Pantoea agglomerans: a mysterious bacterium of evil and good. Part IV. Beneficial effects

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Abstract

Pantoea agglomerans, a gammaproteobacterium of plant origin, possesses many beneficial traits that could be used for the prevention and/or treatment of human and animal diseases, combating plant pathogens, promotion of plant growth and bioremediation of the environment. It produces a number of antibiotics (herbicolin, pantocins, microcin, agglomerins, andrimid, phenazine, among others) which could be used for combating plant, animal and human pathogens or for food preservation. Japanese researchers have demonstrated that the low-molecular-mass lipopolysaccharide of *P. agglomerans* isolated by them and described as 'Immunopotentiator from *Pantoea agglomerans* 1 (IP-PA1)' reveals the extremely wide spectrum of healing properties, mainly due to its ability for the maintenance of homeostasis by macrophage activation. IP-PA1 was proved to be effective in the prevention and treatment of a broad range of human and animal disorders, such as tumours, hyperlipidaemia, diabetes, ulcer, various infectious diseases, atopic allergy and stress-induced immunosuppression; it also showed a strong analgesic effect. It is important that most of these effects could be achieved by the safe oral administration of IP-PA1. Taking into account that *P. agglomerans* occurs commonly as a symbiont of many species of insects, including mosquitoes transmitting the *Plasmodium* parasites causing malaria, successful attempts were made to apply the strategy of paratransgenesis, in which bacterial symbionts are genetically engineered to express and secrete anti-*Plasmodium* effector proteins. This strategy shows prospects for a successful eradication of malaria, a deadly disease killing annually over one million people, as well as of other vector-borne diseases of humans, animals and plants. *Pantoea agglomerans* has been identified as an antagonist of many plant pathogens belonging to bacteria and fungi, as a result of antibiotic production, competition mechanisms or induction of plant resistance. Its use as a biocontrol agent permits the decrease of pesticide doses, being a healthy and environmental-friendly procedure. The application of the preparations of this bacterium efficiently protects the stored pome, stone and citrus fruits against invasion of moulds. *P. agglomerans* strains associated with both rhizosphere and plant tissues (as endophytes) efficiently promote the growth of many plants, including rice and wheat, which are the staple food for the majority of mankind. The promotion mechanisms are diverse and include fixation of atmospheric nitrogen, production of phytohormones, as well as degradation of phytate and phosphate solubilizing which makes the soil phosphorus available for plants. Accordingly, *P. agglomerans* is regarded as an ideal candidate for an environmental-friendly bioinoculant replacing chemical fertilizers. It has been documented that the *Pantoea* strains show biodegradation activity on various chemical pollutants of soil and water, including petroleum hydrocarbons and toxic metals. *P. agglomerans* prevents the penetration of harmful industrial contaminants into deeper parts of soil by biofilm formation, and has an ability to produce hydrogen from waste. Thus, this bacterium appears as a valuable bioremediator which, in some cases, may be acquired as a cheap form of energy. In conclusion, in spite of the proven pathologic role of *P. agglomerans* in causing occupational diseases of allergic and/or immunotoxic background and accidental infections, the beneficial traits of this species, and of related species of *Pantoea* genus, are of great value for potential use in many areas of biotechnology. Hence, any restrictions on the use of these organisms and their products should be declined, providing safety precautions at work with the *Pantoea* biopreparations are maintained.

Key words

Pantoea agglomerans, beneficial effects, LPS, immunostimulation, treatment of tumors, healing properties, vector-borne diseases, paratransgenesis, biocontrol of plant pathogens, antibiotics, plant growth promotion, bioremediation

Introduction

In preceding articles on *Pantoea agglomerans* [1, 2, 3], the deleterious effects of this bacterium were reviewed, including allergenic and immunotoxic effects associated with occupational exposure to organic dusts, as well as

opportunistic infections of humans and plant diseases. However, the pathogenic properties of this bacterium are counterbalanced by the unbelievable beneficial traits exhibited by *P. agglomerans*, and the closely related species of *Pantoea* genus that could collectively be described as '*Pantoea agglomerans* sensu lato'. On the one hand, this group of bacteria is versatile and diverse, but on the other hand, exhibits some common traits associated with an extremely strange biochemical activity and incredible ability of adaptation to a broad range of hosts and various environmental conditions [4, 5, 6]. This creates a unique opportunity for biotechnology

