Uniwersytet Gdański

SUMMARY

of Professional Accomplishments

Development of methods for detecting, identifying and studying the biodiversity of plant pathogens and the application of cold plasma for their eradication

Wojciech Śledź

1. Name
   Wojciech Śledź

2. Diplomas, degrees conferred in specific areas of science of arts, including the name of the institution which conferred the degree, year of degree conferment, title of the PhD dissertation
   2002, PhD diploma in biological sciences in the field of biochemistry, University of Gdansk, Intercollegiate Faculty of Biotechnology University of Gdansk & Medical Academy of Gdansk (now Medical University of Gdansk), Gdansk
   PhD dissertation entitled *"Detection, identification and study of genetic diversity of the Polish collection of bacteria Erwinia carotovora subsp. atroseptica (Pectobacterium carotovorum subsp. atrosepticum)".*
   Thesis supervisor: Professor Ewa Lojkowska
   Thesis reviewers: Professor Adam Jaworski and Professor Piotr Sobiczewski
   PhD dissertation defended with distinction

   1992, Master of Engineering diploma in agriculture, Agricultural and Technical University in Olsztyn (now University of Warmia and Mazury in Olsztyn), Olsztyn
   Master thesis: entitled „The influence of the herbicides Bladex 50WP and Stomp 330EC on the process of mitosis in the selected varieties of field bean *Vicia faba minor* L."
   Thesis supervisor: Dr. eng. Anna Samborska-Ciania

3. Information on employment in research institutes or faculties/departments of arts

2020 – present	academia teacher in the group of research and teaching employees hired on an adjunct position, University of Gdansk, Intercollegiate Faculty of Biotechnology University of Gdansk & Medical University of Gdansk, Laboratory of Plant Protection and Biotechnology
2004 – present	vice-director of the Institute of Biotechnology of Intercollegiate Faculty of Biotechnology University of Gdansk & Medical University of Gdansk

1994 - 2020 academia teacher in the group of research and teaching employees hired on an assistant position, University of Gdansk, Intercollegiate Faculty of Biotechnology at the University of Gdansk & Medical University of Gdansk, Department of Biotechnology, Laboratory of Plant Protection and Biotechnology

4. Description of achievements, set out in art. 219 para 1 point 2 of the Act of 20/07/2018 Law on Higher Education and Science (Journal of Laws of 2021 item 478 as amended). This description should concern a substantive approach to the achievements in question and precisely determine the individual contribution to their creation if a given achievement is a collective work, taking into account the possibility of indicating achievements from the entire professional career.

Title of scientific achievement: ***Development of methods for detecting, identifying and studying the biodiversity of plant pathogens and the use of cold plasma for their eradication***

Publications within the framework of scientific achievement:

[H1] Sledz W., Adamowska A., Piosik J., Łojkowska E. 2012. Detecting live and dead cells of *Pectobacterium atrosepticum* based on immunomagnetics separation and staining. ***Journal of Plant Pathology*** 94(3):681-685 (doi:10.4454/JPP.FA.2012.050)
$IF^a = 2.643$
$IF^b{}_{2012} = 0.688$
$MEiN^c = 40$
$MEiN^d{}_{2012} = 20$
LC^e (WoS) = 2
LC^e (GS) = 1
My contribution to this work included development of the concept of the assay, designing of the experiments and conducting some of the research, substantive and practical supervision of some of the experiments, statistical analysis of the results and preparation of the graphical part of the manuscript, interpretation and partial discussion of the results, formulation of the conclusions, preparation of the preliminary and final version of the manuscript, preparation of the responses to reviewers' comments and improvement of the resubmitted version of the manuscript

[H2] Potrykus[&] M., **Sledz[&]W**., Golanowska M., Slawiak M., Binek A. Motyka A., Zoledowska S., Czajkowski R., Lojkowska E. 2014. Simultaneous detection of major blackleg and soft rot bacterial pathogens in potato by multiplex polymerase chain reaction. ***Annals of Applied Biology*** 165(3) 474-487 (doi:10.1111/aab.12156)
$IF^a = 2.766$
$IF^b{}_{2014} = 2.00$

MEiN[c] = 100
MEiN[d] $_{2014}$ = 40
LC[e] (WoS) = 41
LC[e] (GS) = 72
[&]equal contribution of authors
My contribution to this work included development of the concept of the assay, designing and conducting some of the experiments, substantive and practical supervision of some experiments, participation in the preparation of the first version of the manuscript and discussion on the final content of the manuscript.

[H3] Motyka A., Zoledowska S., **Sledz W.,** Lojkowska E. 2017. Molecular methods as tools to control plant diseases caused by *Dickeya and Pectobacterium spp:* a minireview. ***New Biotechnology*** 39 181-189 (doi:10.1016/j.nbt.2017.08.010)
IF[a] = 6.49
IF[b] $_{2017}$ = 3.733
MEiN[c] = 100
MEiNW[d] $_{2017}$ = 100
LC[e] (WoS) = 33
LC[e] (GS) = 53
My contribution to this work included substantive support in the preparation of the first and final versions of the manuscript, and also included participation in proofreading and discussion of the final content of the manuscript.

[H4] Zoledowska S., Motyka A., Zukowska D., **Sledz W**., Lojkowska, E. 2018. Population structure and biodiversity of *Pectobacterium parmentieri isolated from potato fields in temperate climate.* ***Plant Disease*** 102(1), 154-164 (doi:10.1094/PDIS-05-17-0761- RE)
IF[a] = 4.614
IF[b] $_{2018}$ = 3.583
MEiN[c] = 70
MEiN[d] $_{2018}$ = 70
LC[e] (WoS) = 29
LC[e] (GS) = 38
My contribution to this work included co-creating the concept of this work, participation in conducting the experiments, providing substantive support in working on the initial and final versions of the manuscript, involvement in proofreading and discussion on the contents of the manuscript.

[H5] Motyka-Pomagruk, A., Zoledowska, S., **Sledz, W.,** Lojkowska, E. 2021. The occurrence of bacteria from different species of *Pectobacteriaceae* on seed potato plantations in Poland. ***European Journal of Plant Pathology*** 159(2), 309-325 (doi: 10.1007/s10658-020-02163-x)
IF[a] = 2.224
IF[b] $_{2021}$ = 2.224
MEiN[c] = 100
MEiN[d] $_{2021}$ = 100
LC[e] (WoS) = 13
LC[e] (GS) = 18
My contribution to this work involved co-creating the concept of the research, participation in conducting the experiments, providing substantive support for the preparation of the manuscript, participation in proofreading and discussing the contents of the manuscript.

[H6] Sledz ***W.,** Motyka-Pomagruk A., Zukowska D., Babinska-Wensierska W., Zoledowska S., Lojkowska E. 2023. Genotypic and phenotypic uniformity among the population of *Pectobacterium atrosepticum* strains isolated during three growing seasons from potato fields in Poland. ***European Journal of Plant Pathology***. Opublikowano online 13.05.2023. (doi.org/10.1007/s10658-023-02687-y)
IF[a] = 2.224
IF[b] $_{2023}$ = 2.224
MEiN[c] = 100
MEiN[d] $_{2023}$ = 100
LC[e] (WoS) = 0
LC[e] (GS) = 0
*** corresponding author**
My contribution to this work involved designing and performing some of the experiments, preparing part of the preliminary and final versions of the manuscript, participation in preparation of the responses to reviewers' comments and improving the resubmitted version of the manuscript.

[H7] Sledz*W., Los E., Paczek A., Rischka J., Motyka A., Zoledowska S., Piosik J., Lojkowska E. 2015. Antibacterial activity of caffeine against plant pathogenic bacteria. ***Acta Biochimica Polonica*** 62(3) 605-612 (doi:10.18388/abp.2015_1092)
IF[a] = 2.349
IF[b] $_{2015}$ = 1.187
MEiN[c] = 70
MEiN[d] $_{2015}$ = 40
LC[e] (WoS) = 30
LC[e] (GS) = 52
*** corresponding author**
My contribution to this work involved development of the concept of the research, designing experiments and carrying out some of the experimental work, supervising the experiments, statistical analysis of the results and preparation of the substantive and graphic part of the manuscript, interpretation and discussion of the results, formulation of the conclusions, participation in the preparation of the preliminary and final versions of the manuscript, formulating responses to reviewers' comments and conducting improvement of the resubmitted manuscript.

[H8] Motyka A., Dzimitrowicz A., Jamroz P., Lojkowska E., **Sledz W.[&]**, Pohl P[&]. 2018. Rapid eradication of bacterial phytopathogens by atmospheric pressure glow discharge generated in contact with a flowing liquid cathode. ***Biotechnology and Bioengineering*** 116(6) (1581-1593, doi:10.1002/bit.26565)
IF[a] = 4.395
IF[b] $_{2018}$ = 4.26
MEiN[c] = 100
MEiN[d] $_{2018}$ = 100
LC[e] (WoS) = 11
LC[e] (GS) = 17
[&]equal contribution of the authors
My contribution to this work involved co-authorship of the research concept, designing and performing some of the biological experiments, and participation in the proofreading and discussion of some of the contents of the manuscript.

[H9] Dzimitrowicz A., Motyka-Pomagruk A., Cyganowski P., Babinska W., Terefinko D., Jamroz P., Lojkowska E., Pohl P.[&], **Sledz, W[&]***. 2018. Antibacterial activity of

fructose-stabilized silver nanoparticles produced by direct current atmospheric pressure glow discharge towards quarantine pests. ***Nanomaterials*** 8(10), 751 (doi:10.3390/nano8100751)
IF^{a} = 5.719
IF^{b}_{2018} = 4.039
$MEiN^{c}$ = 100
$MEiN^{d}_{2018}$ = 70
LC^{e} (WoS) = 21
LC^{e} (GS) = 32
& equal contribution of the authors
**** corresponding author***
My contribution to this work involved co-authorship of the concept of the research, designing and performing some of the biological experiments, participation in interpretation and discussion of the results, involvement in partial preparation of the first and final versions of the manuscript, preparation of the responses to the reviewers' comments and correcting the resubmitted version of the manuscript.

