Załącznik nr 3

Autoreferat w języku angielskim

1. First and last name: Włodzimierz Grzegorz Przewodowski

2. Diplomas and academic degrees:

- **16.06.2001** Koszalin University of Technology, Faculty of Mechanical Engineering, Koszalin
  Master's degree in Food Engineering.
  Master's thesis under the supervision of dr hab. Jerzy Lewosz, titled: "Identification of homogeneity and varietal identity of potato with the use of biochemical and genetic markers".
- **17.09.2007** Plant Breeding and Acclimatization Institute at Radzików
  Doctoral degree in agricultural sciences in the field of agronomy.
  PhD thesis under the supervision of dr hab. Jerzy Lewosz, entitled: "Development of diagnostic kits for the detection of *Clavibacter michiganensis* subsp. *sepedonicus* - the cause of ring rot of potato". Reviewers: Prof. Dr hab. Ewa Łojkowska, Professor Dr hab. Piotr Sobiczewski.

Other diplomas

- **23.10.2003** Koszalin University of Technology, Koszalin
  Postgraduate Pedagogical Studies.

3. Information on previous employment in scientific units:

- **01.09.2001 - 30.06.2005** Doctoral studies at the Department of Microbiology (September 1, 2001 - November 30, 2002) and the Department of Biochemistry and Biotechnology (December 1, 2002 - June 30, 2005) Faculty of Mechanical Engineering, Koszalin University of Technology.
- **07.06.2004 - 06.05.2005** Scientific internship at the Membran Modification Laboratory. R&D Department. Faculty of Biotechnology. Sartorius AG, Goettingen, Germany.
- **01.09.2001 - 15.01.2006** Trainee - volunteer, Plant Breeding and Acclimatization Institute of Seed Production Department of Potato Protection and Seed Science (IHAR ZNiOZ) in Bonin.
- **16.01.2006 - 31.12.2006** Engineer intern, IHAR ZNiOZ in Bonin.
- **01.01.2007 - 31.12.2007** Engineer, IHAR ZNiOZ in Bonin.
- **01.01.2008 - 31.10.2009** Assistant, IHAR ZNiOZ in Bonin.
- **01.11.2009 - obecnie** Lecturer, IHAR - PIB, Research Centre at Bonin.

4. Indication of the scientific achievement resulting from Article 16 sec. 2 of the Act of March 14, 2003. on academic degrees and academic title, and on degrees and title in the field of art (Journal of Laws of 2016, item 882, as amended in Journal of Laws of 2016, item 1311):

a) Title of the scientific achievement

„ Development of isolation and identification methods of selected, potato bacterial quarantine pathogens (Solanum tuberosum L.) ".

b) Publications included in the scientific achievement:

The scientific achievement is a monothematic cycle of works including one publication placed in the JCR database and 3 publications in the form of patent patents with a total IF = 3.353, points according to Ministry of Science and Higher Education $_{\text{Year of publication / granting of the patent}}$ = 110 points, according to Ministry of Science and Higher Education 2016 = 125 points. I declare that in the presented works the described research was my own concept, in all my works I was the originator, the author of the text, the author of the correspondence and the main contractor of the research. The presented works were subject to rigorous verification by the reviewers of the publications and the patent assessors in order to meet the specific requirements of the relevant scientific magazines and the originality of the developed solutions on a global scale. I estimate my participation in the creation and publication of the presented publication and patents at 80 and 100%, respectively.

H1 **Przewodowski W.**, Przewodowska A. 2017. Development of a Sensitive and Specific Polyclonal Antibody for Serological Detection of *Clavibacter michiganensis* subsp. *sepedonicus*.PloSONE12(1):e0169785.doi:10.1371/journal.pone.0169785.[**IF=3,535; MNiSW$_{2016}$=35pts**]
My contribution to this work consisted in planning and carrying out experiments, developing a method of antigen preparation, immunizing animals, purifying and assessing the quality of obtained polyclonal antibodies, analysis and interpretation of results, participation in the preparation of the manuscript. I estimate my percentage share at 80%.

H2 **Przewodowski W**. 2013. A method of detecting the presence of bacteria Cms using polycarbonate membranes containing immobilized antibody. Patent of the Polish Patent Office No PL 213857. [**IF[1]; MNiSW$_{2013}$=25pts, MNiSW$_{2016}$=30pts**]
My contribution to the creation of this work consisted in defining the concept of the invention, planning and performing experiments related to confirming the adopted hypothesis, analyzing and interpreting the results of research, writing a patent application and preparing answers to the comments of patent assessors. I estimate my contribution to 100%.

H3 **Przewodowski W**. 2013. Incubation kit for detection or living isolation of selected bacteria, *Clavibacter michiganensis ssp. sepedonicus*. Patent of the Polish Patent Office No PL 213856. [**IF[1]; MNiSW$_{2013}$=25pts, MNiSW$_{2016}$=30pts**]
My contribution to the creation of this work consisted in defining the concept of the invention, planning and performing experiments related to confirming the adopted hypothesis, analyzing and interpreting the results of research, writing a patent application and preparing answers to the comments of patent assessors. I estimate my contribution to 100%.

H4 **Przewodowski W.** 2013. A kit for detecting or isolating living bacteria *Clavibacter michiganensis* ssp. *sepedonicus* w analizowanej próbce. Patent of the Polish Patent Office No PL 213855. [**IF[1]; MNiSW$_{2013}$=25pts, MNiSW$_{2016}$=30pts**]
My contribution to the creation of this work consisted in defining the concept of the invention, planning and performing experiments related to confirming the adopted hypothesis, analyzing and interpreting the results of research, writing a patent application and preparing answers to the comments of patent assessors. I estimate my contribution to 100%.

[1] Due to the lack of influence of IF factor on the above patents, its values have not been included in patent items.

c) Discussing the above scientific goal of the work and results achieved, and discussing their possible use.

Introduction

Potato being one of the most strategically important plant grown worldwide, is also economically very important for Poland, which is ranked, in terms of potato growing area and crops, second in Europe and seventh in the world (FAOSTAT 2017, *http://faostat3.fao.org*). The vegetative method of potato reproduction makes this plant particularly vulnerable to infections originating from numerous bacterial, viral and fungal pathogens occurring in soil environment.. Particularly significant for potato crops are quarantine diseases and, among them, bacterial ring rot of potato caused by one of the most important and, at the same time, most oppressive potato pathogens namely *Clavibacter michiganensis* subsp. *sepedonicus* (Cms) bacteria (Spickermann et Kotthoff) Davis et al. (Lee et al. 1997, Lebecka i Zimnoch-Guzowska 2005; OEPP/EPPO 2006). Cms bacterium was entered in A2 EPPO list of quarantine organisms with zero tolerance (EPPO, *https://www.eppo.int/QUARANTINE/listA2.htm*). This means that detection of any affection results not only indisqualification of plantations and necessity of utilization of the plant material but also in application of phytosanitary restrictions at the production site. This means, in practice, financial problems sometimes leading to a bankruptcy of given farm. The introduction of official phytosanitary regulations regarding ring rot caused by a drastic reduction in potato exports from Poland and a barrier to supply in seed production (Chotkowski and Rembeza, 2013). Occurrence of bacterial ring rot epidemic in North America and Russia caused loss of crops up to 50% (Easton, 1979; Muller and Ficke, 1974). By the end of the seventies, the annual loss worldwide caused by ring rot was estimated at USD 50-100 million (Van der Volf i in., 2005).

Due to high fragmentation of farms and low exchange of seed materials Poland is counted among those countries that suffer from the highest rate of bacterial ring rot affection. Based on the national Cms bacteria detection analysis of the State Inspectorate for Plant and Seed Protection (PIORIN) identified during the period of 2011-2015, occurrence of this dangerous bacteria in all provinces was ascertained (PIORIN Report 2001-2015). As of Poland's accession to the European Union, and the necessity of compliance with strict phytosanitary standards concerning potato health associated therewith, the problem of CmS occurrence in Poland is more and more accented in the international arena. In the opinion of

PIORIN, increased detection of the potato ring rot perpetrator in Poland has been noted by the Standing Committee on Plant Health affiliated to European Commission in Brussels. It was pointed out that the level of potato Cms affection in 2011 in Poland was approx. 12%. This figure was almost nine times higher than the average for all remaining detection of Cms in 26 EU countries. According to the latest report by the EU General Directorate for Health and Food Safety, Poland is still perceived as the country with the highest degree of contamination with ring rot in the EU (Annual Report of DG EU 2016/17). Such situation results in loss of trust in Polish potato producer and in more frequent check ups of seed material exported to many EU countries.