Annals of Agricultural and Environmental Medicine 2016, Vol 23, No 2   207

Jacek Dutkiewicz, Barbara Mackiewicz, Marta Kinga Lemieszek, Marcin Golec, Janusz Milanowski. *Pantoea agglomerans*: a mysterious bacterium of evil and good. Part IV…

to use *P. agglomerans* as an effective drug, biocontrol agent, biofertilizer or bioremediator. It is fascinating that some potentially pathogenic traits of *P. agglomerans*, such as an ability to activate human macrophages causing lung inflammation [1, 2], or an ability to regulate various plant-pathogenic functions of bacterial organisms, depending on population density, known as quorum sensing [7], could be efficiently used for the treatment of human diseases [8] or promotion of plant growth [9], respectively. Below are reviewed the most important beneficial traits of *P. agglomerans* that could be used for the protection of the health of humans, animals and plants, as well as for the marked increase in food supply and sanitation of the human living environment.

Antibiotic production

P. agglomerans has been shown to have unique metabolic capabilities, including antibiotic biosynthesis [10]. These antibiotics could be used for combating plant, animal and human pathogens or for food preservation. They include: • lipopeptide antibiotics – herbicolin A and B, which are active against sterol-containing fungi [11, 12]; • the dapdiamide antibiotic – herbicolin I, produced by *P. agglomerans* and closely related species *Pantoea vagans*, active against *Erwinia amylovora*, a pathogen of apple and pear trees [13]; • pantocins A and B inhibiting growth of various Enterobacteriaceae strains, including *E. amylovora* [14]; • the peptide antibiotic called microcin produced by *P. agglomerans* strain Eh252, targeting also *E. amylovora* [15]; • the acidic antibiotics called agglomerins A, B, C and D which are moderately active against a wide variety of anaerobic bacteria (including *Clostridium difficile*, *C. perfringens*, *Propionibacterium acnes*) and weakly active against aerobic Gram-positive bacteria (including *Streptococcus pyogenes*, *S. pneumoniae*) [16]; • the pseudopeptide antibiotic andrimid which is active against both Gram-negative and Gram-positive bacteria, including methicyllin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE) and *Klebsiella pneumoniae*, and displays also limited antiproliferative activity against human tumor cell lines [17]; • the broad-spectrum peptide antibiotic 2-amino-3-(oxirane-2,3-dicarboxamido)-propanoyl-valine (APV) produced by *P. agglomerans* strain Pa48b which was proved to suppress *E. amylovora*, the soybean pathogen *Pseudomonas syringae* pv. (pathovar) *glycinea*, the agent of crown gall disease in many plants *Agrobacterium tumefaciens* and the human pathogen *Candida albicans* [18]; • multiple antibiotics produced by a clinical isolate *P. agglomerans* Tx10, that target *E. amylovora* and clinically relevant pathogens, including *Staphylococcus aureus*, *Streptococcus epidermidis*, and *Escherichia coli* [10]; • D-alanylgriseoluteic acid (AGA), a potent phenazine antibiotic produced by *P. agglomerans* strain Eh1087 which reveals a broad spectrum of antimicrobial activity and is particularly active against Gram-positive pathogens, such as *Streptococcus pneumoniae* [19, 20]; • the recently isolated in Korea phenazine antibiotic from *P. agglomerans* apple strain R190, active against various spoilage bacteria, including *Pectobacterium carotovorum* subsp. *carotovorum*, *Clavibacter michiganensis*, and *Burkholderia andropogonis*, as well as against foodborne pathogens such as *Escherichia coli* O157:H7 and *Salmonella enterica*, and other human pathogens such as *Klebsiella pneumoniae* and *Yersinia enterocolitica* [21].