$IF^{a)}$ current Impact Factor as of August 29, 2023
$IF^{b)}$ Impact Factor for the year the work was published
$MEiN^{c}$ current score according to MEiN as of August 29, 2023
$MEiN^{d}$ scoring according to MEiN for the year the work was published
LC^{e} number of citations according to Web of Science (WoS)/Google Scholar (GS)

Description of the research objective and achieved results

Introduction

The perspectives for global food security are not optimistic. The size of the human population is constantly growing, and a significant amount of agricultural and food products is lost due to damage resulting from the occurrence of bacterial plant pathogens. It is worth mentioning that aforementioned losses are recorded during vegetation as well as transportation or storage of agricultural products. Literature data suggests that about 40% of the crop yield is wasted due to bacterial, fungal and viral diseases (Ellis and Boehm, 2009). In this context, monitoring the occurrence of plant pathogenic microorganisms, deciphering the paths of their spread, describing thoroughly their biodiversity in addition to proposing innovative, effective methods of combating these microorganisms merits high scientific attention. By addressing these research topics nutritional deficiencies worldwide may be reduced in the future.

In my research work, I focused primarily on pectinolytic bacteria from the genera *Dickeya* and *Pectobacterium*, causative agents of blackleg and soft rot diseases, which infect various crops, ornamentals and vegetables, in particular potato plants (*Solanum tuberosum* L.). It should be mentioned that Poland ranks high as the seventh largest potato producer in the world (FAO, 2012). It is estimated that in our country 2.2 million

growers are engaged in potato cultivation and use for this purpose about 10% of the total planted area. Potato also occupies an important place in international programmes aimed at ensuring global food security, as well as eliminating hunger and malnutrition (International Potato Center, Research for Development). This is why potato is sometimes referred to as the 'crop of the future', even though its global production is even now second to that of maize, rice and wheat (FAO, 2012). Although at least 160 potato diseases have been described so far, Mansfield et al. (2012) listed the 10 most economically significant bacterial plant pathogens; this group included pectinolytic bacteria from the genera *Dickeya* and *Pectobacterium.* It is important to stress that economic losses in potato crops related to blackleg and soft rot differ between countries. For instance in the Netherlands, disqualification of seed potato tubers results in approx. 30 million euros losses annually (Toth et al., 2011). It should be noted that pectinolytic bacteria of the genera *Dickeya* and *Pectobacterium* are broad host range pathogens capable of causing disease symptoms on 50% of angiosperm orders (Ma et al., 2007). Symptoms of blackleg on potato can be observed under field conditions during plant vegetation, while soft rot is recorded not only in the fields, but also during shipment or storage of the crop. Characteristic symptoms of potato blackleg include wilting of the leaves, blackening of the stem base, lack of progeny tubers, and in most severe cases, no plant emergence. The direct causes of these symptoms are assumed to be: degradation of plant tissue by bacterial pectinolytic enzymes and clogging of the vascular system of the plant as a result of the production of exopolysaccharides by the pathogen. In terms of soft rot, a progressive maceration of the tuber tissue is distinctive, which results from the activities of pectinolytic, cellulolytic and proteolytic enzymes produced and secreted by the bacteria from the genera *Dickeya* and *Pectobacterium.*

In the context of my research work to date, it is extremely important that common approaches dedicated for plant protection against bacteria from the genera *Dickeya* and *Pectobacterium* involve only preventive methods (Czajkowski et al., 2011). No effective plant protection products, chemical or biological, have been implemented yet for potato growing sector. Neither has the breeding of potato varieties with increased resistance to these pathogens been proven successful. In addition, the breeding and cultivation of genetically modified plants resistant to phytopathogens meets with public opposition in most European countries, including Poland.

Thus, plant protection against pectinolytic bacteria is based primarily on the use of healthy, certified seed potatoes, avoiding contamination with bacteria by using sanitised agro-technical equipment, and harvesting under optimal weather conditions. Meanwhile, monitoring of the occurrence of pathogens and understanding their spread in the environment becomes of crucial importance. It should be underlined that *Dickeya* and *Pectobacterium* spp. do not cause disease symptoms if they encounter environmental conditions unfavourable for development of the infection process; therefore we often deal with latent infections, which may contribute to further dissemination of the disease. Given the international character of the seed potato market, these infectious agents can be transmitted over long distances, which in turn leads to the effective spread of the so-called "diseases of bacterial origin".

In order to propose new, effective methods to protect plants against bacterial phytopathogens, it is necessary to obtain as much information as possible concerning the causative agent. Pectinolytic bacteria of the genera *Dickeya* and *Pectobacterium* are motile Gram(-) rods with peritrichous flagella. They are facultative anaerobes, incapable of spore formation. Some strains of *Dickeya* and *Pectobacterium* spp. possess fimbriae and haemagglutinins of diameter approx. 7-10 nm (Christofi et al., 1979; Wallace i Pérombelon, 1992). Average cell size of *Pectobacterium carotovorum* subsp. *carotovorum* equals 0.5-0.7 × 1.2-2.2 μm (Ni et al., 2010), while literature data report that *Dickeya* spp. cells reach a size of 1.5-3.6 × 0.6-1.1 μm (Rungnapha et al., 2008). The aforementioned bacteria are now classified within the *Pectobacteriaceae* family, a taxon differentiated from the *Enterobacteriaceae* family.

For the first time these microorganisms were reported in 1926 and described as belonging to the species *Bacillus carotovorus* (Lacey, 1926). Afterwards, these bacteria were reassigned to the genus *Erwinia*, in which the species *Erwinia carotovora* or *Erwinia chrysanthemi* were distinguished (Burkholder et al., 1953). In 1998, these pectinolytic phytopathogens were reclassified from the genus *Erwinia* to the newly differentiated genus *Pectobacterium* as *Pectobacterium carotovorum* or *Pectobacterium chrysanthemi* species, respectively (Hauben et al., 1998). There are currently 22 species classified within the genus *Pectobacterium i.e. Pectobacterium aquaticum* (Pédron et al., 2019), *Pectobacterium aroidearum* (Nabhan et al., 2013), *Pectobacterium atrosepticum* (Gardan et al., 2003), *Pectobacterium betavasculorum* (Gardan et al., 2003), *Pectobacterium cacticida* (Hauben et al., 1998), *Pectobacterium*

colocasium (Klair et al., 2022), *Pectobacterium fontis* (Oulghazi et al., 2019), *Pectobacterium jejuense* (Hong et al., 2023), *Pectobacterium parmentieri* (Khayi et al., 2016b), *Pectobacterium parvum* (Pasanen et al., 2020), *Pectobacterium peruviense* (Waleron et al., 2018b), *Pectobacterium polaris* (Dees et al., 2017b), *Pectobacterium polonicum* (Waleron et al., 2019), *Pectobacterium punjabense* (Sarfraz et al., 2018), *Pectobacterium quasiaquaticum* (Pedron et al., 2021); *Pectobacterium versatile* (Portier et al. 2019), *Pectobacterium wasabiae* (Gardan et al., 2003), *Pectobacterium zantedeschiae* (Waleron et al., 2018a), *Pectobacterium actinidiae, Pectobacterium brasiliense*, *Pectobacterium carotovorum, Pectobacterium odoriferum*, (Gallois et al. 1992, Gardan et al., 2003, Koh et al., 2012, Nabhan et al., 2012, Portier et al., 2019, Skerman et al., 1980).

On the other hand, bacteria from the species *P. chrysanthemi* were reclassified to the genus *Dickeya*, honouring the American phytopathologist Robert S. Dickey by its name. As a result, all strains that belonged to the species *Pectobacterium chrysanthemi* in 2005 were reassigned to this newly distinguished *Dickeya* genus (Samson et al., 2005). There are currently 13 species of *Dickeya*, namely *Dickeya aquatica* (Parkinson et al., 2014), *Dickeya chrysanthemi* (Samson et al., 2005), *Dickeya colocasiae* (Boluk et al., 2022), *Dickeya dadantii* (including *D. dadantii* subsp. dadantii and *D. dadantii* subsp. *dieffenbachiae* (Brady et al., 2012; Samson et al., 2005), *Dickeya dianthicola* (Samson et al., 2005), *Dickeya fangzhongdai* (Tian et al., 2016), *Dickeya lacustris* (Hugouvieux-Cotte-Pattat et al., 2019), *Dickeya oryzae* (Wang et al. 2020), *Dickeya parazeae* (Hugouvieux-Cotte-Pattat et al., 2021), *Dickeya poaceiphila* (Hugouvieux-Cotte-Pattat et al., 2020), *Dickeya solani* (van der Wolf et al., 2014), *Dickeya undicola* (Oulghazi et al., 2019) and *Dickeya zeae* (Samson et al., 2005). It is also interesting that in 2021 the species *Dickeya paradisiaca* (Samson et al., 2005) has been reclassified to the newly created genus *Musicola gen. nov*. (Hugouvieux-Cotte-Pattat et al., 2020), which so far includes two species *Musicola paradisiaca* and *Musicola keenii*.

Bacteria from the genera *Dickeya* and *Pectobacterium* are referred to as pectinolytic due to their ability to degrade pectins, a component of the plant cell wall. The type II secretion system, in response to environmental factors such as: presence of pectin degradation products, limited access to oxygen, changes in pH, temperature, osmolarity, availability of iron ions, lack of complex nitrogen compounds, allows for active secretion of a whole range of virulence factors (Robert-Baudouy et al., 2000).

The key role in pathogenesis is assigned to the enzymes involved in the decomposition of pectins and polygalacturonic acid methylated to varying degrees i.e. methylesterases, acetylesterases, endolyases, and exolyases of pectins and polygalacturonic acid in addition to exopolygalacturonases. It is assumed that endolysases of polygalacturonic acid and pectins are the most important contributors to plant tissue degradation, decomposing these compounds into oligomers used by bacteria as a carbon source (Fagard et al., 2007). Bacteria from the genera *Dickeya* and *Pectobacterium* also have an efficient system for extracting iron ions from the environment, as well as a mechanism for detoxifying free radicals produced by plants in response to the presence of the phytopathogen. The motility of these bacteria and their ability to form a dense biofilm are also of considerable importance, in particular at the early stage of infection. The occurrence of bacteria from the genera *Dickeya* and *Pectobacterium* on the potato, which is the focus of my scientific interest, has been reported in many countries, located in different climatic zones and on different continents. Tsror et al. (2009) estimated the percentage of symptoms caused by bacteria of the genus *Dickeya* in Israel at 10%. In Finland, Degefu et al. (2013) determined that in 2006, as many as 37% of plant samples taken from potato fields showed the presence bacteria from the genus *Dickeya*. In 2008, Pitman et al. described *P. carotovorum* subsp. *carotovorum* as the predominant species of pectinolytic bacteria causes losses on potato in New Zealand. In Chile, on the other hand, it was the *P. atrosepticum* that was the most frequently identified pectinolytic bacterial species in the certified potato seed material (Acuña and Riffo, 1993). In Poland, pectinolytic bacteria are an important economic problem. The research group from the Laboratory of Plant Protection and Biotechnology of the Intercollegiate Faculty of Biotechnology University of Gdansk and Medical University of Gdansk, where I carry out my scientific activity, has been monitoring potato fields and surface waters for the presence of pectinolytic bacteria for many years (Śledź i in., 2000; Sławiak i in., 2009; Potrykus i in., 2015, Żołędowska i in. 2018; Motyka-Pomagruk i in. 2021).