In terms of taxonomy, Cms bacteria is one of five subspecies of *Clavibacter michiganensis* (*C. mildiosus*, *C. m.liganensis, C.m. nebraskensis, C.m. sepedonicus* and *C.m. tessellarius*), highly specialized pathogens of various plant species. On the basis of the latest molecular research based on genomic analysis and MLSA identification (Multi Locus Sequence Analysis, sequencing of many genes of basic metabolism) of individual subspecies of *C. michiganensis*, it was proposed establishing a new separate species *Clavibacter sepedonicus comb. nov*. (Li et al., 2018).

The cause of bacterial ring rot of potato has a number of unique features creating a high risk of uncontrolled propagation of this disease onto new areas. Just like many other subspecies of *Clavibacter michiganensis*, Cms bacteria often cause a latent form of disease (De Boer et al. 2005, OEPP/EPPO, 2006). Low concentration of Cms bacteria in tissues, as well as tolerance of some potatoes varieties, result in absence of visible symptoms of the infection in plants yet it poses an affection hazard to a wide extent even for several vegetation seasons (Pastuszewska 2010). Fully symptomatic form of the disease is also difficult to be diagnosed as it is often confused with other diseases and becomes visible usually by the end of the vegetation period or only during tubers storage (Directive of Commission EC 2006/56, Przewodowski and Treder, 2008).

The most effective method of protection against the potato ring rot is planting of healthy, qualified seed material, quick elimination of disease hotbed and application of hygiene in the entire potato production and storage process. In each of the above cases, it is essential to proceed with a quick and reliable diagnostics confirming phytosanitary purity of seed material and production site. However, due to specific features of potato bacterial ring rot perpetrator, diagnostics of said pathogen is very difficult. **Currently, there is no single diagnostic method that would clearly and reliably confirm occurrence and pathogenicity of Cms bacteria samples taken from relevant environment.** Therefore, according to the adopted

phytosanitary requirements, in order to perform reliable diagnostics of said pathogen, at least two screen tests based on different biological principles, including a pathogenicity test performed on bioindicative plants, must be used (OEPP/EPPO, 2006).

The fundamental impediment in effective Cms bacteria elimination, is lack of proper diagnostic tool that would allow for highly specific separation and identification of Cms bacteria cells taken the from examined environmental samples. This is caused by a number of factors:

- Cms bacteria strains are diversified in terms of the volume of produced mucus coating cells. The high mucoid level has no bearing on virulence of particular strains, but it renders effective identification of Cms bacteria through application of immunological methods impossible. All known commercially available polyclonal anti-Cms antibodies are mainly directed to detect components of bacterial mucus and they often are the cause of false negative result in the case of those strains that produce small volumes of mucus. On the other side monoclonal anti-Cms antibodies characterized by too high specificity also do not recognize many strains of Cms bacteria.
- Additionally, due to the lack of fully selective microbiological growing media for Cms bacteria, occurrence in given sample of any other quickly growing endogenic and saprophytic environmental microorganism, limits considerably the possibility to obtain pure isolates and accomplish a biological test allowing for definition of Cms pathogenicity.
- There is no effective method of Cms bacteria isolation that would allow a possibility of preservation of vitality of obtained bacterial isolates with simultaneous elimination of all impurities (microbiological, chemical, biochemical, biological and physicochemical) occurring in the samples. Such methods play a key role in effective microbiological diagnostics. Isolation of live Cms bacteria is particularly difficult in the case of any samples containing plant tissues, potato tuber remains, remains of soil and environmental water of high volume and low Cms bacteria concentration.
- Limitation of molecular identification methods of Cms bacteria, which despite their high sensitivity and specificity may be disturbed by the action of inhibitors of PCR reactions present in environmental tests. One of the main reasons that influence the correct molecular diagnostics of Cms is the lack of an appropriate method of isolation of genetic material from the tested environmental samples. The methodology recommended by the

EPPO in the European Commission's Directives does not allow the complete removal of impurities and PCR inhibitors in the test sample, which may lead to a false negative result in the molecular analysis.

Purpose of research

To solve the above-mentioned difficulties, the goal of the scientific achievement presented was to develop innovative technological solutions allowing isolation and identification of selected bacterial pathogens of potato (*Solanum tuberosum* L.) from difficult diagnostic environmental probes, with particular emphasis on quarantine Cms bacteria.

The publications making part of my habilitation achievement pertain to development of innovative diagnostic tools that allow for solution of the above-mentioned problems, making possible isolation and identification of selected bacterial potato pathogens (*Solanum tuberosum* L.) from diagnostically difficult environmental samples. Proposed solutions are based, first and foremost, on development of new quality polyclonal antibodies that feature a unique serologic characteristic (H1, Przewodowski and Przewodowska 2017). The greatest advantage of new antibodies, apart from high specificity and sensitivity, is their ability to detect Cms bacteria, independently of mucoid level of the examined strains.

Combination of new antibodies with properly modified immunosorbents allowed for development of diagnostic tests based on immunofiltration techniques (H2, Przewodowski 2013a) and incubation (H3, Przewodowski 2013b, H4, Przewodowski 2013c) The entire achievement makes a comprehensive diagnostic solution allowing for improvement of current standards and circumvention of limitations of current methods applied for the identification of bacterial pathogens from environmental samples and improve current diagnostic standards.

It appears both from the scientific literature and my own investigations, that commercial anti-Cms polyclonal antibodies detect well those strains that produce mucus, but detect poorly, or not at all, any nonmucoid strains. This was proved by Baer and Gudmestad, who used six types of anti-Cms antibodies. They were unable to detect, during their research work, many nonmucoid strains of Cms bacteria through application of ELISA method. However, the authors did not note any problems with detection of those Cms bacteria strains that produce medium and high volumes of bacterial mucus (Baer and Gudmestad 1993). It was proved in my research work evaluating of specificity of commercial antibodies from Loewe company, that high volume of mucus correlates with high content of simple sugars that are chemically

determined by application of the anthrone method. It was ascertained, on that basis, that the cause of abnormalities in Cms bacteria diagnostics, is probably inter-reaction between the above antibodies and components of cell coating bacterial mucus.

It was also ascertained during the research work, that occurrence of bacterial mucus containing acid expolysaccharides, prevents performance of quantitative measurement of bacteria through application of any immunoenzymatic methods. It was proved that absorbance level measured in ELISA test depends, first and foremost, on the volume of mucus produced by particular Cms bacteria strains, and not on the real number of cells occurring in the suspension. Washing of cells with proper buffer solutions allows for elimination of the majority of bacterial mucus components and for standardization of ELISA test results. This effect cannot be achieved when bacterial suspension is washed with water even several times. Development of an efficient method of mucus elimination from bacterial cells through washing with a buffer solution featuring low, and then high, pH, allowed for much better serological assessment of the number of bacterial cells irrespectively of the mucoid level of tested Cms strains.

To obtain properly specific antibodies that would detect all Cms strains, irrespectively of their mucoid level, and to prevent any non-specific reactions with other bacteria occurring in given environment, a method of preparation of a new antigen in form of a mixture of Cms bacteria cells that are completely free from any external bacterial mucus, was developed during this research work. To achieve that, lyophilizates of three extremely mucoidally diversified Cms strains in 1:1:1 weight proportion were used. The bacterial cells were prepared, before immunization, by application of the method described in the invention H1, in which application of the defined concentration, frequency and time of application of particular buffer solutions onto the examined bacterial cells, allowed for total elimination of surface mucus therefrom **(H1**, Przewodowski and Przewodowska, 2017). The newly developed antigen was used to immunize rabbits through application of low invasion adjuvant Gerbu 100, that allowed for increased immunological animal response (Ball et al. (1993).