Tumour treatment with *Pantoea agglomerans* LPS preparation: the Japanese scientists pave the way for efficient therapy

The first systematic use of bacterial products for the treatment of malignant tumors was begun in 1891 by William B. Coley (1862–1936), the creator of Coley's Toxin (*Streptococcus* combined with *Serratia marcescens*). Coley postulated that bacterial products stimulate human's immunity to suppress tumours, and hence he is known as a 'Father of Immunotherapy' [22]. About a hundred years later, his ideas were revived by a group of Japanese scientists [23, 24, 25, 26, 27, 28]. They noticed that the extract of wheat flour revealed anti-tumour effects, and after detailed analysis determined that these effects are due to the presence of *Pantoea agglomerans* [23, 26], the organism commonly associated with grain and flour [2]. Consequently, the Japanese researchers isolated and purified the active substance which was the low-molecular-mass (5 kDa) lipopolysaccharide (LPS) of *P. agglomerans* [26], later defined by them as 'LPSp' [25] or 'Immunopotentiator from *Pantoea agglomerans* 1 (IP-PA1)' [28]. Mizuno and Soma [29] described this new preparation as a 'nontoxic type of Coley's toxin', which could be efficiently used for the treatment of cancer and multiple intractable diseases via oral or percutaneous route, without any side-effects.

Iwamoto et al. [25] demonstrated that intradermal administration of *P. agglomerans* LPS (LPSp) to mice in combination with cyclophosphamide resulted in the complete regression of MH134 haepatoma tumour. They observed in treated mice an extensive necrosis and marked infiltrations of inflammatory cells (CD4+ and CD8+ T-lymphocytes, macrophages, neutrophils) in tumours and an associate increase of cytokine (TNF-α = tumour necrosis factor α, IL-1α = interleukin-1α, IL-6 = interleukin-6, G-CSF = granulocyte colony stimulating factor) serum levels. They came to conclusion that the successful treatment with LPSp was mostly due to enhanced T cell-mediated antitumour immunity, supported by TNF-α and other cytokines. These results were confirmed by Inagawa et al. [30, 31] who noted a complete regression of murine syngeneic tumours, Meth A fibrosarcoma, MH134 haepatoma and Lewis lung (LL) carcinoma after combined treatment with intradermally administered LPSp and cyclophosphamide. The authors underscored an important role of the TNF-α induction in successful therapy, and the low toxicity of the intradermally applied preparation which was 230–380 times smaller compared to intravenous administration.

The enhancement of the potentially anti-tumour cytokines by *P. agglomerans* LPS demonstrated by the Japanese researchers was confirmed by Dutkiewicz et al. [2, 32], who found in the rabbits inhaled with the microvesicular-bound LPS of *P. agglomerans* a significant growth of the levels of circulatory cytokines: total interferon (IFN), interleukin-1 α (IL-1α), and tumour necrosis factor α (TNF-α). In another study, Śpiewak and Dutkiewicz [33] stated that the microvesicular-bound LPS of *P. agglomerans* significantly stimulated the increase of interferon-γ (IFN-γ) and TNF-α content in the culture of the human peripheral blood mononuclear cells.

Hino et al. [34] observed the induction of cell death in lung cancer cells when co-cultured with the rat alveolar macrophages or human monocytes that had been activated by the LPS preparations produced by *Pantoea agglomerans*

208   Annals of Agricultural and Environmental Medicine 2016, Vol 23, No 2

Jacek Dutkiewicz, Barbara Mackiewicz, Marta Kinga Lemieszek, Marcin Golec, Janusz Milanowski. *Pantoea agglomerans*: a mysterious bacterium of evil and good. Part IV…

or *Escherichia coli*. Despite the fact that both preparations exerted similar activation effects, the authors recommended the LPS of *P. agglomerans* for anticancer use because it has a lower molecular weight which enables easy absorption through the skin or mucosa. The LPS-activated rat alveolar macrophages produced large amounts of NO. According to the authors, this reaction was responsible, at least in part, for the lethal effect in cancer cells. In the case of activated human monocytes, the release of a cytotoxic agent was not noted.

More recently, Hebishima et al. [28] documented the usefulness of the IP-PA1 preparation as a supportive drug in melanoma therapy, reporting a significant prolongation of survival period in the melanoma-inoculated mice which were given IP-PA1 orally in combination with intraperitoneally given doxorubicin. The authors stated the increase of serum IFN-γ level, as well as augmentation of the natural killer (NK) cells population, and of the ratio of CD4+ to CD8+ T cells in the spleen, which could be meaningful for final good results of the therapy. As IP-PA1 did not directly affect melanoma cells, the authors suggested that therapy success was related to the enhancement of mice immunity by IP-PA1, which neutralized the immunosuppressive action of doxorubicin.

