✉ Henryk Krukowski, Mariola Bochniarz, Tomasz Piech, Katarzyna Roeske, Zofia Bakula, Łukasz Wlazło, Piotr Woch

First published: 19 March 2019 | <https://doi.org/10.1111/1751-7915.13394> | Citations: 12

Funding Information:  
 The study was financed by the National Science Centre 'SONATA' Programme (2014/15/D/NZ7/01797).

Mycopathologia 137: 33–36, 1997.  
 © 1997 Kluwer Academic Publishers. Printed in the Netherlands.

**Epidemiologic study of environmental sources in a *Prototheca zopfii* outbreak of bovine mastitis**

E.O. Costa, P.A. Melville, A.R. Ribeiro, E.T. Watanabe and M.C.F.F. Parolari  
 Department of Preventive Veterinary Medicine, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Research on Mammary Gland and Milk Production (NAP-GAMA), Brazil

(Received 15 October 1996; accepted in final form 19 April 1997)



**Kurze Charakteristik einiger niederer Organismen im Saftflusse der Laubbäume.**  
 Von Dr. W. Krüger.

1. Ueber einen neuen Pilztypus, repräsentirt durch die Gattung *Prototheca* (*Pr. muriformis* et *Pr. Zopfii*).

**Estudio morfológico y fisiológico de una nueva especie de *Prototheca*: *Prototheca Ciferrii* n.sp., aislada de epidermis de papa**

por los  
 PABLO NEGRONI Y RAÚL BLAISTEN  
 (Inst. Bacteriológico D.N.H. de Buenos Aires)  
 Con láminas V y VI.  
 (16.III.1940.)

**Studies in the Genus *Prototheca*.**  
 II. Taxonomy  
 WM. BRIDGE COOKE<sup>1</sup>  
 U. S. Department of the Interior  
 Federal Water Pollution Control Administration, Cincinnati, Ohio

Mycopathologia 94: 67–73 (1986)  
 © Martinus Nijhoff/Dr. W. Junk Publishers, Dordrecht – Printed in the Netherlands

**The association of *Prototheca* spp. with slime flux in *Ulmus americana* and other trees**

R. S. Pore  
 Department of Microbiology, West Virginia University, School of Medicine, Morgantown, WV 26506, USA

Mycopathologia 81, 49–62 (1983). 0301-486x/83/81/0049-14/\$00.20/0.  
 © Dr. W. Junk Publishers, The Hague. Printed in The Netherlands.

***Prototheca* ecology**

R. S. Pore<sup>1</sup>, E. A. Barnett<sup>2</sup>, W. C. Barnes, Jr.<sup>3</sup> & J. D. Walker<sup>4</sup>

<sup>1</sup>Department of Microbiology, West Virginia University School of Medicine, Morgantown, WV 26506, USA  
<sup>2</sup>Bio-Systems Research, Salida, CO 81201, USA  
<sup>3</sup>Monsanto World Headquarters, St. Louis, MO 63130, USA  
<sup>4</sup>Office of Toxic Substances (TS-778) USEPA, Washington, DC 20460, USA

Available online at [www.sciencedirect.com](http://www.sciencedirect.com)  
**MYCOSCIENCE**  
 ISSN 1340-3540 (print), 1616-2545 (online)  
 Journal homepage: [www.elsevier.com/locate/myc](http://www.elsevier.com/locate/myc)

**Short communication**  
***Prototheca tumulicola* sp. nov., a novel achlorophyllous, yeast-like microalga isolated from the stone chamber interior of the Takamatsuzuka Tumulus**

Yuka Nagatsuka<sup>a,1</sup>, Tomohiko Kiyuna<sup>a,1</sup>, Rika Kigawa<sup>b,2</sup>, Chie Sano<sup>b</sup>, Junta Sugiyama<sup>a,2</sup>

<sup>a</sup> TechnoSuruga Laboratory Co., Ltd., 330 Nagasaki, Shimizu-ku, Shizuoka-shi, Shizuoka 424-0065, Japan  
<sup>b</sup> Independent Administrative Institution, National Research Institute for Cultural Properties, Tokyo, 13-43 Ueno Park, Taito-ku, Tokyo 110-8713, Japan  
<sup>c</sup> TechnoSuruga Laboratory Co., Ltd., Chiba Branch Office, 3-1532-13 Hasama-cho, Funabashi-shi, Chiba 274-0822, Japan