Another important aspect of my research work is studying the transmission pathways of pectinolytic bacteria. Blackleg and soft rot are considered as seed-borne diseases. Nevertheless, it should be underlined that bacteria from the genera *Dickeya* and *Pectobacterium* spp., besides from the infected mother tubers (latently infected seed potatoes) may originate from surface waters, aerosols, biological vectors like insects or nematodes. These bacteria can penetrate into the tissues of stems or tubers through

naturally occurring openings (stomata and spiracles) or through mechanical damages (Huang, 1986).

Human activities contribute greatly to the spread of these microorganisms. This is related not only to international networks of the seed potato trade, but also to the effects of local infiltration of microorganisms through mechanical damage caused by agricultural machinery (especially potato tuber harvesters), but can also be the outcome of "transmission" of bacteria by people or animals (van der Wolf and Kastelein, 2014). The occurrence of these microorganisms have been described in sewage, drainage ditches, streams, rivers and lakes in upland and arable areas, though no isolates have yet been obtained from groundwater. Even rainwater, snow and sea water have been found not to be free of these phytopathogens (McCarter-Zorner et al., 1984). With regard to survival in the soil, it is presumed that these pathogens are incapable of overwintering in soil deprived of plant residues and that they tend to have a higher viability in moist soil than in dry soil (J. van der Waals, oral report EUPHRESCO Workshop, Gdansk 2015). Nonetheless, these bacteria can overwinter on the remains of plant tissues as saprophytes (Czajkowski et al., 2011). Phytopathogens of the genera *Dickeya* and *Pectobacterium* can also be transmitted by vectors, which can be insects and nematodes. As early as the 1920s, Leach (1926, 1931, 1933) discovered that insects of the species *Delia platura* can be vectors for bacteria from the genera *Dickeya* and *Pectobacterium*. Molina et al. (1974) reported that *Drosophila melanogaster* transmits *P. atrosepticum* to healthy potato plants under greenhouse conditions. Particularly interesting is the case of the insect *Acyrthosiphon pisum*, that, according to Gernier et al. (2006), is not only a vector of *D. dadantii* 3937 but also its alternative host. It has also been proven that *Dickeya* and *Pectobacterium* spp. can be transmitted by the nematodes *Caenorhabditis elegans* (Nykyri et al., 2014) and snails (Charkowski, 2007).

To conclude, pectinolytic bacteria of the genera *Dickeya* and *Pectobacterium* are important pathogens of crops including potatoes and a range of vegetable and ornamental plants. They cause significant yield losses ranging from 5 to 40% each year. It should also be taken into account that diseases caused by these bacteria may pose an even greater threat to crops in Europe in the future. It is predicted that the warming climate will favour the spread of bacterial pathogens. The second reason may be drastic restrictions in pesticide use. In 2022 the European Commission proposed regulations (a draft reached the Council of Member States and the European Parliament in 2022), in

which a 50% reduction in the use of certain pesticides is planned by 2030, as well as a complete ban on their use on the so-called "sensitive" areas. At the same time, it is anticipated that there will be a total ban on synthetic plant protection products from 2035. The European Parliament Committee on the Environment, Public Health and Food Safety (ENVI) is currently working on the project and advocates an amendment thereof and a reduction in the use of certain pesticides from 50% to 80% by 2030. Mandatory national pesticide reduction targets and a ban on the use of pesticides in the so-called 'sensitive areas', which, according to representatives of member states, have been defined in the abovementioned project in such a way that they sometimes account for 100% of the agricultural areas of a given country will lead to substantial reduction in pesticides use since 2035.

The data provided above show how important it is to monitor the occurrence and spread of bacterial pathogens in the environment and to develop new methods for fast and reliable identification of the pathogen in addition to innovative and environmentally-friendly efficient plant protection products.

Aim of the research

The main goal of my research, which led to the scientific achievements presented in this application, was to undertake three very important research topics related to the protection of crops (mainly seed potatoes) against pectinolytic bacteria, including:

- development of methods for detecting, identifying and examination of the spread of pectinolytic bacterial pathogens,
- characterization of the biodiversity of pectinolytic bacteria from the selected species classified within the genera *Dickeya* and *Pectobacterium*,
- development of innovative methods for control of bacterial phytopathogens.

Therefore, I have divided this application into three parts, consistent with the topics indicated above, in which I will present the detailed objectives of the research and their results, which constitute the reported achievements.

Presentation of the detailed goals and results of the research work included in the scientific achievement

Development of methods for detection, identification and examination of the spread of pectinolytic bacterial pathogens.

In research on plant pathogens, the methods undertaken by the researcher to identify pathogens in a quick and unambiguous manner are of crucial importance. There are many methods and techniques for detecting and identifying bacterial pathogens including, among others: immunological methods based on antibodies and molecular methods relying on the detection of specific DNA or RNA sequences. These techniques, however, have both limitations and advantages. Therefore, new solutions useful for detection of bacterial phytopathogens are constantly being sought. In this part, I present the research results leading to the development of methods for identification and detection of pectinolytic bacteria from the genera *Dickeya* and *Pectobacterium*.

Presentation of the work: **I**. Detecting live and dead cells of *Pectobacterium atrosepticum* based on immunomagnetics separation and staining

The aim of this work was to develop an assay for detection and identification of living and dead cells of bacterial plant pathogens from the species *P. atrosepticum* (Pba) in potato tuber tissue. The basis of the test was combing immunomagnetic separation (IMS) of bacterial cells with their selective staining using LIVE/DEAD BacLight™ Bacterial Viability kit (Invitrogen, USA). The developed assay was named IMS/LD. LIVE/DEAD BacLight™ Viability Kit contains Syto9 dye (exhibiting green fluorescence at maximum excitation at a wavelength of approximately 480 nm and a maximum emission at 500 nm) and propidium iodide (PI) (showing red fluorescence at maximum excitation at wavelength 490 nm and a maximum emission at 635 nm). Syto9 has many properties, which make it useful for the so-called "barcoding" (staining) of living bacterial cells among others, including high ability to penetrate cell membranes, low cytotoxicity and enhanced fluorescence after penetrating DNA structures. On the other hand, PI dye penetrates only into dead cells with damaged membranes and stains DNA and RNA as a result of intercalation into their molecules (Monis et al., 2005). It should be emphasized that PI has a stronger affinity than Syto9 for nucleic acids, so when both dyes penetrate the cell (which is only possible in the case of dead cells with damaged membranes), Syto9 is "eliminated" from the structures of nucleic acids by PI. For the IMS method, we used two types of magnetic beads: Epoxy M-450 and M-280 (Dynabeads; Invitrogen, USA) and monoclonal antibodies for the most frequently represented serogroup I Pba (Pba Z-Eca04A-01, Prime Diagnostics PRI Wageningen, The Netherlands).

Research outcomes: The best linkage, separation and detection of Pba cells with Epoxy M-450 beads were achieved when the beads were coated in the first stage with 5.0 μg ml^{-1} of protein A (Sigma, USA). Coating of Epoxy M-450 beads with monoclonal antibodies (Pba Z-Eca04A-01) was the most effective if IgG antibodies were applied at 6.0 μg ml^{-1} concentration and the process took place at a temperature of 22°C. We found that it was necessary to use 0.1% BSA for assuring the binding of IgG to the cell wall antigen. Bacterial cell separation was performed using the Dynal Magnetics bead separation system (Dynal MX4 Mixer; Invitrogen, USA). Staining of bacteria with the previously mentioned dyes was performed while the cells remained attached to the M-450 beads. The optimal dye concentrations were determined to be 8.35 μM for Syto9 and 0.1 mM for PI. The same experimental conditions were optimal for the preparation of M-280 beads, but it was not necessary to use protein A for better attachment of antibodies to the surface of the microspheres. Epoxy M-450 or M-280 microspheres coated in this way (at a final concentration of 2 x 10^6 microspheres per ml) were used to separate Pba bacteria from the infected plant material. During staining, the samples were incubated in the dark for 20 min at a room temperature. These samples were placed on standard diagnostic microscope slides and observed using a fluorescence microscope (ECLIPSE TE2000, Nikon with a filter system: filter G-2A, EX 510-560 nm, DM 575, BA 590 nm; B-2A filter, EX450-490 nm, DM 505, BA 520 nm, laser system 488–515 nm) (Fig. 1).

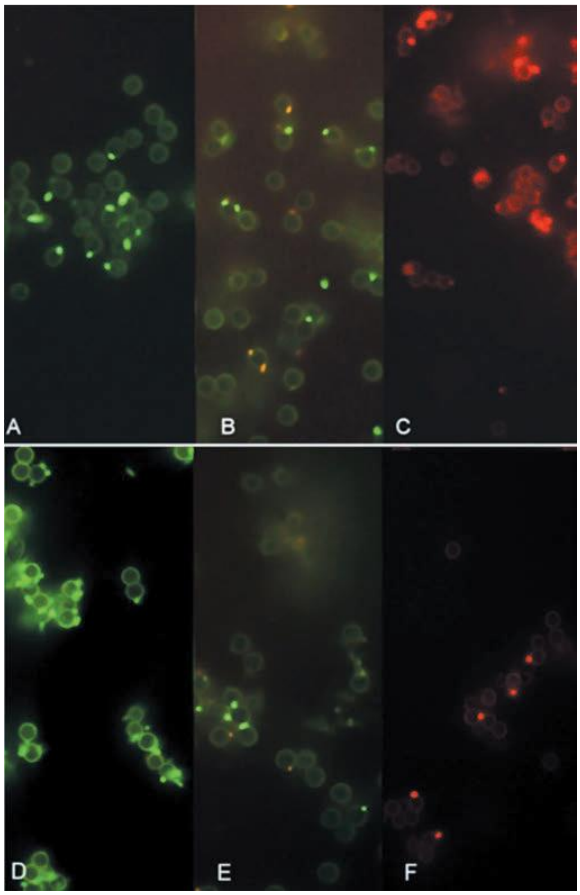

Fig. 1. Isolation and detection of live and dead *Pectobacterium atrosepticum* (Eca SCRI 1043) cells in potato tissue homogenate (peel) by immunomagnetic separation and IMS/LD staining. Bacteria were extracted from the homogenate using 2×10^6 M-280 ml^{-1} beads (A, B, C) and 2×10^6 M-450 ml^{-1} beads (D, E, F); the beads were coated with anti-Pca antibodies (6 µg ml^{-1}). A, D: living cells; B, E: a mixture of live and dead cells in a 1:1 ratio; C, F: dead cells. Green fluorescence indicates living cells (excitation at 480 nm and emission at 500 nm); B, D, F: red fluorescence indicates dead cells (excitation at 490 nm and emission at 635 nm).