Japanese researchers also successfully used the *P. agglomerans* LPS preparation for cancer treatment in humans. Kasugai et al. [24] applied the intradermal administration of LPSp in combination with transarterial intermittent chemotherapy (with 5-Fluorouracil, Farmorubicin, and Mitomycin C) for treatment of a patient suffering from advanced gastric cancer with multiple liver metastasis (H3). Therapy ended with full recovery: the computer tomography examination and endoscopy showed regression of the tumour and the patient was discharged from hospital. With the exception of fever, no serious side-effects were observed. The serum TNF-α levels were elevated and, as shown by histological examination, CD8+ lymphocytes dominantly infiltrated around the tumour. According to the authors, the achieved results clearly indicated the immunostimulative anti-tumour effect of LPSp, which appeared as a promising new adjuvant in cancer therapy. In a pilot clinical study, Goto et al. [35] applied intradermal administration of LPSp in combination with intravenous dosing of cyclophosphamide in the treatment of 10 patients with advanced cancer. Five of them had evaluable tumour, which had failed earlier to respond to conventional chemotherapy. After a 4-month therapy, a significant size reduction was noted in 3 of 5 evaluable tumours. Intradermally administered, LPS was well tolerated in high doses which contributed to the significant and continuous rise of cytokine (TNF-α, IL-6, G-CSF) level. In conclusion, the authors recommended the use of *P. agglomerans* LPS for cancer treatment in humans.

Hebishima et al. [36] found the IP-PA1 preparation selectivity for normal and cancer cells. They demonstrated that IP-PA1 protects normal macrophages from cell growth inhibition induced by chemotherapeutic agent, but did not affect the cytotoxicity of the chemotherapeutic agents in carcinoma cells. According to the authors, the protective effect was mainly due to the activation of transcription factor NF-κB factor (nuclear factor-κB), an expression of anti-apoptotic proteins and the production of NO and TNF-α, the important mediators in the killing of tumour cells. The authors concluded that IP-PA1 can be used as an edible drug for the efficient support of chemotherapy.

Other healing properties in humans and animals: a panacea?

Broad spectrum of healing properties due to macrophage activation. Japanese researchers have demonstrated the extremely wide spectrum of healing properties revealed by the *P. agglomerans* LPS (IP-PA1), which is mainly due to its ability to activate macrophages that play an essential role in the maintenance of homeostasis in all multi-cellular animals [26]. By activation of macrophages, IP-PA1 assures homeostasis, protects multi-cellular organisms from external hazards, and contributes to recovery from a broad range of diseases, such as hyperlipidaemia, diabetes, ulcer, herpes and atopic allergy [26, 29]. It can also stimulate bone resorption and egg-laying, and exerts a strong analgesic effect that even allows a release from drug addiction [29]. Another advantageous property of IP-PA1 is its effectiveness when administered by the oral route which proved to be safe in large doses up to 600 mg/kg, without any side-effects [23, 36, 37, 38, 39, 40]. Kohchi et al. [26] and Inagawa et al. [40, 41] proposed that the mechanism for maintaining homeostasis by oral administration of *P. agglomerans* LPS includes a signal transfer system via cell-to-cell contact, termed the macrophage network system. According to these authors, oral application of IP-PA1 had preventive and curative properties against various diseases, including allergic and lifestyle-related diseases.

Hebishima et al. [39] demonstrated that IP-PA1 is a promising edible immunomodulator which could be used in the prevention and treatment of a wide range of disorders either caused or exacerbated by stress-induced immunosuppression in many animals and in humans.

According to Nakata et al. [42], the primary reason for the development of a stress-induced disease is suppression of macrophage function, which plays a pivotal role in innate immunity and maintenance of homeostasis. Hence, the activation of macrophages by priming with the *P. agglomerans* LPS (IP-PA1) prevents various kinds of stresses, such as chronic psychological stress, or surgery stress. Application of IP-PA1 prior to surgery by the oral route is both safe and useful for the prevention of infectious diseases and tumour metastasis.