# Research Questions

- ⑧ **What is the prevalence of *Prototheca* spp. in the environment and among animals?**
- ⑧ **What is the major reservoir of the pathogen?**
- ⑧ **What is the infectious cycle of *Prototheca* spp.?**
- ⑧ **What are the sources and transmission pathways of the disease?**
- ⑧ **What are the conditions promoting saprophytic → parasitic conversion?**
- ⑧ **What are the virulence factors?**
- ⑧ **How does the pathogen interact with the host?**

# Environmental reservoir and infectious cycle of pathogenic algae of the genus *Prototheca*. The occurrence of *Prototheca* spp. in soil environments.



**Project no.: BOB-IDUB-622-164/2021**

**Funding agency: University of Warsaw - "Excellence Initiative – Research University (2020-2026)"**

**Leader: Tomasz Jagielski, PhD, DSc**

**Duration period: 1<sup>st</sup> December 2021 – 30<sup>th</sup> November 2022**




**Budget: 99,951.81 PLN**

## AIMS OF THE PROJECT:





- ☉ **To assess the occurrence of *Prototheca* spp. in soil environments.**
- ☉ **What is the isolation rate of *Prototheca* spp. in different types of soils?**
- ☉ **If/how do soil characteristics correlate with the occurrence of *Prototheca* spp.?**
- ☉ **Is there a link between soil infestation by *Prototheca* spp. and the prevalence of bovine mastitis?**