Produced IgG antibodies, directed against bacterial cells without mucus, featured high affinity with relation to the tested Cms bacteria strains with simultaneously poor reaction with other bacteria. Said antibodies did not react with any other examined potato pathogens except Cms. For comparison antibodies directed against mucus-coated bacteria perfectly detected all Cms strains, however, they featured also non-specific reaction with relation to other examined potato pathogens *Pseudomons fluorescens* and *Pectobacterium carotovorum* subsp.

atrosepticum, which makes them useless for serological diagnostics. According to Miller 1984 and Kokoskowa and Pankova (1998, 2002) *Pseudomonas fluorescens* bacterium very often yields cross reactions in serological tests directed against Cms bacteria. Furthermore, it was ascertained that none of the commercially available kits (Loewe, Agdia, Adgen) detects all Cms bacteria strains. The commercial Loewe kit based on usage of polyclonal antibodies, failed to detect as many as 5 out of 29 tested Cms strains and detected only one strain from the group of dry strains (strain 527). Better features has, to that effect, a kit with monoclonal antibodies from Agdia and polyclonal kit for PTA-ELISA Adgen, which failed to detect only two of the examined Cms strains.

Summing up, from among tested commercially available kits based both on monoclonal and polyclonal anti-Cms antibodies, none detected 100% of all examined Cms strains. The best results were obtained for application of the newly developed, under (H1) invention igG polyclonal directed against nonmucoid Cms bacteria. They detected all examined Cms strains irrespectively of their mucoid level. Development of such type antibodies featuring simultaneously a wide spectrum of reaction and not reacting non-specifically with other potato pathogens, allowed for development of new immunodiagnostic tests for said bacteria identification. Fast and sensitive specific immunodiagnostic tests attract permanently interest and will be developed in future (Danks and Barker 2002). Production of new polyclonal antibodies for detection of Cms bacteria featuring relatively high specificity, shows a new trend allowing for development of a serological method to detect bacteria that are extremely different in terms of their mucoidal features.

Despite many years of research work, no proper solutions/diagnostic tools that would allow for efficient live bacteria isolation and highly specific identification of Cms bacteria cells obtained in such type tests, were developed. The EPPO diagnostic methods that are currently recommended, also do not provide such opportunities. They are based mainly on bacteria thickening by cell centrifugation in the centrifugal force field or by filtration of bacterial suspension in anti-bacterial filters (OEPP/EPPO 2006). This results in Cms cells concentration together with other impurities contained in the tested sample.

The inventions/patents mentioned in the hereby presented scientific achievement (H2, Przewodowski 2013**; H3,** Przewodowski 2013**; H4,** Przewodowski 2013**) comprise effective solutions of current problems with diagnostics of Cms bacteria from environmental samples.**

Many of the previously mentioned problems with isolation and identification of *Clavibacter michiganensis* ssp. *sepedonicus* were solved based on methodical studies within the first invention framework **(H2**, Przewodowski 2013).

The invention comprises a method of detection of Cms bacteria occurrence using polycarbonate membranes coated locally with antibodies. The principle of operation of such solution consist in that the analysed sample (potentially) containing Cms bacteria (e.g. extract from potato tubers or plant tissue) is hanged in a buffer solution. Such mixture is then filtered through a membrane with antibodies covalently set thereon that are specific for the tested bacteria. The method of constructing the test and the use of highly specific antibodies allows fast and highly selective binding of only Cms cells, with the simultaneous possibility of removing the remaining impurities present in the test sample. The latter, being components of the extract and cells of other environmental microorganisms, as not recognized by the antibodies, are removed flowing through the pores in the membrane. At the stage of the evaluation of the operation of the invention, the high efficiency of anti-Cms antibodies immobilized on the surface of the polymer has been demonstrated.

One of the most important solutions of this invention was development of proper buffer conditions, which allowed, on one hand, on preservation of proper ionic force essential for efficient operation (recognition and binding) of IgG and, on the other hand, did not cause loss of vitality of tested Cms bacteria cells.

Equally important was achievement of correct properties of semi-permeable polycarbonate matrix allowing for high effectiveness of immunoconcentration and elimination of impurities. Polycarbonate, of which the membrane is made, has low chemical reactivity and relatively high physical resistance, whereas membrane pore diameter is several times bigger than bacteria and extract components. These properties allow for quick and efficient filtration originating from good membrane permeability and low non-specific sorption of impurities at its surface.

A negative feature of applied polycarbonate, like of other materials showing low chemical reactivity, is lack of a possibility of direct setting of antibodies on such type material surface. Therefore, it was necessary to find a method, within this research achievement framework, which would allow for a permanent, covalent immobilization of IgG type biological microparticles on membrane surface.

Experience gained during traineeship in biotechnological company Sartorius, when I worked on chemical modification of membrane surfaces, appeared to be very useful. I worked on

membranes made of regenerated cellulose, which were fairly “easy” to modify due to abundant contents of chemically reactive amine and aldehyde groups, to which examined IgG could “easily” be attached. This method, just as any method mentioned in literature, despite blocking of membrane surface with proteins used to avoid non-specific sorption, caused, however, binding of many impurities, which disqualified it for Cms diagnostics. Therefore, a new method of modification of surface using aniline polymer rich in amine groups and chemical activation of obtained polymer with glutaraldehyde or gold colloid nanoparticles, was developed within my scientific achievement framework. The applied polycarbonate - polyaniline - IgG system allowed isolation of all tested Cms strains, regardless of the degree of mucidity. On the other hand, the high specificity of the IgGs used, characterized by the absence of cross-reacting with components of the extract and mucus of other bacteria, allows easy removal of impurities from the test sample.

The aniline polymerization method itself was known (Y Wei i in., 1989;Stejskal i in., 1999) at the moment of this solution development, but it was used usually in the entire solution volume. However, it was not used for modification of polycarbonate membranes, which were a novelty at that time. The method of polymerization developed within the invention framework allows for local activation of the polycarbonate matrix with a polymer. This allowed, on the other hand, chemical durable attachment of antibodies precisely in the place of such modifications after application of the glutaraldehyde or gold nanoparticles as IgG connector.

An unquestionable advantage of such solution is a quick and selective concentration of Cms bacterial cells from big volume of the examined sample on small area, therefore, increase of detection sensitivity.

The developed method allows for further analyzing of Cms cells caught at membrane surface in several ways:

1. The first method is based on a visual analysis of immunocaught Cms bacteria under a classical or epifluorescent microscope following coloration of cell surfaces with an anti-Cms antibodies conjugate with colloidal gold or a fluorescent dye.
2. The second, slightly more time consuming method, which allows for achievement of higher detection sensitivity, is based on identification of live bacteria preceded by bioenrichment consisting in setting and incubation of the membrane with its active side directly on microbiological medium thus allowing for multiplication of Cms

bacteria for further examination. Thus obtained, concentrated and pure isolate of live cells can easily be analyzed using any EPPO technique (IF, PCR or live bacteria test on a bioindicative plant) without any risk of getting a false negative result.

The method developed within the invention framework is quick, simple and functional. It allows for both live and visual assessment of morphology of identified bacterial cells. Versatility of the test allows for diagnostics of diseases caused by other pathogens after previous change of antibodies.

Another solution developed within the presented achievement framework is the incubation kit **(H3,** Przewodowski 2013) used for detection and isolation of live selected bacteria. The principle of kit operation is as follows: the analyzed sample containing examined Cms bacteria (e.g. extract from plant tissue and potato tubers, soil and water) is suspended in a buffer solution and incubated in a vessel containing a locally activated immunolayer. During incubation, bacteria contact the antibodies placed on the polymer surface. Due to high specificity of anti-Cms antibodies, only Cms cells are identified and caught from the solution whereas any other microorganisms, as well as remaining components and impurities contained in the sample, are efficiently eliminated at the washing stage. To prevent any accidental deposition of impurities or other microorganisms that could distort the analysis result, the incubation vessels are made of materials featuring low chemical reactivity such as polystyrene, polyethylene, polycarbonate and glass. Activation of the vessels in a precisely defined place, and on a very small surface, allows for catching Cms bacteria from big volume and to concentrate them strongly. It is important, in the case of very big volumes, that antibodies located on the polymer surface should feature the highest possible effectiveness being arranged not in an accidental way, but in the best possible way, with their active side towards the antigen. The space-oriented method of immobilization was achieved by oxidation of carboxylic groups of antibodies and covalent bonding with polymer amine groups by reduction of bonds with $NaCNBH_4$. This solution allowed for considerable increase of the method reaction sensitivity, thus curtailing the analysis performance time.