Efficiency depending on structure. Inagawa et al. [38] examined the protective effect of various lipopolysaccharides prepared from 6 species of bacteria (*Escherichia coli, Pantoea agglomerans, Serratia ficaria, Enterobacter cloacae, Bordetella pertussis, Alcaligenes faecalis*), and from wheat flour on gastric ulcer in mice using parenteral as well as the oral route. LPS of *Pantoea agglomerans* appeared to be most effective among other examined LPSs. The biological activity of LPS depends on the chemical structure of its molecule composed of lipid A, core oligosaccharide and O-antigen polysaccharide, and is determined mostly by lipid A moiety, although according to recent studies, O-specific chains could be also important in this respect [43, 44, 45]. According to Kohchi et al. [26], the O-specific chain of *P. agglomerans* LPS (IP-PA1) is shorter compared to LPS of typical *Escherichia coli* and has a different composition. IP-PA1 consists of rhamnose and glucose, whereas *E. coli* LPS consists of colitoses, glucosamine, glucose and galactose. The overall molecular weight of IP-PA1 is much smaller than *E. coli* LPS. Therefore, the small amphipathic IP-PA1

Annals of Agricultural and Environmental Medicine 2016, Vol 23, No 2

209

Jacek Dutkiewicz, Barbara Mackiewicz, Marta Kinga Lemieszek, Marcin Golec, Janusz Milanowski. *Pantoea agglomerans*: a mysterious bacterium of evil and good. Part IV…

easily forms a small-diameter micelle which easily penetrates through mucosa and/or skin, enhancing the positive effects of treatment after oral or intradermal administration [26]. This is noteworthy, that the safe and stable production process of IP-PA1, which is a water extract of *P. agglomerans* culture on a medium containing wheat flour, water and minerals [26, 41], reveals some similarities with the simple production process of the CA-S *P. agglomerans* preparation used by our group in numerous experiments [2]. The CA-S is obtained by the extraction of bacterial mass in saline and was found to exhibit strong immunostimulatory properties, including macrophage activation [2].

Anti-allergic effects of IP-PA1. Yoshida et al. [46] postulated that the anti-allergic effects of *P. agglomerans* LPS (IP-PA1) are due to shifting the Th1/Th2 balance towards Th1 response after activation of macrophages in the epidermis by LPS. As a result of this shift, the cytokines of Th1 type are produced instead of excessive reactions of Th2 type that cause allergic dermatitis or other IgE-dependent allergies. This view is in accordance with the studies performed in Germany and Switzerland which proved that allergic atopic diseases are less common among children raised on farms and exposed to early contacts with bacterial lipopolysaccharides (endotoxins) [47]. The anti-allergic action of *P. agglomerans* LPS has been confirmed recently by an experimental study by Wakame et al. [48], who demonstrated that the oral administration of IP-PA1 to NC/Nga mice exhibited inhibitory effects on atopic dermatitis symptoms induced by the topical application of the body extract of *Dermatophagoides farinae*, a known mite allergen associated with house dust (Fig. 1). The disease process alleviation has been evidenced by the significantly decreased severity of inflammatory skin lesions and lowered serum levels of the allergic type I disease markers: IgE, periostin, and TARC (thymus and activation-regulated chemokine), indicating a Th2 to Th1 shift. The authors expressed an opinion that IP-PA1 could be effective for the prevention of atopic dermatitis and inhibition of its exacerbation. Thus, the same *P. agglomerans* species which causes occupational, non-atopic allergic diseases in workers exposed to organic dusts [2], may be very efficient in the treatment of atopic, IgE-mediated allergic diseases.
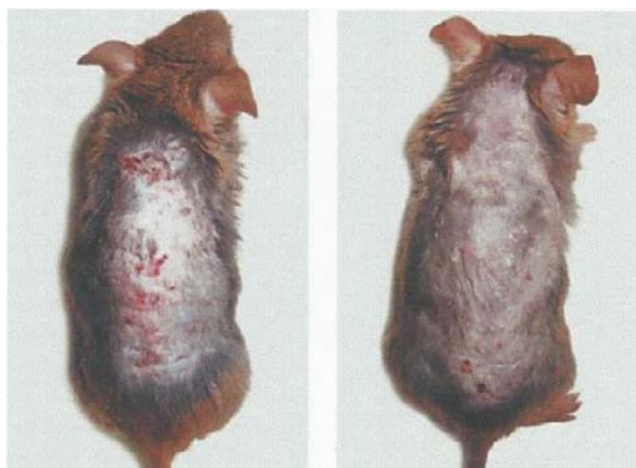