# Algorithm of Research Work

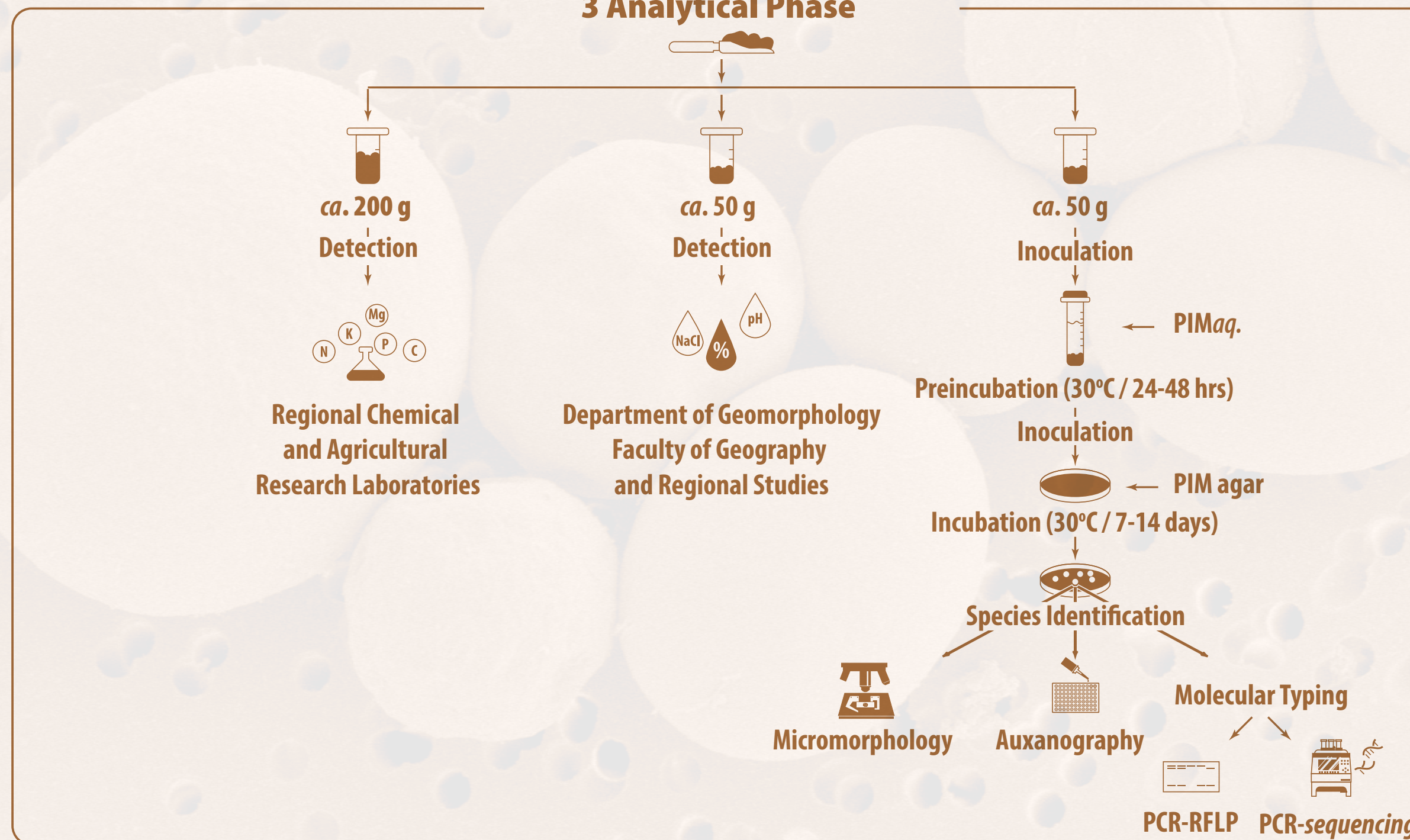
## 1 Preparatory Phase

-  Selection of sampling sites
-  Contracting services with the Regional Chemical and Agricultural Research Laboratories
-  Purchase of reagents and materials

## 2 Sample Collection

-  Questionnaire and photographic-based survey
-  Sample questionnaire
-  Epidemiological questionnaire (*mastitis protothecosa*)
-  Soil sample