The detection level of the IMS/LD test for the identification of Pba bacterial cells was determined in homogenized potato tissue. The detection threshold for Pba in the IMS/LD test was determined as 10^5 cfu/ml of sample with M-280 beads and 10^4 cfu/ml of sample with Epoxy M-450 beads.

Summary of the achievement: The outcome of this research was development of an assay dedicated for isolation and detection of live and dead Pba cells in potato tuber tissue. The detection levels in the IMS/LD assay are similar to those obtained by ELISA with the same antibodies (van der Wolf et al., 1996). An additional advantage of the IMS/LD test is its execution time. The results can be obtained within 1 hour (using previously prepared coated magnetic beads).

Comment on the achievement: The assumption behind the work on IMS/LB was to propose a test supporting other methods of identification of Pba bacteria in "difficult"

environmental samples in which, for example, the presence of PCR inhibitors does not allow for obtaining unambiguous results. In addition, this test is useful when we want to quickly obtain information whether a given plant material is infected with alive Pba bacteria cells or only dead cells are present, which is significant for predicting the possible yield losses.

Presentation of the work: **II.** Simultaneous detection of major blackleg and soft rot bacterial pathogens in potato by multiplex polymerase chain reaction

The main goal of the presented research was to develop a quick laboratory assay useful for detection and identification of pathogenic pectinolytic bacteria that cause diseases called blackleg and soft rot on potato in Poland and Europe. This group included bacteria of the following species: *Pectobacterium atrosepticum* (Pba), *Pectobacterium carotovorum* subsp. *carotovorum* (Pcc) (now *Pectobacterium carotovorum*) together with *Pectobacterium wasabiae* (Pwa) and *Dickeya* spp. (Dsp). I assumed that this assay should enable detection of the above-mentioned bacteria simultaneously (in one sample) and would be based on the so-called polymerase chain reaction (PCR). To develop the multiplex PCR assay, the previously described specific primers Df and Dr for the detection of Dsp (Laurila et al., 2010), primers Y45 and Y46 for identification of Pba (Frechon et al., 1998) and primers ExpccF and ExpccR for detection of Pcc (with Pwa) were used (Kang et al., 2003). The research was carried out to optimize the composition of the amplification mixture and the conditions of the amplification reaction in order to obtain specific DNA products from the three primer pairs used in one multiplex PCR reaction. The specificity of the multiplex PCR test was confirmed during testing 71 bacterial strains, including 48 belonging to the *Pectobacterium* or *Dickeya* genera. The other 23 strains represented species that could be potentially present in the same environmental niche, where potatoes are grown. The sensitivity of the polymerase chain reaction (multiplex PCR) was determined for pure bacterial cultures and in plant material (homogenate). More than one species of pectinolytic bacteria may be present in environmental samples taken from potato tubers. **The developed assay enables the detection and identification of bacteria from the three above-mentioned species, causing single or mixed infections.**

Research outcomes: A specialized original protocol for the isolation of bacterial DNA from potato stem and tuber homogenate was also proposed for this research procedure.

The developed procedure allows getting rid of compounds (including humic acids and starch) that negatively affect the PCR amplification reaction. Here, specific amplification products were obtained for each tested group of bacteria from the genera *Pectobacterium* or *Dickeya* (Fig. 2).

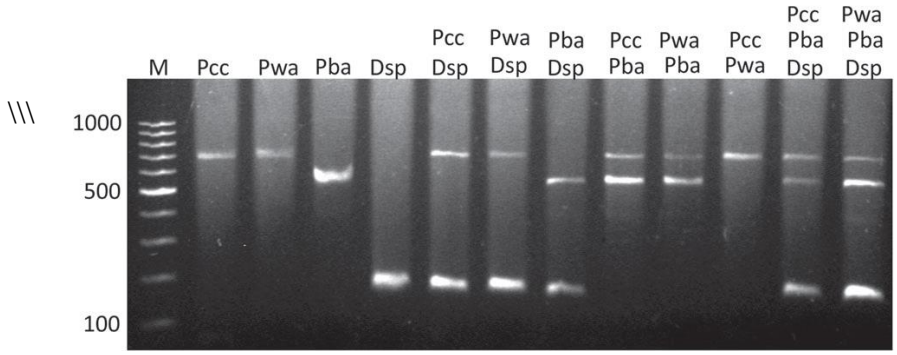

Fig.2. Multiplex PCR – an assay for detection and identification of the main pathogens causing soft rot and blackleg: *P. carotovorum* subsp. *carotovorum*, *P. wasabiae*, *P. atrosepticum* and *Dickeya* spp. The assay was performed using bacterial cell lysates, both single component and mixtures; size of amplification products for each group of the tested pathogens: 550 bp (Pcc/Pwa), 420 bp (Pba), 130 bp (Dsp). Pwa – *P. wasabiae* 3193, Pcc – *P. carotovorum* subsp. *carotovorum* Ecc71, Pba – *P. atrosepticum* SCRI 1043, Dsp – *Dickeya* spp. IFB0099, M – 100 bp DNA standard (Fermentas)

The sensitivity of the developed *in vitro* assay was determined for Dsol bacteria and equalled 0.01 ng μL^{-1} DNA per reaction mixture, while the sensitivity of detection of Pcc and Pba was 0.1 ng μL^{-1}. The sensitivity of the assay for detecting bacteria in plant extracts was determined (in the extract from potato tuber tissue) to be 10^3 cfu/mL for Pwa (10^4 cfu/g tissue), 10^2 cfu/mL (10^3 cfu/g tissue) for Pba and 10 cfu/mL (10^2 cfu/g tissue) for Dsol; (in the case of the potato haulm tissue extract). The detection levels were very similar to those presented above, *i.e.* 10^3 cfu/mL for Pwa, 10^2 cfu/mL for Pba and 10^2 cfu/mL for Dsol. In the case of potato extracts that were infected with more than one species of the pathogen (combinations Pwa+Pba, Pwa+Dsol, Pba+Dsol and Pwa+Pba+Dsol), sensitivity of the assay decreased on average 10-100 times, but in the case of Dsol it remained the same or decreased only tenfold. Evaluations were also carried out to detect the targeted bacteria in samples of potato tubers showing no disease symptoms, which could have been latently infected. The obtained outcomes from the detection of target pathogenic bacteria were consistent with the results of PCR tests involving single primer pairs in 85.5% of the included samples.

Summary of the achievement: **a multiplex PCR assay was developed and optimized for detection and identification of potato bacterial pathogens from the *Pectobacterium* species (*P. atrosepticum* species, *P. carotovorum* and *P. wasabiae*) and *Dickeya*. The developed method is useful for routine testing of seed potatoes for the presence of these bacteria.**

*A) The developed method has been patented: "Method of preparing plant material and method of detecting and identifying bacteria of the species Pectobacterium carotovorum subsp. carotovorum, Pectobacterium atrosepticum and bacteria of the genus Dickeya spp. Patent application (UP RP) No. 397896 from 2012-01-25. Exclusive right number Pat. 223540 from 2016-01-04. Authors: Potrykus Marta, **Śledź Wojciech**, Łojkowska Ewa. Submitted by: University of Gdańsk.*

B) The method described in the publication, after additional validation, was implemented for routine diagnostics in the Research & Development Laboratory IFB UG & MUG. In 2020, the above-mentioned laboratory obtained accreditation (no. AB 1760) for accordance with standard ISO 17025:2018-02 of this method from the Polish Center for Accreditation.

Presentation of the work: **III.** Molecular methods as tools to control plant diseases caused by *Dickeya* and *Pectobacterium* spp: A minireview

This is one of the publications that was prepared during my supervision as an auxiliary supervisor of Agata Motyka's doctoral dissertation.

The aim of the research was to describe important characteristics of bacteria from the genera *Dickeya* and *Pectobacterium*, relevant for their identification process, and to summarize modern and newly developed specific methods most often used for detection and identification of these phytopathogens. In the study, the importance of 20-year research on monitoring the population of pectinolytic SRP (Soft Rot *Pectobacteriaceae*) bacteria on potato plants in Poland was indicated as an example of the successful implementation of molecular diagnostic methods to assess the health of seed potato material in this country. The first large-scale studies on the SRP population in Poland were carried out on strains isolated from cabbage plants and/or potato plants affected with soft rot in the growing seasons 1996 and 1997. Based on biochemical methods, physiological tests and PCR reactions, it was found that *P. atrosepticum* constituted 57% of the SRP population and the remaining identified isolates were designated as *P.*

c. carotovorum (Sławiak M et al., 2009). Majority of the obtained *P. atrosepticum* isolates originated from potato stems with blackleg symptoms and not from the analyzed tubers. Later on, a method based on *recA* PCR-RFLP analysis was implemented for genotyping *Dickeya* and *Pectobacterium* spp. (Waleron et al. 2002), which were isolated also from other vegetables than potatoes. It was found that high genomic heterogeneity was characteristic for the strains *P. c. carotovorum* (18 specific genomic profiles identified) and *Dickeya* spp. (15 profiles) in comparison to bacteria from the species *P. atrosepticum* (only 2 profiles were identified). Afterwards, sequencing of the *recA* gene was implemented for reassessment of the taxonomic position of SRP isolates deposited so far in the collection of the Intercollegiate Faculty of Biotechnology University of Gdańsk and Medical University of Gdańsk (MWB UG & GUMed), which resulted in reclassification of 14% of *P. c. carotovorum* isolates (Waleron et al. 2002) at first to the species *P. wasabiae* and then to *P. parmentieri* (Waleron et al., 2013). The conducted analyzes clearly showed that *P. parmentieri* isolates were an important constituent of the SRP population in Poland already in 1996, but they remained "unnoticed" as incorrectly classified as *P. c.* subsp. *carotovorum*. Apart from Poland, an analogous trend was observed throughout Europe. A similar situation also concerned *P. c. carotovorum* isolates obtained from potato plants or tubers (Waleron et al. 2002), as some of these strains were later reclassified either as *P. c. brasiliense* or *P. c. odoriferum* based on the analysis of gene sequences coding for 16S rRNA, *recA* and *rpoS* (Waleron et al., 2014, 2015).