Just like in the case of the previous H2 solution, the incubation kit allows for confirmation of occurrence of immunocaught Cms cells in several ways:

1. The first method allows for observation and analysis of immunocaught Cms cells morphology on the layer, or its fragment, located under a classical microscope, following previous dyeing of cells with anti-Cms antibodies conjugate with colloidal gold or

enzymatic marker (e.g. alkaline phosphatase). After performance of enzymatic reaction with the substrate (BCIP/NBT), an insoluble product is obtained at the caught cells place.

2. There is also a possibility to observe the immunocaught bacteria under an epifluorescent microscope following previous cell marking with anti-Cms antibodies conjugate wit fluorochrome (e.g. indocarbocyanine, fluoroscein or quantum dots). In this case, not only physico-chemical properties of polyaniline as a medium for IgG, but also its optical features, are used. Said polymer is able to attenuate UV light exciting glowing of fluorochrome used for bacteria marking. Therefore, the image obtained become more contrasting. For this purpose, a dark (black) background, or proper filter, is used as a standard.

3. The developed kit provides also an opportunity for live identification of immobilized Cms cells. Optimized conditions of elimination (desorption) of Cms from immunosorbent surface allow for preservation of examined cells vitality. Therefore, a strongly alkaline buffer solution (e.g. 0.1M glycine-NaOH pH 10.5) was used for this purpose; it reduces the antibody-antigen bonding force and releases bacteria from the immunosorbent surface. This, in turn, allows for setting a culture on growing medium and multiplication of examined cells. Obtaining of vital cells is greatly important in examination of virulence of given bacterial strain during a biotest with use of indicative plants as it allows for ascertainment if there occurs any level of virulence of the isolated bacterial strain. Such multiplicated bacteria can also be analyzed by application of any EPPO recommended technique (IF, PCR test).

Compared with an immunofiltration kit, the kit developed by me requires no extra devices and is less vulnerable to occurrence of any components and impurities contained in the examined environmental samples. Furthermore, as any vessel capacity can be used, my kit allows, apart from examination of plant tissues, for examination of such samples as environmental water and soil of much higher volume and high degree of contamination. Research performed within the invention framework using other potato pathogens, such as *Pectobacterium carotovorum subsp. carotovorum* and *Pectobacterium carotovorum subsp. atrosepticum* has shown high usability of the kit in isolation and identification of the above-named bacteria coming from potato tuber extracts.

The final invention that makes part of the achievement, which is particularly useful for Cms bacteria identification in high volume and strongly contaminated samples, is a kit for

detection and isolation of live *Clavibacter michiganensis* ssp. *sepedonicus* bacteria in analyzed sample **(H4,** Przewodowski 2013).

The principle of kit operation is as follows: fine-grained microsphere immunosorbent is placed in the analyzed sample containing examined Cms bacteria (soil extract, environmental water). Cms bacteria are caught, during incubation, by antibodies located on the immunolayer surface and slow microsphere decantation at the bottom of the vessel containing the examined solution proceeds. Due to high specificity of anti-Cms antibodies only Cms bacteria are identified and caught, whereas any other microorganisms, as well as remaining sample components and impurities, are effectively eliminated at the washing stage.

Compared with the other two inventions, this particular invention allows for isolation of Cms bacteria from the entire sample volume. Attempts made by various commercial companies to develop and implement kits for Cms isolation from the entire volume using a magnetic bed appeared to be hardly effective due to absence of properly specific antibodies and low durability of the magnetic matrix. However, use of EPPO proposed earlier described methods for Cms isolation results in occurrence of high amount of impurities together with isolated Cms bacteria. A key solution appeared to be development of a granular and properly durable immunosorbent having properly developed sorption surface, which allowed for quick and specific separation of Cms cells from big volume of strongly contaminated samples.

As a medium for isolation, commercially available, dextran microspheres that are commonly used in column/gel chromatography as Sephadex (Bilkova et al. 1999, Kaseda et al. 2001, Karmarkar et al. 2006,) were used. Despite many beneficial physico-chemical properties of dextran, said material as a hydrogel has low specific gravity and remains in the solution for a very long time. This makes direct use of dextran microspheres, as immunolayer for isolation of bacteria cells from strongly contaminated high volume and density samples, impossible. Therefore, it was necessary to find a method that would make it possible, on one hand, to increase microsphere weight, and on the other hand, to get a surface that would be capable of a permanent covalent deposition of antibodies on its surface. Despite universal use of dextran microspheres, none of the current modification methods allowed for achievement of both parameters at the same time.

This effect was achieved, however, by application of chemical dextran modification, as per this invention (through ammino silanization, glutaraldehyde and chitosan or ammino cysteine activation), and covalent deposition of gold colloid and anti-Cms antibodies on its surface.

Particularly important appeared to be development and use of gold colloid nanoparticles, presence of which on microsphere surface allowed for considerable increase of the medium sorption surface and provided a possibility of space-oriented immobilization of antibodies as well as provided proper weight to dextran grains. This solution allowed for temporary, lasting several minutes, keeping of microspheres in the examined solution and then their decantation at the vessel bottom, which considerably simplified and facilitated production of pure Cms isolates without any necessity to use additional facilities and procedures recommended by EPPO.

The methodology developed under this invention allows for assessment of presence of Cms cells immunoconcentrated on microsphere surface by application of several detection techniques. The first method is based on immunoenzymatic analysis following previous use conjugate of anti-Cms antibodies with an enzyme (e.g. peroxidase, alkaline phosphatase etc.) which allows, after application of proper substrate, in presence of Cms cells on microsphere surface, for getting a colorful immunoenzymatic reaction product. Thanks to use of dextran properties and development during isolation of proper conditions allowing to keep Cms cells live, also accomplishment of live bacteria identification preceded by a bioenrichment stage, is possible. Placing and incubation of microspheres directly on the microbiological growing medium, allows for multiplication of Cms bacterium colonies for further examination. Such produced isolate of multiplicated and live Cms cells may be analyzed after use of the conjugate of anti-Cms antibodies with colloidal gold, enzyme or fluorochrome as well as any EPPO technique (IF, PCR or live bacteria test on a bioindicative plant) without any risk of getting a false negative result.

The immunoenzymatic and live test sensitivity using dextran grains coated with gold colloid nanoparticles was 500 and 50 CFU/ml in the case of Cms detection. On the other hand, change of anti-Cms antibodies on dextran surface for antibodies directed against gram negative *Erwinia carotovora* subsp. *carotovora* bacteria (a synonym of *Pectobacterium carotovorum* subsp. *atrosepticum*), allowed for detection of cells of the potato wet rot perpetrator by application of the immunoenzymatic method, thus allowed for showing usability and versatility of the developed kit for isolation and identification of other bacterial pathogens in potato tuber extract.

Summary

Summing up, it is worth to note that the solutions developed within the hereby described achievement, have many important advantages that allow for solving of currently existing problems with isolation and identification of the ring rot perpetrator in environmental samples.

One of the most important elements of the achievement was development of highly specific polyclonal anti-Cms antibodies combining features of both high specificity of monoclonal antibodies as well as high sensitivity of polyclonal antibodies. (**H1**, Przewodowski i Przewodowska, 2017). Combination of these properties allowed for detection of Cms bacteria cells irrespectively of their mucoid level and for avoidance of unspecific reactions with other bacteria and impurity components contained in examined environmental samples.