## 3 Analytical Phase



## 4 Integration and analysis of data

 Statistical analysis

1 XII.2021 r. - II.2022 r.   2 III.2022 r. - VIII.2022 r.   3 III.2022 r. - VIII.2022 r.   4 IX.2022 r. - XI.2022 r.

# 1 Preparatory Phase



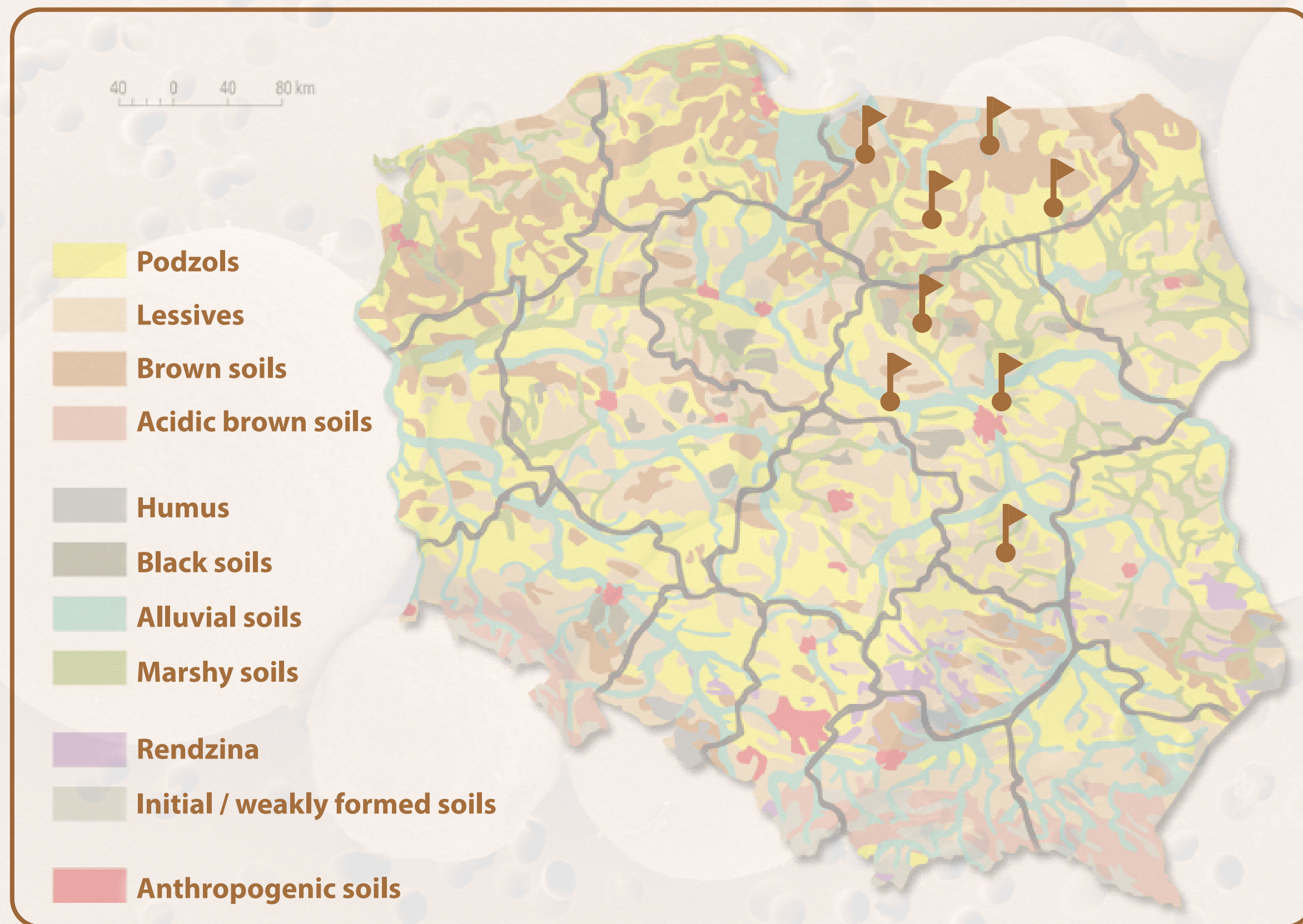
Selection of sampling sites



Contracting services with the Regional Chemical and Agricultural Research Laboratories