In 2005, bacteria from the species *D. solani* were detected for the first time in Poland by our research group (Sławiak et al., 2009). This fact encouraged our research group to conduct screening of seed potato plantations in 2009, 2010, 2011 and 2013 and to monitor surface waters in 2010-2013 for the presence of these pathogens. Bacteria classified as *Dickeya* spp. (*D. dianthicola* or *D. solani*) accounted for 5% of the isolates obtained from potato samples. In water samples, strains belonging to *Dickeya* spp. (but only *D. zeae* and *D. chrysanthemi*) were isolated from 0.4% of the aquired samples, which indicated that *Dickeya* spp. do not constitute an important component of the microflora of Polish waters (Potrykus et al., 2016). The performed genomic profiling based on the RFLP-PFGE methodology showed that *Dickeya* spp. isolates from water samples are more diverse than those obtained from potato plants. It is also worth

emphasizing that the isolates from the *Dickeya* genus were detected in most of the studied Polish voivodeships.

Summary: **the conducted research clearly shows that effective methods of detecting and identifying pathogens are very important in the context of monitoring the presence of bacteria from the genera *Dickeya* and *Pectobacterium* on plants and in inland waters.** Thanks to applying molecular methods based on PCR and sequencing of highly conserved housekeeping genes, we identified most of the Polish SRP isolates. Assigning the correct taxonomic position to phytopathogenic bacteria is very important in the case of studies on controlling the spread of particular species of pectinolytic bacteria and taking actions to reduce losses in plant cultivation caused by diseases such as blackleg and soft rot.

Studies on the biodiversity of pectinolytic bacteria from the genera *Dickeya* and *Pectobacterium* present in Poland

In this part of presentation, I describe research on unveiling biodiversity of pectinolytic bacteria. In the following years, the diversity of bacteria from the species *P. atrosepticum*, *P. carotovorum* and P. parmentieri isolated from seed potato plantations in Poland was examined. Describing the frequency of occurrence of pectinolytic bacteria from the genera *Dickeya* and *Pectobacterium* in Poland, their population structure in addition to phenotypic and genomic variability leads to better understanding of the processes related to the pathogenesis of these microorganisms, and may also constitute theoretical basis for development of new methods for detection, identification and monitoring the above-mentioned pathogenic bacteria.

Presentation of the work: **IV.** Population structure and biodiversity of *Pectobacterium parmentieri* isolated from potato fields in temperate climate

The aim of this study was to monitor the occurrence and *characterize P. parmentieri* strains isolated from seed potato plantations in Poland. *Pectobacterium parmentieri* is a recently established (Khayi et al., 2016) species of pectinolytic bacteria belonging to the *Pectobacteriaceae* family. Until recently, strains from this species were classified as *P. wasabiae*, but after a comprehensive genome analysis based on comparative DDH (DNA-DNA Hybridization) and ANI (Average Nucleotide Identity) methods, the *P. wasabiae* strains originating from potatoes were reclassified as *P. parmentieri*.

Although the occurrence of *P. parmentieri* has been confirmed in many countries, no comprehensive studies on the population structure and biodiversity of strains from this species have been carried out so far.

Research outcomes: *P. parmentieri* has been isolated from 13% of seed potato samples collected in 2013 and 2014 in Poland. Bacteria from this species have been also acquired from weeds and soil samples. It was also found that *P. parmentieri* occurs in almost all regions of Poland. Genomic profiling based on repetitive DNA sequences using the REP-PCR method showed the presence of five distinct genomic profiles among the isolated *P. parmentieri* strains. The REP I profile turned out to be the most frequently identified (44%). The phylogenetic analysis based on the *recA* gene sequence revealed the presence of two separate clades among the tested *P. parmentieri* isolates. Both clades included strains isolated either in 2013 or 2014. Phenotypic analysis of features highly important for the virulence of pectinolytic bacteria, including determination of the activities of pectinases, cellulases and proteases, siderophore production and efficiency in maceration of potato tissue, pointed to differences between the characterized *P. parmentieri* strains.

Summary of the achievement. **As part of the conducted research, for the first time the genomic structure of the *P. parmentieri* population originating from seed potato plantations was thoroughly characterized.** It is worth emphasizing that the strains were isolated during two consecutive growing seasons under temperate climate conditions. High heterogeneity within this newly established species was confirmed based on the genotypic and phenotypic characteristics of the included *P. parmentieri* strains. Since the measures to combat soft rot and blackleg on potato implemented so far include only preventive methods, it now seems extremely important to conduct monitoring studies and expand knowledge about biodiversity among strains of pectinolytic bacteria, including *P. parmentieri* species.

Presentation of the work: **V.** The occurrence of bacteria from different species of *Pectobacteriaceae* on seed potato plantations in Poland

The aim of this research was to analyze the frequency of occurrence of various groups of bacteria from the genera *Dickeya* and *Pectobacterium* on seed potato plantations in Poland in 2013 and 2014. The origins of bacteria, seasonal variability in the presence and the type of fertilization applied to the tested seed potato plantations were examined.

The plant material was acquired from 58 different potato varieties collected in 13 voivodeships in 2013 and 12 in 2014.

Research outcomes: In 2013 and 2014, 101 and 104 strains of bacteria belonging to the *Pectobacteriaceae* family were isolated, respectively. SRP bacteria were detected in samples from 11 voivodeships in 2013 and from 12 voivodeships in 2014. *Pectobacterium* spp. were isolated much more often than bacteria from the *Dickeya* genus. The most common species of pectinolytic bacteria on seed potato plantations in Poland turned out to be *P. carotovorum* (36% in 2013, 31% in 2014), *P. atrosepticum* (26% in 2013, 38% in 2014) and *P. parmentieri* (32% in 2013, 23% in 2014). While comparing the collected results with the previous works of our research group, *Pectobacterium* spp. were the main cause of soft rot and blackleg on potato in Poland in the years 1996-2014, in contrast to bacteria from the *Dickeya* genus, whose population turned out to be small and stable since the first detection of these microorganisms in our country in 2005 (as already mentioned earlier).

The tested samples (potato stems, potato tubers and the so-called accompanying weeds) were collected from seed potato fields over a period of 5 months in 2013 (May-September) and 4 months in 2014 (June-September). Interestingly, in the case of samples collected in August and September 2013, a high percentage of detection of the *Pectobacteriaceae* of interest was noted. It should also be emphasized that the *Pectobacteriaceae* bacteria were mainly isolated from potato stems (65% on average), while from weeds only about 6.5% of isolates was acquired.

Summary of the achievement. **As a result of the conducted research, I showed that rods from the species: *P. atrosepticum*, *P. carotovorum* and *P. parmentieri* were widespread in our country since 1997 and outnumbered the population of bacteria from the *Dickeya* genus, which were first detected in Poland in 2005.** Based on the results of our previous research, it can be concluded that bacteria from the *Dickeya* spp. have never played a dominant role in the population of pectinolytic bacteria in Poland. A very interesting fact was that during both analyzed growing seasons, the highest number of strains from the *Pectobacteriaceae* family was isolated in July and it was the only month in which the presence of bacteria of **all the analyzed species was confirmed on seed potatoes in Poland. The research also confirmed the possibility of co-infections of potato plants with bacteria belonging to 2 or 3 different SRP species.** The most common co-infections were these caused by *P. parmentieri* and *P.*

carotovorum. Moreover, it was shown that *Pectobacteriaceae* strains were isolated from most of the potato varieties tested. Studies focused on the sources and occurrence of pectinolytic phytopathogens may contribute to a more effective risk assessment of infections caused by various species of *Pectobacteriaceae*.

Presentation of the work: **VI.** Genotypic and phenotypic uniformity among the population of *Pectobacterium atrosepticum* strains isolated during three growing seasons from potato fields in Poland

The aim of this study was to characterize strains belonging to the species *Pectobacterium atrosepticum* isolated from seed potato fields in Poland. Samples of *S. tuberosum* plants with symptoms of blackleg (blackening of the stem base leading to wilting of the entire plant) and/or soft tuber rot (maceration of the inner tuber tissue) and accompanying weeds were collected from the fields and/or storage facilities and sent by the employees of the State Plant Protection and Seed Inspection to ZOBR laboratory. In total, 118 isolates were characterized (35 from 2013, 41 from 2014 and 42 from 2016), which were identified as bacteria belonging to the species *P. atrosepticum* using a multiplex PCR reaction with primers specific for this species, *i.e.* Y45 and Y46 described by Frechon et al. (1998). These strains were subjected to genomic profiling tests based on rep-PCR method using BOX, ERIC and REP primers. Also phylogenetic and phenotypic studies were carried out for Polish isolates belonging to this species.

It was found that the most effective genetic differentiation method for *P. atrosepticum* isolates was the one based on the presence of repetitive BOX sequences. Application of this method allowed me to classify 118 strains of *P. atrosepticum*, isolated from potato plants in Poland, into 6 different BOX profiles groups. The BOX-PCR genomic profile IV was the one most frequently observed (almost 60% of the isolates showed it). Basing on the results of genomic profiling with BOX primers, 23 isolates from the *P. atrospeticum* species exhibiting genetic diversity and isolated in different years were selected for further research.