Another important stage was development of materials, bacterial cell isolation and identification methods described in three presented inventions (**H2**, Przewodowski, 2013; **H3**, Przewodowski, 2013; **H4**, Przewodowski, 2013). Application of antibodies in preparation of immunosorbents+ on porous structure surfaces (membranes), vessels (dishes) or microspheres allowed for quick and highly specific concentration of Cms bacteria cells on a small area from high probe volume with simultaneous elimination of presence of other bacterial cells growing on microbiological media for Cms and substances, occurrence of which in PCR and IF tests creates a high risk of result distortion.

The developed solutions allow to identify immunopressed Cms cells in many ways. One of them is a direct visual analysis of the morphology of immunocaught Cms bacteria under a classical or epifluorescent microscope following coloration of cell surfaces with an anti-Cms antibodies conjugate with colloidal gold or a fluorescent dye. Slightly more time consuming, yet allowing for achievement of higher identification sensitivity, method is the live bacteria detection preceded by the bioenrichment stage, which allows for obtaining a pure concentrated isolate of highly vital Cms cells for further examination. Such obtained material can be analyzed using a conjugate of anti-Cms antibodies with colloidal gold, enzyme or fluorochrome or any EPPO technique (IF, PCR or live bacteria test on a bioindicative plant) without any risk of getting a false negative result.

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Li X., Tambong J., Kat (Xiaoli) Yuan,1 Wen Chen,2 Huimin Xu,1 C. Andre Levesque2 and Solke H. De Boer. 2018. International Journal of Systematic and Evolutionary Microbiology 68: 234-240.

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Przewodowski W., Treder K. 2008. Difficulties related to the diagnosis and elimination of ring rot of potato. Ziemn. Pol. 2008(4): 1-3.

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5. Discussion of other scientific and research achievements.

I started my research work in 2001 under the supervision of dr hab. Jerzy Lewosz at the Faculty of Mechanical Engineering at Koszalin University of Technology. The main research issue that I dealt with at that time was the differentiation and identification of potato varieties using biochemical and molecular techniques. The work carried out was related to the use of biochemical and molecular markers of respectively protein trypsin inhibitors isolated by affinity chromatography and analyzed by polyacrylamide electrophoresis and DNA sequences from potato tubers obtained by ISSR-PCR (Inter Simple Sequence PCR) using AC type intersatellite primers and AG, analyzed by agarose gel electrophoresis. The research allowed us to develop a fast and specific methodology for differentiation and identity testing of tested potato varieties and was the basis of my master's thesis: "Identification of homogeneity and potato varietal identity with the use of biochemical and genetic markers", which I defended in 2001 with a very good result.

The obtained results, which are of great practical importance, especially in the identification of potato varieties, were presented during a scientific and training conference (Przewodowski et al., 2006) and in the form of a publication (Przewodowski et al., 2007):

PUBLICATIONS:

1. Przewodowski W., Lewosz J., Treder K., Pilecki T., Barnyk A. 2007. Identification of potato varieties by electrophoresis. Biul. IHAR 243: 151-157.

CONFERENCES:

1. Przewodowski W., Lewosz J., Treder K., Pilecki T., Barnyk A. 2006. Identification of potato varieties by biochemical methods. [In:] Seed production and potato protection. Conf. science-schools. Kołobrzeg, March 30-31, 2006. IHAR ZNiOZ Bonin: 96.

After defending my master's thesis, I started doctoral studies at the Mechanical Department of the Koszalin University of Technology. In the initial period of doctoral studies, i.e. from September 2001 to November 2002, I conducted research at the Department of Microbiology. They mainly concerned the use of classical microbiological techniques in the isolation and identification of microorganisms found in food as well as plant pathogens, animals and humans. After moving to the Department of Biochemistry and Biotechnology, my research was additionally enriched with a number of different methods of chemistry, biochemistry and chemical and instrumental analysis of food. The work carried out focused on the development of methods for the production and modification of the surface of a new type of biosensor matrices for use in the detection and analysis of microorganisms. The result of my work from that period was, among others, the making of two patent applications for inventions regarding the method of production and modification of bio-membranes (Przewodowski and Lewosz 2010a, 2010b). The research concerned the development of new

methods of producing and modifying the surface of biologically active, semipermeable polyphenol membranes based on viscose films as a result of enzymatic copolymerization of phenolic substances with simultaneous inoculation of biologically active biological molecules. The developed modification of the membrane surface enabled covalent binding to the matrix surface of various types of biological particles, such as enzymes, antibodies. As a result, a wide potential application spectrum was obtained in the laboratory analysis, sensing, biotechnology, and technological processes in the field of chemistry, food technology and environmental engineering. The possibilities of using inventions were presented at two nationwide congresses, biotechnology in Lodz and biochemical Olsztyn (Lewosz and Przewodowski 2003, Przewodowski i Lewosz, 2008), and XXXVII Scientific Session of the Committee of Food Sciences of the Polish Academy of Sciences, during which the work was distinguished by a committee composed of PAN members for innovation, originality and functionality (Przewodowski and Lewosz 2006).

PATENTS:

1. Przewodowski W., Lewosz J. 2009. Method of production of bio-membranes. Patent No. P 204512.
2. Przewodowski W., Lewosz J. 2010. Method of bio-membrane modification. Patent No. P 206271.

CONFERENCES:

1. Lewosz J., Przewodowski W. 2003. Preparation of polyphenol membranes by enzymatic polymerization of phenolic derivatives. [In:] II National Biotechnology Congress, Announcement PD10 Łódź June 23-27, 2003. Summary: 98.
2. Przewodowski W., Lewosz J. 2006. Methods of producing polyphenol membranes as substrates for enzyme immobilization and potential possibilities of their use in the production and analysis of food. [In:] XXXVII Scientific Session of the Food Sciences Committee PAN Improving the quality of food and nutrition in the perspective of the needs of the 21st century consumer. Gdynia, 26-27 September 2006. Abstract: 306.
3. Przewodowski W., Lewosz J. 2008. The way of fabrication and food analysis of polyphenolic membranes. (Poster) [In:] Session L. Biochemistry and Food Biology, Olsztyn, Poland, September 7-11 2008, Abstracts, Acta Biochimica Polonica, 55 (3):280.

As a beneficiary of the 11-month scientific internship under the Leonardo da Vinci Program, I participated in research carried out in the biotechnology company Sartorius (Göttingen, Germany), specializing in the development and production of filtration membranes. I participated in the research conducted as part of the international project AIMs (Methods for Material Performance and Evaluation) in the 6th EU Framework Program, which consisted in developing new and improving existing types of filtration membranes used in the purification of bio-molecules (antibodies and other proteins), surface modification these membranes, instrumental analysis and the implementation of developed membranes as ready-made products on an industrial scale. Performed work allowed me to learn about modern techniques of membrane surface modification and to gain experience in the field of chemical

and biochemical modification of filter matrices of regenerated cellulose and its derivatives. The results of solutions developed by me during the internship were highlighted by the department's management for the usefulness and originality and implemented for the production of new membranes for the purification of biological molecules of potential use in medicine, diagnostics and the pharmaceutical industry.

Starting my doctoral studies, at the same time as a volunteer I started over four years of practice at the IHAR Potato and Potato Plant in Bonin. By participating in the work carried out then at the Laboratory of Serology and Laboratory of Molecular Diagnostics and Biochemistry, I carried out research that concerned the development of polyclonal antibodies to potato viruses and immunoassays for their detection. It allowed me to get acquainted with various techniques of immunization of animals, purification of antibodies from blood serum and methods of quality analysis (titre and specificity) of obtained antibodies. One of the tasks was to develop conjugates of obtained antibodies with enzymes (eg alkaline phosphatase, horseradish peroxidase, etc.) allowing to obtain immunoenzymatic markers for detection of potato viruses by ELISA. After recruitment in January 2006, I continued to participate in the work related to virus diagnostics, performing, among others, tasks within research projects, including Biological Progress in Plant Production (PBwPR), MRiRW (2008-13) and NCN (2011-14) . The results of the team's work were presented during two conferences (Pilecki et al., 2006, Pilecki and others 2007) and in the form of four publications (Treder and Przewodowski, 2009, Treder et al., 2009, Pilecki and others 2009, Treder i in . 2015).