Purchase of reagents and materials



**Total: 65 sampling sites**

## 2 Sample Collection



Questionnaire and photographic-based survey



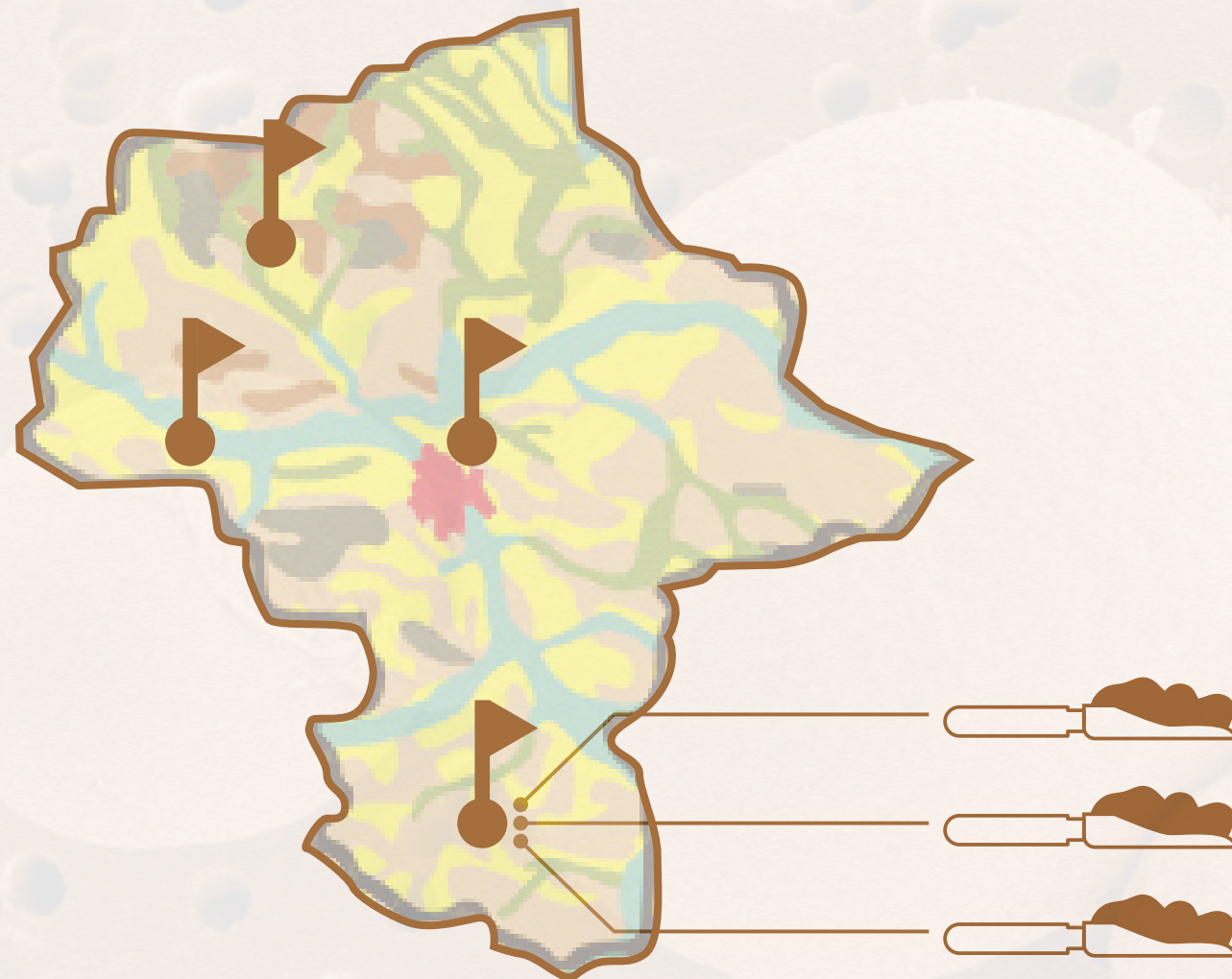
Sample questionnaire



Epidemiological questionnaire (*mastitis protothecosa*)



Soil sample



### SOIL SAMPLE INFORMATION SHEET

Form no. ....

1. Sample Code: .....

2. Location (province, powiat, gmina etc.) .....

3. Size of Area Sampled [Has.] .....

4. Topography (natural/artificial water sources, domestic buildings, farm infrastructure etc.) .....

5. Type of Soil .....

6. Primary Crop .....

7. Manure/Fertilizer Information .....

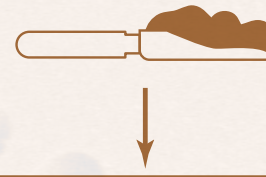
8. Animal breeding .....

9. Others .....

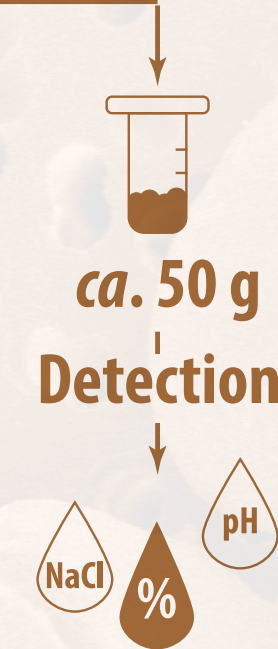
Date: .....