Phylogenetic analysis on the Polish population of *P. atrosepticum* was performed relying on the sequences of highly conserved genes encoding proteins involved in basic metabolism of the cell, *i.e. recA* (encoding recombinase A), *gyrA* (encoding the A subunit of gyrase) and *rpoS* (encoding one of the forms of sigma factor: σ^{38} (RpoS),

which is a subunit of the RNA polymerase complex). The *recA* gene sequence turned out to be identical in all included *P. atrosepticum* isolates, while for the *gyrA* sequence one single nucleotide polymorphism (SNP) was identified, distinguishing one isolate (belonging to the I BOX profile group) from the other *P. atrosepticum* strains. Analysis of the sequence of *rpoS* gene revealed the occurrence of 16 single nucleotide polymorphisms among the tested *P. atrosepticum* isolates. A phylogenetic analysis was performed on the *rpoS* sequences, which revealed that majority, *i.e.* 19, of the *P. atrosepticum* environmental isolates included in the study and the reference strain *P. atrosepticum* LMG 2386^{TS} grouped within one clade. Interestingly, the next clade consisted of only two *P. atrosepticum* strains IFB5660 and IFB5661, both exhibiting the BOX profile V.

Phenotypic analysis of *P. atrosepticum* isolates was based on studying features important for virulence among the tested *P. atrosepticum* strains. There were demonstrated variabilities in bacterial abilities to macerate potato tissue at diverse temperatures, *i.e.* 20°C, 28°C and 37°C, in growth in an environment of increased salinity, as well as activities of enzymes that cause disease symptoms on the plant tissues. The capacity of *P. atrosepticum* isolates to macerate potato tuber tissue was investigated at two different temperatures, *i.e.* 20°C and 28°C. As a result of this research, I found that *P. atrosepticum* strains isolated in Poland macerate potato tuber tissue more efficiently at the lower temperature; the maceration potency of the included isolates was at a similar level even though these strains were classified to different BOX profiles groups. None of the Polish *P. atrosepticum* isolates showed an ability to grow at 37°C. Polish strains were incapable of multiplying in conditions of increased salinity (5% salinity). The study on pectinases indicated minimal differences in the activities of these enzymes between Polish isolates of *P. atrosepticum*. Concerning cellulases, the vast majority of *P. atrosepticum* isolates showed low activity of this group of enzymes. Polish isolates of *P. atrosepticum* showed more deviations when they were examined for protease activity. Approximately one third of *P. atrosepticum* isolates had a low ability to produce proteases. The level of protease activity, similarly to the cases of pectinases and cellulases, was not correlated with the observed genomic BOX profiles among Polish *P. atrosepticum* isolates. In terms of efficiency in the production of siderophores, which are necessary to control iron levels in the bacterial cell, some

differences were observed for some *P. atrosepticum* isolates, but these deviations were statistically insignificant.

Summary of the achievement. **The conducted detailed and extensive characterization of *P. atrosepticum* bacterial isolates acquired in Poland during three growing seasons provided evidence of low genetic diversity in this species and notable importance of this phytopathogen for cultivating potato crops under temperate climate conditions.** Since the current approaches utilized to control SRP are based solely on prevention (as mentioned in the introduction), proper identification of the pathogen, in addition to gathering knowledge on the biodiversity, activities of virulence factors and mechanisms of interaction with the host plant, is of crucial importance for building perspectives for proposal of procedures for combating this pest and developing new, more advanced methods of detection and identification.

Development of innovative methods for eradication of the selected phytopathogenic bacteria

Below I will discuss publications, whose aim was to develop alternative and most of all pro-ecological methods of controlling/combating bacterial pathogens infecting economically important crops. Taking into account much greater acceptance of the society for the so-called "green" control methods, which is also related to the dominating tendency to choose bio-products instead of protection products manufactured in a traditional way, I decided to assess the antibacterial action of a natural compound such as caffeine, as well as to examine the possibility of using a reaction-discharge system generating cold atmospheric pressure plasma of the dc-APGD type (Direct Current Atmospheric Pressure Glow Discharge) to eliminate bacterial cells of plant pathogens. The subsequent research was focused on demonstrating the antimicrobial properties of silver nanoparticles obtained by an ecological method based on the use of dc-APGD, against quarantine plant pathogens subjected to compulsory eradication.

Presentation of the work: **VII.** Antibacterial activity of caffeine against plant pathogenic bacteria.

The aim of this study was to examine antibacterial properties of a secondary plant metabolite – caffeine, as well as to evaluate the possibility of using this substance to

protect plants. Caffeine (1,3,7-trimethylxanthine, CAF) is a purine alkaloid synthesized by over 100 species of plants, the most famous of which are shrubs and trees from the madder family, *i.e.* coffee trees (Ashihara, 2006). The antibacterial properties of caffeine were tested against the following bacterial species: *P. atrosepticum*, *P. c.* subsp. *carotovorum* (currently *P. carotovorum*) and *D. solani*, but also towards bacteria from the species: *Ralstonia solanacearum* (Rsol), *Clavibacter michiganesis* subsp. *sepedonicus* (Cms) (now *Clavibacter sepedonicus*), *Pseudomonas syringae* pv. *Tomato* (Pst) and *Xanthomonas campestris* subsp. *Campestris* (Xcc) (now *Xanthomonas campestris* pv. *campestris*); some of them are a subject of compulsory eradication.

Research outcomes: **It has been shown that caffeine possesses antibacterial properties against all tested plant pathogenic bacteria. Caffeine inhibited the growth of all studied microorganisms, depending on the concentration and dose applied.** The determined values of Minimal Inhibitory Concentration (MIC) were in the range from 5 to 20 mM caffeine. The values of the defined Minimal Bactericidal Concentrations (MBC) ranged from 43 to 100 mM caffeine. I also found the impact of caffeine supplementation on deviations in the morphology of bacterial cells (Fig. 3 AB). Addition of this alkaloid forced Xcc cells to form long chains (Fig. 3 CD), while Cms cells exhibited abnormal morphology and likely underwent lysis (Fig. 3 EF).

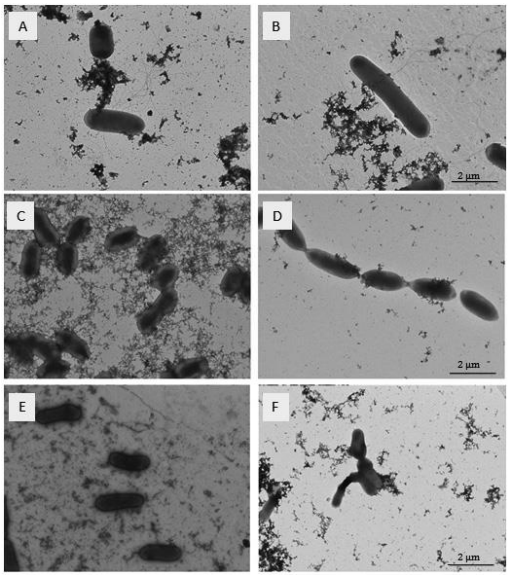

Fig 3. Morphology of bacterial cells growing on a medium containing 8 mM caffeine compared to the control samples: (A) *Dickeya solani* - 0 mM caffeine, (B) *Dickeya solani* - 8 mM caffeine, (C) *Xanthomonas campestris* subsp. *campestris* – 0 mM caffeine, (D) *Xanthomonas campestris* subsp. *campestris* – 8 mM caffeine, (E) *Clavibacter michiganensis* subsp. *sepedonicus* - 0 mM caffeine, (F) *Clavibacter michiganensis* subsp. *sepedonicus* - 8 mM caffeine.

I also revealed the impact of caffeine on inhibition of biosynthesis of DNA, RNA and proteins in the cells of the tested bacteria; the described effect of caffeine supplementation was estimated by measuring incorporation of radioactive precursors to the mentioned compounds according to a procedure described by Węgrzyn et al., 1991. I showed that the process of DNA replication tended to be inhibited during 90 min exposure to a 5 mM caffeine solution for Dsol, Rsol and Pba, but not for Pcc, Pst or Xcc. However, RNA transcription rate was significantly reduced by 5 mM caffeine solution after just 15 minutes of treatment with this compound of Dsol, Pba and Pcc bacterial cells. We did not observe any significant changes in the protein biosynthesis rates even after 120 minutes of bacterial exposure to the concentration of caffeine used. I also discovered that treatment of Pba with 8 mM caffeine solution for 336 h does not lead to induction of bacterial resistance to this compound.

Summary of achievement. **I demonstrated that caffeine shows antibacterial properties against a broad spectrum of plant pathogenic bacteria and therefore can be used as an environmentally friendly, natural biopesticide.**

*Based on the results obtained in the above-mentioned project, a national patent was obtained: "The agent and methods for protecting plants against bacteria and the method of obtaining an agent for protecting plants against bacteria (application of caffeine)". Patent application (UP RP) No. 404115 from May 28, 2013. Exclusive right number Pat. 233502 from 2019-10-31. Authors: **Śledź Wojciech**, Łoś Emilia, Banecki Bogdan, Łojkowska Ewa. Submitted by: University of Gdańsk*

Presentation of the work: **VIII.** Rapid eradication of bacterial phytopathogens by atmospheric pressure glow discharge generated in contact with a flowing liquid cathode

The aim of this research was to determine the applicability of a reaction-discharge system generating a cold plasma as a result of generation of a direct current glow discharge under an atmospheric pressure (dc-APGD) for eradication of the selected bacteria pathogenic to crops. The study was carried out on reference strains from the following bacterial species: *C. m.* subsp. *sepedonicus* (now *C. sepedonicus*), *D. solani*, *X. c.* pv. *campestris*, *P. atrosepticum* and *P. c.* subsp. *carotovorum* (now *P. carotovorum*). Importantly, the bacteria of the above-mentioned species can spread in various ways, *e.g.* together with post-industrial waters remaining after washing fruits

and vegetables. In this research an original reaction-discharge system was developed, by our group in cooperation with scientists from the team of Prof. Ph.D. eng. Paweł Pohl from Wroclaw University of Science and Technology, which shows a high level of innovation. This device operates in a flow-through mode and the glow discharges are generated at an atmospheric pressure in contact with the flowing bacterial suspension acting as a liquid cathode. To illustrate the developed reaction-discharge system, its construction scheme is depicted (Fig. 4).