PATENTS:

1. Przewodowski W., Lewosz J. 2009. Method of production of bio-membranes. Polish Patent No. P 204512.
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PUBLICATIONS:

1. Treder K. Zacharzewska B., Przewodowska A., Przewodowski W., Otulak K. 2015. Ion-exchange chromatography membrane chromatography membrane chromatography Y. Plant Breeding and Seed Science 72: 56-67
2. Treder K., Przewodowski W., 2009. The simple method of increasing microplates. ELISA. Threshold. Plant Prot. 49, 4: 1767-1769.
3. Treder K., Przewodowski W., Barnyk A. 2009. Potato extracts in potato tuber extracts. Plant Breed. Seed Sci. 59: 65-74.
4. Pilecki T., Barnyk A., Przewodowski W., Treder K. 2009. The influence of insects on contaminants. Threshold. Plant Prot. 49, 2: 691-695.

PROJECTS:

1. "Developing a procedure for detecting viral infections in potato tubers immediately after harvest or in a state of rest". 2008-2013. Research project MRiRW 4-3-00-7-03. Performer
2. 'Development of a procedure for the isolation of plant viruses using ion-exchange membrane chromatography'. 2011-2014. Scientific project of the National Science Center No. N N310 728540. Main contractor.

CONFERENCES:

1. Pilecki T., Lewosz J., Treder K., Barnyk A., Wi. W. W. 2006. Detection of PLRV, PVM, PVS, PVX and PVY in potato tubers. (Poster) [In:] Seed production and potato protection. Scientific and training conference. Kołobrzeg, March 30-31, 2006. IHAR ZNiOZ Bonin: 98.
2. Pilecki T., Lewosz J., Treder K., Przewodowski W., Barnyk A. 2007. Possibilities of PLRV, PVM and PVY detection in potato tubers. (Poster) [In:] Third National Congress of Biotechnology. Biotechnology - man and the environment. Materials T3: 92.
3. Lewosz J., Przewodowski W. 2003. Preparation of polyphenol membranes by enzymatic polymerization of phenolic derivatives. [In:] II National Biotechnology Congress, Announcement PD10 Łódź June 23-27, 2003. Summary: 98.
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5. Przewodowski W., Lewosz J. 2008. The way of fabrication and food analysis of polyphenolic membranes. (Poster) [In:] Session L. Biochemistry and Food Biology, Olsztyn, Poland, September 7-11 2008, Abstracts, Acta Biochimica Polonica, 55 (3): 280.

Based on the experience gained during the work related to virological diagnostics, I began research for the immunodiagnostics of potato bacterial pathogens, with particular reference to the quarantine bacteria Clavibacter michiganensis ssp. sepedonicus, the cause of ring rot of potato disease. Due to the problems with the diagnosis of Cms bacteria described in the self-report, the pathogen required a special methodological approach enabling the development of diagnostic solutions to avoid false results, often associated with currently recommended methods of Cms detection.

One of the important research topics was the development of appropriate anti-Cms antibodies with greater specificity for Cms strains and a lower impact unspecific with other potato pathogens and environmental microorganisms. For this purpose, a method for obtaining antigen for immunizing rabbits and obtaining anti-Cms antibodies based on it was developed and claimed. The result of the research was the granting of two patents of the RP (Przewodowski and Lewosz, 2012, and 2012) and the implementation of five research projects, including three awarded by the Local Ethics Committee (in 2008, 2013 and 2018) and two from the PBWPR of the Ministry of Science and Higher Education (in 2008- 13 and 2014-20). A large part of the work related to the development of anti-bacterial antibodies focuses on the search and development of new techniques for isolation and improvement of quality (purity, titre and specificity) of antibodies obtained. One of the techniques widely used in the conducted research are affinity techniques, especially column and membrane chromatography. The results of the works were presented at three conferences (Barnyk et al 2006, Przewodowski i Stochła, 2015, Przewodowski i Przewodowska, 2017) and in the form of four publications (Barnyk et al 2008, Stochła et al 2014, Stochła et al. 2015 a & b) .

PATENTS

1. Przewodowski W., Lewosz J. 2012. The method of removing bacterial mucus.lish Patent No. P 210394.
2. Przewodowski W. 2012. Immunological test for bacteria. Polish Patent No. P 210395.

PUBLICATIONS

1. Barnyk A., Lewosz J., Treder K., Przewodowski W., Pilecki T. 2008. Use of thiophilic chromatography to isolate polyclonal antibodies from rabbits serum. Biul. IHAR, 248: 87-95.
2. Stochła W., Przewodowski W., Przewodowska A. 2014. Selected methods of obtaining antibodies for detection and identification of potato pathogens. Ziemn.Pol. 3: 46-49.
3. Stochła W., Przewodowska A., Przewodowski W. 2015. Usefulness of rabbit antibodies obtained by two methods to detect Clavibacter michiganensis subsp. sepedonicus by DAS-ELISA. Progress in Plant Protection / Advances in Plant Protection 55: 352-357. DOI: 10.14199 / ppp-2015-060
4. Stochła W., Przewodowska A., Przewodowski W. 2015. Detection of bacteria Clavibacter michiganensis subsp. sepedonicus using rabbit antibodies obtained by two different methods. Threshold. Plant Prot. 55, 3: 352-357

PROJECTS:

1. "Production of rabbit polyclonal antibodies to potato pathogens and vegetable proteins". 2018-2023. Research project LKE No. 10/2018 implemented based on the consent of the Local Ethics Committee for animal experiments in Poznań. Manager.
2. "Generation of polyclonal antibodies to potato pathogens and vegetable proteins". 2013-2017. Research project LKE No. 10/2013 implemented based on the consent of the Local Ethics Committee for animal experiments in Szczecin. Main constructor.
3. "Production of polyclonal antibodies to potato pathogens". 2008-2013. LKE research project no. 10/2008 implemented based on the consent of the Local Ethics Committee for animal experiments in Szczecin. Main constructor.
4. Biological Progress Program in Plant Production financed by the Ministry of Agriculture and Rural Development. Duration of the project 2008-2013, Research project No. 4-3-00-6-01 entitled: "Development of procedures and production of diagnostic materials for the detection of Clavibacter michiganensis ssp. sepedonicus". Contractor 2008-2010. Manager 2011-2013.
5. Research on the development of methods for selective isolation and sensitive identification of *Clavibacter michiganensis* ssp. *sepedonicus* in diagnostically difficult environmental trials. 2014-2020. Research project MRiRW No. 4-3-00-6-01 pt. Implemented as part of the Biological Progress Program in Plant Production, IHAR-PIB Branch in Bonin. Manager.

CONFERENCES

1. Przewodowski W., Stochła W. 2015. Purification of rabbit antibodies by affinity. (Poster) [In:] Potato seeding and protection, 48 Science and training conference. Dźwirzyno, 13-15 May, Materials: 87.
2. Przewodowski W., Przewodowska A. 2017. Development of a new quality of polyclonal antibodies detecting quarantine bacteria Clavibacter michiganensis subsp. sepedonicus. (Presentation) [In:] Nasiennictwo i Ochrony Potato, 50th jubilee scientific and training conference, Dźwirzyno, 7-9 June, Abstracts: 37.
3. Barnyk A., Lewosz J., Treder K., Pilecki T., Przewodowski W. 2006. Application of thiophilic chromatography in the process of production of diagnostic kits. (Poster) [In:] Seed production and potato protection. Conf. nauk.-schools. Kołobrzeg, March 30-31, 2006. IHAR ZNiOZ Bonin: 95.

In addition to immune diagnostics, an important element of my scientific activity are activities aimed at improving the molecular diagnostics of bacterial pathogens of potato. Previous studies conducted in this direction were related to the development of various methods of isolating nucleic acids from environmental tests, allowing the removal of inhibitors that inhibit the action of PCR polymerases, investigating the influence of bacterial mucus-differentiated mucus bacteria on the sensitivity of the PCR test, studying the effect of lytic enzymes on DNA isolation and the sensitivity of the PCR test, as well as the development of modern methods of isothermal amplification of nucleic acids. The developed solutions were presented at five conferences (Przewodowski and others 2014 aib, Salamońska and others 2016, Salamońska and Przewodowski, 2017, Salamońska and others 2018) and in

the form of four publications (Chołuj and Przewodowski, 2014, and Przewodowski et al 2014; Salamońska and others 2016, Salamońska and Przewodowski, 2018).