Figure 1. Dorsal skin of mice topically exposed to the extract of *Dermatophagoides farinae* with no treatment (left) and oral treatment with *P. agglomerans* LPS (IP-PA1) (right). The non-treated mice developed atopic dermatitis symptoms (erythema, haemorrhages) which were remarkably reduced by the treatment with IP-PA1. According to Wakame et al. [48], with permission

Analgesic and psychotropic effects. IP-PA1 showed also an analgesic effect in mice, rats and humans [40, 49]. Kamei et al. [50] demonstrated that subcutaneous injection of the *P. agglomerans* LPS (LPSp) suppressed hyperalgesia in streptozotocin-induced diabetic mice, and concluded that LPSp may be effective in relieving the pain associated with diabetic neuropathy. Okutomi et al. [51] and Okutomi and Suzuki [52] reported that LPSp showed a relief effect on the physical dependence of morphine and psychic dependence of cocaine by induction of endogenous opioid, beta-endorphin. The LPSp was more effective than LPS from *E. coli* or *Bordetella pertussis*, and thus was considered to be applicable for clinical use [51]. These results were confirmed by Suzuki et al. [53], who found that LPSp blocks both the reinforcing and the locomotor enhancing effects of cocaine, and therefore might be useful in pharmacotherapy for the prevention of recurrent cocaine abuse.

Prevention and treatment of infectious diseases in humans and animals. Besides the antibiotics produced by *P. agglomerans*, which were characterized in one of the preceding chapters of this article, the bacterium may be efficient in the prevention and treatment of various infectious diseases of humans and animals by other mechanisms, including the protective action of the lipopolysaccharide preparation from *P. agglomerans* (LPSp, IP-PA1) isolated by the Japanese scientists [26, 41]. The protective action is mainly due to the increase of innate immunity by macrophage activation.

Very significant results were recently obtained by Fukasaka et al. [54], who demonstrated that the LPS preparation from *P. agglomerans* (denoted by the authors as LPSpa) caused a strong immune response in mice when given sublingually together with influenza vaccine. LPSpa proved to be a potent adjuvant that induced both systemic (IgG-mediated) and mucosal (IgA-mediated) antibody responses by activating innate immune cells (macrophages and dendritic cells) via a Toll-like receptor 4 (TLR4). This was followed by cytokine production, migration of dendritic cells to cervical lymph nodes and presentation of antigen to T cells, leading to the activation of the adaptive (acquired) immune system. It is noteworthy that LPSpa induced a stronger cytokine response than MPL (Monophosphoryl lipid A), one of the few adjuvants commonly used in human vaccination. The potent immune response resulted in a significant increase in the mice survival rate against lethal influenza virus challenge, compared with subcutaneous vaccination. The authors concluded that sublingual vaccination with LPSpa (as TLR4 ligand) associated with the specific viral antigen might be an ideal vaccination strategy against the influenza virus, and probably also against other viruses.

Feeding animals with food containing IP-PA1 protects them against infection by increasing innate immunity and limits the overuse of antibiotics. Komori et al. [55] have recently shown that oral administration of IP-PA1 to broiler chickens significantly reduced their mortality, induced cytokine expression, and stimulated phagocytic activity and nitric oxide (NO) production by peripheral blood monocytes. According to the authors, all these effects were due to priming of macrophage activation as a result of the innate immunity enhancement by *P. agglomerans* LPS. Hebishima et al. [56] demonstrated that oral pretreatment of chickens with IP-PA1 inhibited the stress-induced immunosuppression,