### 3 Analytical Phase



**Regional Chemical  
and Agricultural  
Research Laboratories**



**Department of Geomorphology  
Faculty of Geography and Regional Studies  
University of Warsaw**

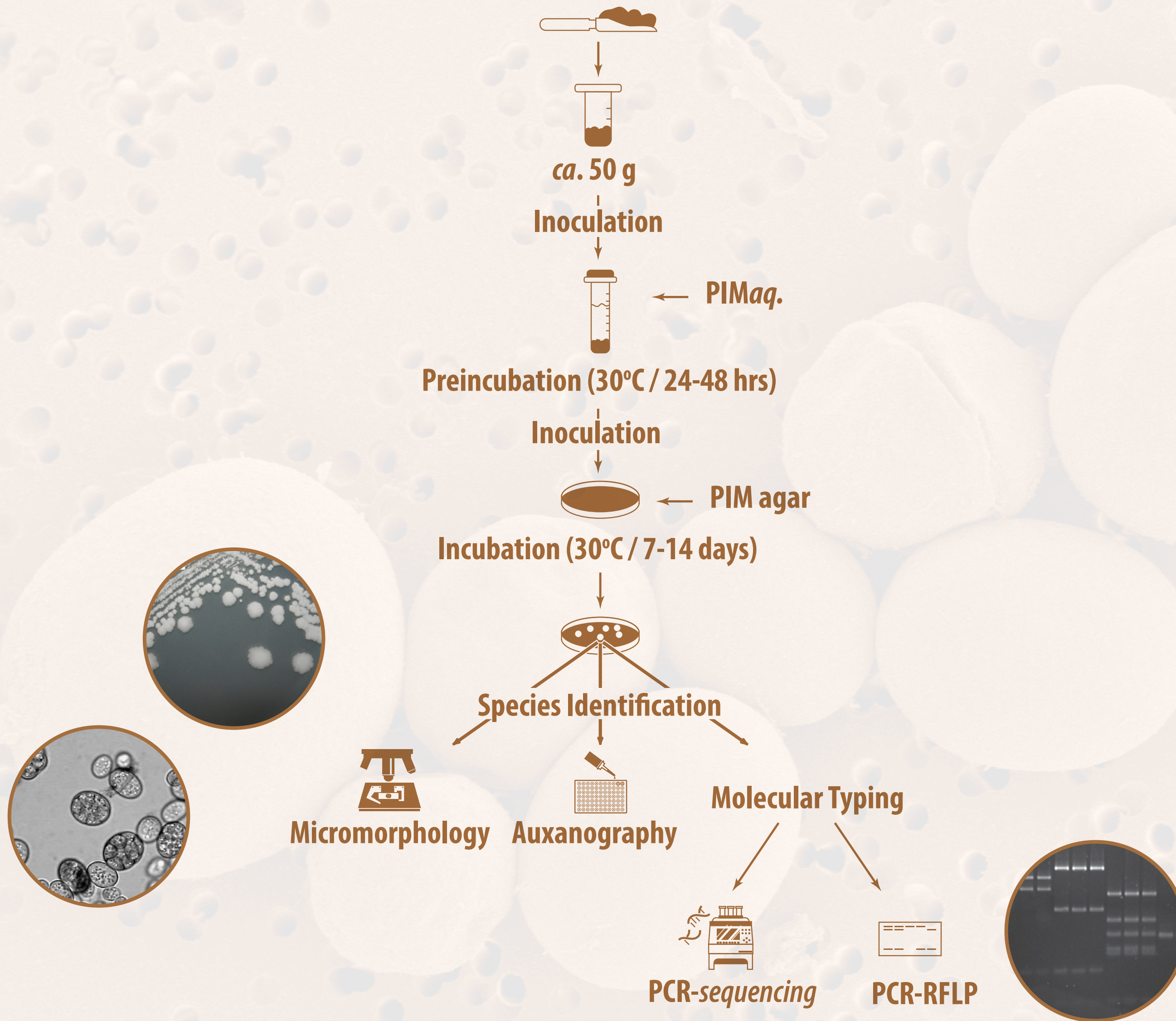


- ⊗ **Total Organic Carbon (TOC, Tiurin's Method)**
- ⊗ **Total Nitrogen Content (TNC, Kjeldahl's Method; Total Kjeldahl Nitrogen, TKN)**
- ⊗ **Macroelements**
  - **Phosphorus (P, Ammonium Molybdate Spectrophotometric Method)**
  - **Magnesium (Mg, FAAS)**
  - **Potassium (K, AES)**

- ⊗ **Soil texture (Particle-Size Distribution Analysis)**
- ⊗ **Moisture (Gravimetric Analysis)**
- ⊗ **Salinity (Electrical Conductivity)**
- ⊗ **pH (Potentiometric Measurement)**



### 3 Analytical Phase



# Significance

- 🌀 The project is conceived as the most extensive study ever performed on the natural habitat of *Prototheca* spp.
- 🌀 The results of the project will provide a substantial insight into the ecology of *Prototheca* spp.
  - Description of the genetic structure of *Prototheca* soil isolates.
  - Identification of factors influencing the occurrence of *Prototheca* spp. in soils.
- 🌀 By combining the results with data on the prevalence of animal protothecosis in the regions surveyed, the project will add importantly to our understanding of the epidemiology of the protothecal disease. It will allow to better recognize:
  - environmental reservoir of the pathogenic species.
  - risks of contracting and transmission (and its drivers) of the disease.
- 🌀 → Development of control and preventive strategies to contain the spread of *Prototheca* infections.



The project will pave the way for embedding a new research field in ZMMed – environmental epidemiology of infectious diseases.

**Thank you for your attention!**