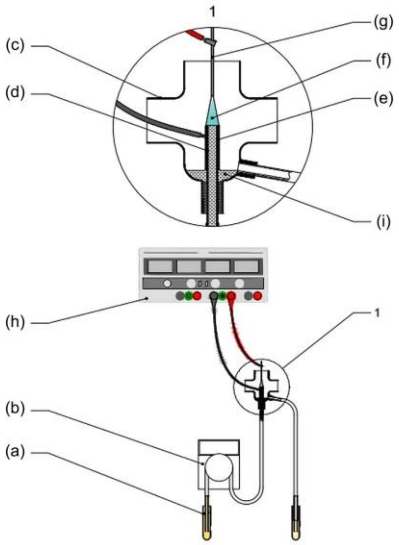

Fig 4. Schematic illustration of a dc-APGD-type plasma-generating reaction-discharge flow-through system designed for rapid elimination of phytopathogenic bacteria from aqueous solutions. The bacterial suspension (a) (enlarged in the circle at the top and marked as 1) is pumped into the system, where the decontamination process takes place. a) bacterial suspension, b) peristaltic pump, (c) quartz chamber, (d) quartz capillary, (e) graphite tube, (f) dc-APGD, (g) pin-type tungsten electrode, (h) dc-HV generator , (i) dc-APGD-treated solution

Research outcomes: The logarithm of the reduction in the number of living cells of plant pathogenic bacteria ranged from 3.43 to complete eradication of phytopathogens. In percentage terms, the bacterial eradication efficiency ranged from 99.996% to 100%. The differences have been noticed between bacteria from different species in their sensitivity to plasma. The most sensitive bacteria included the species: Cms, Dsol and Xcc, where complete elimination of living bacterial cells was observed. However, bacteria from the species Pcc and Pba turned out to be more resistant to plasma treatment. It is worth emphasizing that the reported effectiveness of the dc-APGD generating system was achieved after a short (60 s) plasma exposure period. The conducted research showed that the plasma-liquid interactions induce formation of

reactive oxygen and nitrogen species (NOx, NH, H_2O_2, O_2, O, and OH) and UV radiation and these factors were considered responsible for the observed antibacterial properties of the generated discharge.

Summary of achievement. **It has been demonstrated that the developed reaction-discharge system based on dc-APGD-type plasma is an effective method for controlling/combating plant pathogenic bacteria, including Xcc, Cms, Pcc, Pba and Dsol from aqueous solutions. The proposed innovative reaction-discharge system operates in a flow-through mode and it is the first original solution to the scientific problem related to combating bacterial phytopathogens from liquids by using plasma technology.** The system based on dc-APGD generated in a continuous flow can be used, for example, to disinfect water used to irrigate crops in the fields or greenhouses, to eliminate bacteria from post-industrial waters (remaining after washing vegetables and fruits), agricultural sewage, the used up hydroponic media or liquid waste from microbiological laboratories. It is worth underlining that the developed reaction-discharge system is characterized by a very low energy consumption (~55 W).

The above described achievement has been patented: "Method of eradicating bacterial phytopathogens using constant current glow discharge". Patent application (UP RP) No. P.419246 from 2016-10-26. Exclusive right number Pat. 236055 from 2020-07-24. Authors: Łojkowska Ewa, ***Śledź Wojciech****, Motyka Agata, Anna Dzimitrowicz, Piotr Jamróz, Paweł Pohl. Submitted by: Wrocław University of Science and Technology and University of Gdańsk.*

Presentation of the work: **IX** Antibacterial Activity of Fructose-Stabilized Silver Nanoparticles Produced by Direct Current Atmospheric Pressure Glow Discharge towards Quarantine Pest

The aim of this research was to develop an ecological method for synthesis of fructose-stabilized silver nanoparticles (FRU-AgNPs) by using cold atmospheric pressure plasma of the dc-APGD type in addition to determination of the antimicrobial activity of the nanoparticles obtained in this manner against economically important bacterial plant pathogens such as: *Erwinia amylovora* (Eam), *C. michiganensis* (Cm), *R. solanacearum* (Rsol), *X. c.* pv. *campestris* (Xcc) and *D. solani* (Dsol). The nanoparticles synthesis method based on dc-APGD is economically advantageous and does not require addition of toxic reducers.

As mentioned earlier, new, alternative and, most of all ecological solutions are currently being sought to handle the issue of plant diseases. Therefore, my research group undertook studies on the possibility of using dc-APGD, generated in a continuous flow mode, for the synthesis of stable AgNPs. The flowing solution of the AgNPs precursor ($AgNO_3$) and stabilizer (D-fructose) constituted a liquid anode (Flowing Liquid Anode; FLA), in which reactive oxygen and nitrogen species (RONS) and hydrogen radicals (H) were formed as a result of plasma-liquid interactions. These interactions subsequently led to reduction of Ag(I) ions and were responsible for the production of AgNPs. The properties of the synthesized FRU-AgNP nanoparticles were analyzed using UV/Vis spectrophotometry, Transmission Electron Microscopy (TEM) supported by Energy Dispersive X-Ray Scattering (EDX) and electron diffraction at a selected area (Selected Area Electron Diffraction, SAED) in addition to the dynamic light scattering method (DLS). In order to confirm the functionalization of the AgNP surface with FRU (selected as a stabilizer that induces chemotaxis in the selected phytopathogenic bacteria), Attenuated Total Reflection-Fourier Transformation Infrared Spectroscopy (ATR FT-IR) was used. The antibacterial properties of FRU-AgNPs against bacterial phytopathogens were determined on the basis of two parameters, *i.e.* minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) in relation to the selected plant pathogenic bacterial species listed above.

Research outcomes: Spherical and stable over time FRU-AgNP nanoparticles of average dimensions 14.9 ± 7.9 nm and 15.7 ± 2.0 nm were synthesized. The analyses showed that the generated nanoparticles possessed antibacterial properties against all the included phytopathogens. The strains of the Eam, Cm and Xcc species turned out to be more susceptible (MIC equalled 1.64 mg L^{-1}) to the formed nanostructures than the other investigated bacterial species. On the other hand, Dsol strain showed the highest resistance to FRU-AgNPs with a MIC of 13.1 mg L^{-1}. There was an interesting case of a Rsol strain, for which both MIC and MBC values were even and amounted 6.58 mg L^{-1}.

As a result of the conducted research, FRU-AgNP nanostructures were obtained by using a "green" method of their synthesis relying on dc-APGD plasma. Application of silver nanoparticles acquired by the developed procedure may be an alternative method

useful for combating bacterial plant pathogens, including microorganisms subjected to compulsory control.

Summary of achievement. **An efficient, fast, environmentally-friendly and cost-effective method for synthesis of uniform and stable in time FRU-AgNPs nanoparticles was developed. The obtained nanostructures showed antibacterial properties against all the tested bacterial phytopathogens.** The described achievement is in line with the plant protection policy applied by the European and Mediterranean Plant Protection Organization (EPPO).

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5. Presentation of significant scientific or artistic activity carried out at more than one university, scientific or cultural institution, especially at foreign institutions

My significant scientific activity outside the parental unit was carried out in the Laboratory of Mycology and Bacteriology, Plant Research International, Wageningen, the Netherlands (currently Wageningen University and Research, Bioscience Department) in cooperation with Dr. Jan van der Wolf (I woked in this institution for 6 months in 2005 and 2006).
Throughout my internships, I have participated in the studies focused on the application of flow cytometry and its modification (Luminex xMAP®) for fast detection of bacterial pathogens in plant material. My research led to development of a procedure for preparation of plant material for flow cytometry analysis among others. As a result, it was possible to describe a method for detection and identification of viable cells of the pathogenic bacterium *Ralstonia solanacearum* at the level of approx. 10^2 jtk/mL. On the other hand, by application of Luminex xMAP® technology I focused my research on the detection of bacteria *Pectobacterium atrosepticum* (Pca) and *Dickeya dianthicola* (Dcd). With the use of this technology, we developed an immunoassay on microspheres (MIA) coated with antibodies of different colours and secondary antibodies conjugated with Alexa Fluor® 532 (the so-called reporter dye), for simultaneous detection of *Pectobacterium atrosepticum* (Pca) and *Dickeya dianthicola* (Dcd) in potato plant extracts.

The results of these studies were published in three publications (**presented in**: *List of scientific achievements constituting a significant contribution to the discipline of biotechnology/ List of published scientific monographs/chapters in a monograph (After obtaining a Ph.D. degree), item 5; List of published articles in scientific journals (After obtaining a Ph.D.) items 21 and 22*) **and four conference reports** as presentations and posters (**presented in:** *List of scientific achievements constituting a significant contribution to the discipline of biotechnology (After obtaining a doctoral degree)/National conferences item 24, International conferences items 29, 30, 33).*

My significant scientific activity carried out at more than one national University is carried out in long-term cooperation with the research team of the Department of Analytical Chemistry and Chemical Metallurgy of the Faculty of Chemistry, Wroclaw University of Science and Technology. The research that I conduct together with this group concerns the direct or indirect application of various types of cold plasma, among others for:
- plants protection to eradicate bacterial phytopathogens,
- plants protection to obtain post-plasma solutions used to eradicate pathogenic bacteria and stimulate growth of crops,
- synthesis of nanostructures for eradication of bacterial pathogens,
- inactivation of antibiotics and endocrine-active compounds in environmental protection,
- in veterinary medicine to eradicate bacteria that cause skin diseases.

The results of this research have been published in 8 publications and a book chapter. (**presented in*:*** *List of published articles in scientific journals (After obtaining a doctoral degree)*, items 1, 3, 6, 8, 11, 12; *List of published scientific monographs/chapters in the monograph*, item 2). Two publications are part of the scientific achievements presented as a part of this habilitation procedure: publications H8 and H9.

Research on the use of cold plasma also resulted in obtaining 5 patents and 5 patent applications that I indicated in *List of scientific achievements constituting a significant contribution to the discipline of biotechnology/List of obtained industrial property rights, including obtained national or international patents*, items 1, 2, 4, 5, 6; *Patent applications* item 1, 2, 3, 4, 5)

Currently, my cooperation with the above-mentioned team is carried out as realisation of two NCN projects (in the Opus 17 project I am the principal investigator, in the Sonata 15 project I am the principal investigator from the partner side, *i.e.* University of Gdańsk).