210 Annals of Agricultural and Environmental Medicine 2016, Vol 23, No 2

Jacek Dutkiewicz, Barbara Mackiewicz, Marta Kinga Lemieszek, Marcin Golec, Janusz Milanowski. *Pantoea agglomerans*: a mysterious bacterium of evil and good. Part IV…

caused by intramuscular administration of dexamethasone, a synthetic stress-eliciting glucocorticoid. The *P. agglomerans* immunopotentiator protected the birds against weight loss, excessive apoptosis of lymphocytes and decrease of antibody response to *Salmonella enteritidis* vaccine and sheep erythrocytes. The authors concluded that IP-PA1 could be useful in controlling immunosuppression in poultry husbandry, which may be caused by overcrowding stress, and could result in an enhanced risk of food-borne salmonellosis. Kohchi et al. [26] reported that mortality among chickens fed with food containing IP-PA1 was significantly lower compared to those which received Calspollin, or Calsporin (a probiotic produced in Japan containing viable spores of *Bacillus subtilis*).

In other studies, Japanese researchers demonstrated in experiments on aquatic animals that feeding with IP-PA1 protected kuruma shrimp (*Penaeus japonicus*) against penaeid acute viraemia (white spot virus), ayu fish (*Plecoglossus altivelis)* against *Pseudomonas* and *Flavobacterium* infections, and carp (*Cyprinus carpio*) against koi herpesvirus [26, 41, 57]. Kadowaki et al. [58] demonstrated that oral administration of IP-PA1 to carp induced activation of M1 type macrophages which are responsible for antibacterial and anti-tumour defence, and caused the significant enhancement of the bactericidal activity of the head kidney leukocytes against a fish pathogen *Aeromonas hydrophila*. Carbajal-González et al. [59] report that *P. agglomerans* occurring in trout as a natural constituent of microbiome, protects the fish against *Saprolegnia parasitica*, the oomycete fungus-like protist which evokes saprolegniosis, a severe disease in trout.

Suzuki et al. [60] demonstrated that an oral administration of a small amount of the lipopolysaccharide from wheat flour (LPSw), equal to *P. agglomerans* LPS (LPSp), significantly decreased mouse mortality and prevented animal weight loss in acute infection with *Toxoplasma gondii*, a deadly sporozoan parasite. A similar effect was achieved by the administration of LPS from *Bordetella pertussis*, whereas LPS from *Escherichia coli* and synthetic lipid A did not show a significant decrease in mortality. Wang et al. [61] proved that LPSp can act as the adjuvant of the hepatitis B virus (HBV) antigen, and effectively increased the production of anti-HBV antibodies in the mice immunized by the HBV antigen.

Mushtaq et al. [62] have recently demonstrated that *Pantoea* could be included in probiotics, as a *Pantoea* sp. strain isolated from human gut was found to produce colicin that inhibited the growth of *Escherichia coli* O157:H7, which is a known enteric pathogen. The *P. agglomerans* endophytic strains isolated by Rafat et al. [63] from *Centella asiatica*, an important Asian medicinal plant, inhibited the growth of the pathogenic bacterium *Pseudomonas aeruginosa*, but did not affect the growth of *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus*.

Other healing properties. Okutomi et al. [64] found in experiments on rabbits that the serum levels of cholesterol and apolipoprotein B (a constituent of low-density lipoprotein, described as LDL or 'bad' cholesterol) decreased with uptake of IP-PA1 contained in water. Conversely, the level of high-density lipoprotein (described as HDL or 'good' cholesterol) did not change. Iguchi et al. [65] showed that the intradermal administration of IP-PA1 had a suppressive effect on diabetes progression in mice. More recently, Nakata et al. [66] demonstrated that a combination of soy milk and

P. agglomerans LPS (LPSp) given orally to premenopausal women may be useful for osteoporosis prevention.

Apart from curative effects, IP-PA1 could be efficiently used for various preventive purposes, e.g. as a constituent of skin-care cosmetics and health foods to prevent and improve metabolic syndromes [26, 41]. It was also proved that IP-PA1 applied as an additive to human skincare cream was effective for moisture retention and curing of burns, pressure sores and atopic dermatitis [26]. Suzuki et al. [67] evidenced the enhancement effect of IP-PA1 on hen egg-laying and the breaking strength of eggshells.

Pantoea agglomerans was among 9 bacterial species which have been recently isolated by Nicoletti et al. [68] from spring water samples collected in Comano, Italy. The Italian researchers hypothesize that these bacterial isolates may contribute to the regenerative and wound healing properties of Comano spring water [68].