PUBLICATIONS

1. Salamońska K. and Przewodowski W., 2018. The problem with the eradication and occurrence in Poland of Ralstonia solanacearum bacteria - the perpetrator of myxoma. Potato Polish 2: 28-32.
2. Salamońska K., Stochła W., Przewodowski W. 2016. Modern diagnostic methods in the molecular identification of potato quarantine bacteria. Potato Polish 4: 41-45.
3. Przewodowski W., Chołuj J., Przewodowska A. 2015. Influence of different methods of isolating nucleic acids from bacteria on the sensitivity of the PCR test. Threshold. Plant Prot. 55, 3: 321-326.
4. Chołuj J., Przewodowski W. 2014. PCR technique and its modifications in the identification of potato pathogens. Peanuts. Half. 2: 40-45

CONFERENCES

1. Salamońska K., Przewodowski W., Szarek D., Stochła W., Przewodowska A. 2018. Evaluation of the presence of bacterial slimes in the molecular diagnostics of mucoid differentiated strains of Cms bacteria. (Presentation) [In:] 51 Scientific and training conference "Seed production and protection", Dźwirzyno, 6-8 June, IHAR-PIB ZNiOZ, Bonin, Abstracts: 23
2. Salamońska K., Przewodowski W. 2017. The effect of DNA isolation conditions on the sensitivity of the molecular test in the diagnosis of potato ring bactermia agent. (Presentation) [In:] 50 Scientific and training conference "Seed production and protection", Dźwirzyno, 7-9 June, IHAR-PIB ZNiOZ, Bonin, Abstracts: 39
3. Salamońska K., Przewodowski W., Przewodowska A., Stochła W. 2016. Effect of lytic enzymes on DNA isolation and sensitivity of the PCR test in the diagnosis of quarantine potato bacteria. (P): Seed Production and Potato Protection. Scientific and training conference. Dźwirzyno, 11-13 May. IHAR, ZNiOZ, Bonin: 72-73.
4. Przewodowski W., Chołuj J., Przewodowska A., Treder K. 2014. Detection of *Clavibacter michiganensis ssp. sepedonicus* using Loop Mediatel Isothermal Amplification (LAMP). (Poster) [In:] Conference of molecular Plant-Microbe Interactions by the International Society of Molecular Plant-Microbe Interactions. 6-11.06.2014 Rhodes, Greece: P209.
5. Przewodowski W., Chołuj J., Przewodowska A., Treder K. 2014. Isothermal method of amplification of LAMP nucleic acids in modern diagnostics of potato ring bacteremia agent. (Paper) [In:] Seed production and potato protection. Scientific and training conference. Dzwirzyno, 15-16 May, IHAR-PIB ZNiOZ, Bonin, Abstracts: 24-25.

Another research issue aimed at improving the diagnosis of bacterial and viral pathogens of potato was the development of modern materials and unconventional methods for the isolation and identification of Cms bacteria, allowing for faster and more sensitive diagnostics of potato bacteria. As part of the research, materials such as synthetic and natural polymers, colloidal metal nanoparticles (including gold, silver, copper and platinum) were developed, silica microspheres modified with gold colloid nanoparticles. The developed nanoparticles were used both as a vehicle for antibodies, as well as a marker (label) of developed antibodies. One of the most important stages of the research was the development of new methods of chemical and biochemical modification of the surface of the developed materials. This allowed for the covalent and targeted deposition of developed antibodies on the surface of the materials at the site of the activation of the substrate. Such a method of fixing antibodies allows faster and more effective action of antibodies to the tested antigen (bacterial cells, virus particles, etc.).

The above research was carried out as part of two research projects, PBWPR from the National Science Center project (2008-11) and PBWPR from the Ministry of Science and Higher Education (2008-13) and presented at five conferences (Lewosz et al., 2005, Przewodowski W., 2012, Przewodowski et al., 2013 and Przewodowski W. 2013, 2017).

PROJECTS

1. Optimization of new immunological methods for detection of Clavibacter michiganensis subsp. sepedonicus. " 2008-2011. Research project NCN No. N N310 144235. Head.
2. Development of procedures and production of diagnostic materials for the detection of *Clavibacter michiganensis ssp. sepedonicus* ". 2008-2013. Research project MRiRW 4-3-00-6-01. Contractor: 2008-2010. Manager 2011-2013.

CONFERENCES

1. Przewodowski W. 2017. Development of innovative materials for isolation and identification of the potato ringrobial agent from diagnostically difficult environmental tests. (Presentation) [In:] 50 Scientific and training conference "Seed production and protection of potatoes", Dźwirzyno, 7-9 June.
2. Przewodowski W. 2013. Modern diagnosis of Clavibacter michiganensis ssp. sepedonicus based on colloidal gold as sensitive marker. (Presentation) [In:] Classical and molecular approaches in plant pathogen taxonomy. Symposium, Warsaw, Poland, September 10-11, 2013. WULS-SGGW. Book of Abstracts: 30.
3. Przewodowski W., Przewodowska A. and Treder K. 2013. Application of colloidal metal nanoparticles in diagnosis of quarantine bacteria - Clavibacter michiganensis subsp. sepedonicus. (Poster) [In:] 10th International Congress of Plant Pathology. Beijing, China. 24-31 September 2013. ActaPhytopathologicaSinica 43 (suppl.): 370.
4. Przewodowski W. 2012. Application of colloidal metals in virus diagnostics. (Presentation) [In:] XVI Symposium of the Virological Section of the Polish Phytopathological Society and XXXVIII Conference of Plant Viruses of the Committee of Plant Protection of the Polish Academy of Sciences. Skierniewice, 18-19 September 2012: 33-34.
5. Lewosz J., Pilecki T., Przewodowski W. 2005. Nanoparticles as immunosorbents and markers for detection of potato pathogens. (Poster) [In:] Meeting COST 853, Agricultural biomarkers for array-technology. Sobieszewo June 19-21: 56.

One of the most important achievements in my scientific and research activity is the development of a number of new diagnostic methods and tests for the detection of bacterial pathogens. For the construction of these tests, previously developed, highly specific antibodies were used that, depending on the design of the test, were placed on appropriately modified materials to provide functional substrates for fast and highly specific bacterial cell isolation. An appropriate method of modification of the tested materials allowed for a targeted method of antibody deposition, the active side of the antibody towards antigen, which allowed for rapid isolation and identification of potato pathogens from large volumes, highly contaminated environmental samples such as water, soil and plant tissue extracts. The development of appropriate methods for surface modification of materials made it possible to use these materials both in the diagnosis of bacterial and viral pathogens, whereas the special construction of materials allows to remove all unwanted contaminants in one step, such as PCR inhibitors, other environmental bacteria overgrowing microbial media for Cms multiplication and tissue components of plants that cover the examined bacterial cells under the microscope, give a false negative result in the IFAS test. The use of developed

immunomodules in developed diagnostic tests allows to avoid these difficulties, allowing also to maintain the viability of isolated bacterial cells. Isolation of living cells of bacteria is very important in the case of the need to conduct research on the pathogenicity of the pathogen under investigation. An additional advantage of the developed tests is their versatility, characterized by the possibility of diagnostics of any pathogen, depending on the antibodies used.d obtaining patents, I received two awards from the Director of the IHAR-PIB in 2009 and 2013, respectively. Novelty of the developed tests have been confirmed by the granting of happiness patents, including four Polish (2013) and two international, respectively American (2014) and European (2016). The inventions were presented at the International Fair "BIO International Convention" in Chicago in 2010, while the results of the research were presented at five conferences and in the form of eight publications.

I did research on the verification of the tests I developed as part of the LIDER project (NCBiR), while I obtained patent protection through the implementation of the OPI project financed from the Innovative Economy Operational Program (POIG). Research carried out as part of the LIDER project allowed us to confirm the functionality and usefulness of the developed solutions in the diagnosis of the ring rot of potato. The developed inventions were also appreciated both on the scientific and practical side at the nationwide forum in 2010 by awarding distinctions twice for the POIG project implemented in two national competitions "Funds and Science" and "Quality of the Year 2010". In addition, for the work related to the scientific activity and obtaining patents, I received two awards from the Director of the IHAR-PIB in 2009 and 2013, respectively.