6. Presentation of teaching and organizational achievements, as well as, achievements in popularization of science or art

Teaching achievements

I started my teaching activity at IFB UG & MAG (currently MUG) in 1994 by giving lectures and practical courses in the subjects "Basics of Molecular Biology" and "Basics of Microbiology with Elements of Environmental Protection" for the students of Biotechnology IFB UG & MUG and students of the Faculty of Chemistry UG. Since 2010 until now I have been giving lectures and practical classes in the subject Engineering of Bioprocesses (since 2022 as a part of the block: "Biotechnology in Industry and Agriculture - Bio-Technologies-Foundations") for students of Biotechnology IFB UG & MUG, as well as lectures and practical classes in the subject Biotechnology for students of the Faculty of Chemistry UG. Starting from 2021 I have also been delivering lectures with a practical classes in the subject of Drug Engineering in a block: "Biotechnology in Medicine - Therapies and Technologies" for students of Biotechnology at IFB UG & MUG. In 2010 I was nominated by votes of the students of our Faculty for the "Best Lecturer MWB UG and GUMed" competition.

As part of my didactic activity from 1994 to the present, I have been a supervisor of 20 master's theses, a reviewer of 15 master's theses, and a supervisor and reviewer of bachelor's theses.

In 2015-2019, I was an auxiliary supervisor of the doctoral dissertation of MSc Sabina Elzbieta Zoledowska entitled. "Characterization of the biodiversity and pan-genome of plant pathogenic bacteria from *Pectobacterium parmentieri* species" (thesis was defended with distinction on 24.05.2019). Supervisor: Professor Ewa Lojkowska, co-supervisor: Professor Alessio Mengoni, auxiliary supervisor: Dr. eng. Wojciech Sledz, Reviewers: Professor Katarzyna Brzostek, Dr. Andrzej Mazur, Professor of Maria Curie-Sklodowska University.

Also in 2015-2019, I was an auxiliary supervisor of doctoral dissertation of MSc Agata Motyka-Pomagruk entitled " Genotypic and phenotypic characterization of bacteria from *Dickeya solani* species and development of novel control methods against phytopathogens " (thesis was defended with distinction on 20.09.2019), Supervisor: Professor Ewa Lojkowska, co-supervisor: Professor Alessio Mengoni, auxiliary

supervisor: Dr. eng. Wojciech Sledz, Reviewers: Dr. Monika Beata Janczarek, Professor of Maria Curie-Sklodowska University, Professor Katarzyna Dorota Lisowska.

Both doctoral dissertations were awarded with distinction by the Council of the IFB UG & MUG, and Dr. Agata Motyka-Pomagruk additionally received the Prime Minister's Award for an outstanding doctoral dissertation (2020).

Organizational Achievements

During my employment at Intercollegiate Faculty of Biotechnology UG & MUG, I have been involved in the organisation activities at our faculty:

- In years 1994-2002 and 2004-2008 I was a member of the Intercollegiate Faculty of Biotechnology Council,
- In years 1996-1998, I participated in the works of the Committee for Social and Living Conditions of the UG employees,
- In years 2000-2002, I was a member of the Committee for Misconduct of UG Teaching and Research Staff,
- In years 2005-2008, I worked as a member of the UG Student Offences Committee team and the Committee for Internet Recruitment of Candidates to the UG
- In years 2012-2016, I actively participated in the works of the committee for the design and supervision of the construction of the UG Institute of Biotechnology building. For my involvement in the work, together with the other members of the committee, I received the UG Rector's First Degree Award in 2016.

Since 2017, I have been a chairman of the GMO and GMM Committee at IFB UG & MUG. Thanks to our efforts, we have obtained approval to operate GMM and GMO category II genetic engineering facilities. At the moment, we are working towards obtaining approval to operate a III category GMM genetic engineering facility.

In 2013, I co-founded the first University of Gdansk spin-off company producing liquid brewing yeast. I carried out my activities at Fermentum Mobile spin-off company as a person responsible for the collection of yeast from 2013 until 2018.

Since 2016 I have been the technical manager of the Research & Development Laboratory (RDL) of IFB UG & MUG. Through the efforts of the team I am leading, in 2018 we implemented in RDL a quality management system in accordance with the PN-EN ISO/IEC 17025:2018-02 standard confirming the competence of testing and calibration laboratories. In 2020, the team of RDL, as the first in the history of the University of Gdansk, and to date the only one, was awarded with accreditation from the Polish Centre for Accreditation (AB 1760). Moreover, in 2023 the team obtained an environmental management certificate in accordance with the ISO 14001 standard.

In addition to confirming the competence of the team and acting in accordance with best laboratory practice, the certificates obtained substantially affect and enhance contacts and cooperation with industry in addition to boost the effectiveness of co-realised projects.

Popularization of science or art

In years 2003-2012 I was the main coordinator of the Baltic Science Festival at IFB UG & MUG. For my involvement in the organisation of the above-mentioned event, I received, together with my support team, the UG Rector's First Degree Award in 2011.

In years 2012-2015 I was the main coordinator of the Night of the Biologists at

IFB UG & MUG.

In 2017, I organised the interactive stand "Bioreactor" during the 2017 Night of the Biologists.

I supervised Students Research Group BioMed at IFB UG & MUG as an assistant supervisor in years 2013-2015. During this period, two projects were executed:

- forensic project "Determination of relationship using DNA analysis" - a series of four-hour meetings (learning: sampling, DNA isolation, PCR method, electrophoresis, analysis of results),
- biotechnological project: "Biofuels" (a series of four-hour meetings): extraction of oils from oilseeds and obtaining esters (bio-diesel), determination of the quality of the obtained fuel using industrial methods.

Within the framework of the cooperation I established with the Johannes Hevelius Public Secondary School No. 2 in Zukowo, school students together with their teacher, supervisor MSc Anna Borowicz, participated in the above-listed projects. The middle school students actively participated in the Night of Biologists, by organizing under my supervision a joint science stand with IFB UG & MUG students in the years 2013-2015.
From 2003 to 2017, I delivered popular science lectures entitled "Genes on the menu" for the Gdansk University of the Third Age.
In the years 2014-2015, I provided teaching and research supervision to student Sara Berent from LO III in Gdynia (since 2015 a student of the University of Gdansk) under the programme “Mentorship for gifted students”. Her research project was carried out in the Laboratory of Plant Protection and Biotechnology IFB UG & MUG under my supervision and dealt with the effects of essential oils on selected bacterial and fungal microorganisms.
The research results obtained enabled Sara Berent to:
- qualify for the semi-final of the E(x)plory 2015 competition, Torun: the prize was the participant’s qualification for the final of E(x)plory in Gdynia,
- win the Special Prize *i.e.* accreditation to attend INESPO in Amsterdam, in the final of the E(x)plory 2015 competition Gdynia 2015,
- win the 1st prize (gold medal and statuette for the best research project) at the INESPO 2015 Amsterdam,
- qualify for the Special Edition of E(x)plory 2015 in Warsaw,
- win the 2nd prize at the EUCYS 2015 Warsaw and a promotion to EUCYS 2015 competition in Milan,
- win the 1st prize at the EUCYS 2015 competition in Milan: "EXPO donated prize European Union Competition for Young Scientists 2015" at the EUCYS 2015
- be promoted to Finalist of Expo Sciences Europe Forum Toulouse 2016

Since 2013, as a vice-president of the Polish-Israeli Society MORESHET MESHUTEFET in Gdansk, I have been a co-founder and co-organizer of the initially Shalom Polin Jewish Film Review at the University of Gdansk, and now the Gdansk Jewish Culture Festival at the University of Gdansk (edition years: 2013-2018, 2021-2023).

7. Apart from information set out in 1-6 above, the applicant may include other information about his/her professional career, which according to he/she seem important.

Awards and distinctions received for scientific and organisational activities:

2022, Silver medal for invention (co-inventor): “Method of deactivation of endocrine active compounds from aqueous solutions” (Patent application to the Polish Patent Office No. P.438832) at the International Warsaw Invention Show 2022

2022, Grand Prize for invention (co-inventor): "Method of deactivation of antibiotics in aqueous solutions" at the 9th edition of the Eureka! Competition of the Journal Gazeta Prawna

2020, Distinction for invention (co-inventor): "Method of eradicating bacterial phytopathogens" based on the use of cold atmospheric plasma-activated solutions to combat plant disease-causing bacteria at the 7th edition of the Eureka! Competition of the Journal Gazeta Prawna

2020, Distinction for invention (co-creator): "Method of producing a plant growth stimulation preparation, preparation obtained by this method and use of the preparation to stimulate the growth of plants, in particular those of economic importance" Special award of the President of the Patent Office of the Republic of Poland at the 10th anniversary edition of the "Student-Inventor" Competition organized by the Kielce University of Technology (patent application in the Polish Patent Office P.430866)

2018, UG Rector's Award: First Degree Team Award for the implementation of a quality management system compliant with the PN-EN ISO/IEC 17025:2005 standard at the Research and Development Laboratory of IFB UG & MUG

2016, UG Rector's Award: First Degree Team Award for outstanding performance in the leadership of organisational units and the development of the teaching facilities at the University of Gdansk

2015, UG Rector's Award: First Degree Team Award for 6 publications published in 2014 on the understanding of the mechanisms determining the pathogenicity of bacterial plant pathogens of the genera *Dickeya* and *Pectobacterium*

2014, Best Poster Award at the 11th conference of EFPP "Healthy Plants – healthy people" for a Poster "Monitoring of pectinolytic bacteria originating from potato (*Solanum tuberosum* L.) plants and water samples" session: Pathogen identification, detection and monitoring (Motyka M., **Sledz W.**, Potrykus M., Golanowska M., Zoledowska S., Butrymowicz J., Kolodziejska A., Czajkowski R., Lojkowska E). Cracow, 8-13/09/2014.

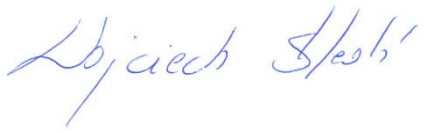
2011, UG Rector's Award: First Degree Team Award for many years of leadership in the organisation of the Science Festival at the Intercollegiate Faculty of Biotechnology of the University of Gdansk and Medical University of Gdansk

2010, Award of the Rector of Warsaw University of Life Sciences in Warsaw: Team Award for co-authorship of the next edition of the academic book "Biotechnology of plants" published by Polish Scientific Publishers

Attachment 3

2002, Distinction of doctoral dissertation by the Faculty Council of the Intercollegiate Faculty of Biotechnology University of Gdansk and Medical University of Gdansk

2001, Scholarship from the Dutch Ministry of Agriculture and Fisheries



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(Applicant's signature)