PATENTS

1. Przewodowski W. 2016. Immunological tests for the presence of specific methods. European patent granted by EPO No. EP 2 205 735. Patent protection in the European Union
2. Przewodowski W. 2014. Immunological tests for the presence of specific methods. The American patent granted by USPTO No. US 8,642,275 B2. Patent protection in the USA.
3. Przewodowski W. 2013. The method of detecting the presence of *Clavibacter michiganensis ssp. sepedonicus* in the analyzed sample. Polish PatentNo. P 213858. Patent protection in Poland.
4. Przewodowski W. 2013. A method of detecting the presence of Cms bacteria using polycarbonate membranes containing immobilized antibodies. Polish PatentNo. PL 213857. Patent protection in Poland.
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7. Przewodowski W. 2010. Determination of viability of Cms bacteria in various media used in diagnostic methods. (Poster) [In:] XL Scientific Session IOR ¬¬- PIB, Poznań, 4-5 February 2010, Abstracts 50: 257.
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PROJECTS

1. "New immunoassays for widespread use detecting potato tuberculosis". 2009-2011. POIG project no. OPIE.01.03.02-14-013 / 08 implemented under Sub-measure 1.3.2 of the Priority Axis of the Innovative Economy Operational Program, financed by OPI. Manager.
2. "A new diagnostic tool with high sensitivity and specificity for the detection and identification of quarantine bacteria Clavibacter michiganensis ssp. sepedonicus". 2012-2015. Research project LIDER nr Lider / 28/199 / L-3/11 / NCBR / 2012. NCBiR project as part of the third edition of the LIDER competition. Manager

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2. Przewodowski W. 2012. Detection of mulotally differentiated strains of *Clavibacter michiganensis* ssp. *sepedonicus* against screening tests recommended by EPPO. (Presentation) [In:] Seed production and potato protection. Sci and training conferenceDarłówko, May 24-25, 2012. IHAR - PIB ZNiOZ Bonin: 18-19.
3. Przewodowski W. 2011. Development and use of the Lateral Flow type test strip in the diagnosis of potato ring rot. (Presentation) [In:] Seed production and potato protection. Sci and training conferenceDarłówko, 19-20 May 2011. IHAR - PIB ZNiOZ Bonin: 40.
4. Przewodowski W. 2009. Fast immunological test for the identification of Clavibacter michiganensis ssp. sepedonicus. (Presentation) [In:] Seed production and potato protection. Sci and training conferenceDarłówko, 21-22 May 2009. IHAR ZNiOZ Bonin: 78.
5. Przewodowski W. 2009. Modern methods in the diagnosis of bacteria Clavibacter michiganensis ssp. sepedonicus. (Presentation) [In:] XLIX Scientific Session IOR ¬¬- PIB, Poznań, 19-20 February 2009, Abstracts 49: 125.

AWARDS

1. Award of the Director of the Plant Breeding and Acclimatization Institute. Awarded in 2014 by the letter of the Director of IHAR-PIB No. RN-23412014 of 9 December 2014. for obtaining five patents in 2013 in the Patent Office of the Republic of Poland and one patent in the US Patent Office in 2014.
2. Title 'Quality of the Year 2010'. Awarded in 2011 in the nationwide Quality of the Year competition by the Chapter composed of experts from the Polish Center for Testing and Certification (PCBC) in the category "Commercialization of research" for the project "New immunoassays for widespread use detecting potato bacteriosis".
3. Distinction "Funds and Science" Granted in 2010 in the national competition "Funds and Science" for the project entitled; "New immunoassays for widespread use detecting potato bacteriosis" carried out at the IHAR - PIB as part of Sub-measure 1.3.2 of the Priority Axis of the Innovative Economy Operational Program.
4. Award of the Director of the Institute of Plant Breeding and Acclimatization. Adjudicated in 2009 with the letter of the Director of IHAR-PIB No. D - 195 / S / 2009 from December 14, 2009. for activity, commitment and outstanding results in scientific work in 2009.

6. Summary of scientific achievements

My publication output after obtaining the doctoral degree is 28 items, including 4 works on scientific achievement (Table 1). 18 of them are original, published creative works, some of which have been published in major foreign journals, such as the American Journal of Potato Research, European Journal Plant Pathology and PLOS One. I am the author and co-author of 10 national and international patents. All inventions so far developed were considered original, which resulted in the granting of patent rights for all applications submitted for which I applied. I also disseminated the results of my work by participating in scientific conferences, training sessions and seminars. After obtaining the doctoral degree, I took part in 27 scientific conferences, including 18 national and 10 international ones, on which I gave a total of 21 papers and presented 22 posters. I am a beneficiary of two scientific internships, including one (11-month) foreign and one (6-month) national.

I prepared and realized total of 8 trainings for employees of Main Inspectorate of Plant Health And Seed Inspection (PIORiN) and potato producers, as well as 9 seminars

I worked in 4 expert teams and led research teams participating in the implementation of 12 national and foreign projects, including six as a manager and eight as a contractor or main contractor. The implementation of LIDER (NCBiR) and POIG (OPI) projects was important, as it allowed to gain a lot of valuable experience and knowledge in managing research teams and proper implementation of projects. I received a total of 6 awards and prizes for scientific and organizational activity as well as implemented projects and patents.

The performed nature of the research also required self-education and / or acquisition of appropriate qualifications, which results in the previous participation in 13 refresher courses in Poland and abroad, as well as obtaining 7 permits required in the case of research with quarantine organisms and laboratory animals. Currently, I am a member of 3 scientific organizations, six research networks / consortia, the Young Scientists' Council of the IHAR-PIB and the Advisory Council of the Pomeranian Agricultural Advisory Center. On June 20, by the decision of the IHAR-PIB Scientific Council, I became the auxiliary promoter in the PhD thesis M. Pietraszko, who prepares the work on the epidemiology of ring rot of potato.

Table 1. Numerical list of scientific achievements before and after obtaining the doctoral degree.

Specification	Number		
	Before PhD	After PhD	Sum
Original published creative works (including on the Philadelphia Institute of Scientific Information list)	1	18	19
Review works and popular-scientific-dissemination studies	-	9	9
Monographs (author and / or co-author)	-	1	1
Patent applications and patents obtained, included	4	15	19
• national applications & patents	4 (2 & 2)	12 (6& 6)	16 (8 & 8)
• international applications & patents	-	3 (1 & 2)	3 (1 & 2)
Points for publications and patents (according to Ministry of Science and Higher Education) (Year of publication)	33	506	539
Points for publications and patents (according to Ministry of Science and Higher Education)) (2016)	35	575	610
Impact factor of the publications (according to Ministry of Science and Higher Education) (Year of publication)	-	6,700	6,700
Impact factor of the publications (according to Ministry of Science and Higher Education) (2016)	-	6,823	6,823
Number of citations for publications according to the WoS database	13		
Hirsch Index according to the WoS database	1		
Number of citations for publications according to the GS database	20		
Hirsch Index according to the WoS database	2		
Research and reports published in publishing houses from congresses and conferences, including:	10	46	56
• reviewed	3	12	15
• placed in magazine supplements	1	4	5
• summaries	6	30	36
Lectures and papers delivered at seminars and conferences:	14	48	62
• international	-	10	10
• nationwide	9	18	27
• at universities and other scientific units	3	12	15
• training seminars	2	8	10
Membership in scientific organizations	-	3	3
Membership in scientific councils and other non-scientific organizations	-	2	2
Grants from EU funds	1	2	3
National grants (management and/or performance), including:	2	10	12
• from the funds of the Ministry of Science and Higher Education	2	3	5
• from other means	-	7	7
Participation in expert and competition teams (including participation in expert opinions):	-	4	4
Reviews made:	-	11	11

1. Due to the lack of influence of IF factor on the above patents, its values have not been included in patent items.

Włodzimierz Rozwadowski