Fighting of diseases transmitted by arthropod vectors and arthropod pests with use of paratrangenesis

Common occurrence in arthropods. *Pantoea agglomerans* strains are widespread, usually as symbionts, among arthropods which form the largest phylum in the animal kingdom. As indicated in the preceding article [3], they occur mostly in insects, but were isolated also from species belonging to other arthropod taxa. This article also reviewed examples of insects exerting deleterious effects as vectors of plant diseases [69, 70].

Combating malaria using paratransgenesis strategy. In the light of the recent publications, combating malaria appears to be the most promising field for the efficient use of engineered *P. agglomerans* strains. *Pantoea* was identified as the most prevalent symbiotic bacterium living in the mid-gut of anopheline mosquitoes [71, 72, 73] which transmit *Plasmodium* protozoans causing malaria, one of the most lethal infectious diseases in the world, with about 300–500 million cases and 1.2 million deaths annually [73]. The common occurrence of *Pantoea* symbionts in malaria vectors allows for the development of a strategy termed paratransgenesis in which the symbiont is engineered to express and secrete anti-*Plasmodium* effector proteins in mosquito mid-gut. The final step in the strategy is to introduce these transgenic symbionts into the vector populations in the wild [72, 73]. The use of this strategy by Wang et al. [73] appeared successful: the engineered *P. agglomerans* strains inhibited development of the human malaria parasite *Plasmodium falciparum* and rodent malaria parasite *Plasmodium berghei* by up to 98%, which creates new prospects for combating the deadly disease.

Combating other diseases or pests with the use of paratransgenesis. *Pantoea agglomerans* also occurs commonly in the gut of sand flies *Lutzomyia longipalpis* and *Phlebotomus papatasi* which are vectors of leishmaniasis, a disease caused by the protozoan parasites belonging to *Leishmania* genus [74, 75]. Paratransgenesis with the use of *P. agglomerans* is therefore also considered in the case of this vector-borne disease.

The common occurrence of *P. agglomerans* as symbionts inside arthropods or inside plants affected by arthropod

Annals of Agricultural and Environmental Medicine 2016, Vol 23, No 2

211

Jacek Dutkiewicz, Barbara Mackiewicz, Marta Kinga Lemieszek, Marcin Golec, Janusz Milanowski. *Pantoea agglomerans*: a mysterious bacterium of evil and good. Part IV…

pests, also creates promising possibilities for combating arthropod pests or pathogens transmitted by arthropod vectors with the use of paratransgenesis, as genetically modified symbiotic strains of *Pantoea* have a much greater chance to develop inside arthropods than the exogenous strains of bacteria routinely used to kill the pest. Thus, Quecine et al. [76] recently demonstrated that an endophytic *P. agglomerans* 33.1 strain, identified earlier as a promising sugarcane growth promoter and living in the same niche as the larvae of the sugarcane borer *Diatraea saccharalis* (Lepidoptera: Pyralidae), proved to be efficient as a biocontrol agent of this insect pest after genetic modification causing expressing of the insecticide Cry protein. Paratransgenesis appears also as a promising strategy in the control of such insect pests as locusts (*Schistocerca gregaria*) [77] or blueberry maggot fly (*Rhagoletis mendax*) [78] in which *P. agglomerans* commonly exists in the gut as a symbiont.

A field application of paratransgenesis requests a safe delivery of transformed microbes into the natural environment, addressing appropriate regulations. Major progress in this area has been recently achieved by Arora et al. [79], who successfully used a biopolymer (calcium-alginate) microencapsulation system for the containment and safe introduction of genetically engineered *P. agglomerans* E325 strain to the gut of glassy-winged sharpshooter (*Homalodisca vitripennis*), a leafhopper pest transmitting the plant pathogenic bacteria *Xylella fastidiosa*. This strategy decreases the risk of environmental contamination, horizontal gene transfer, and competition with native species by acting as a barrier between recombinant bacteria and the environment, thereby opening a real perspective for the application of paratransgenesis with the use of *P. agglomerans* for control of vector-borne diseases such as malaria and leishmaniasis.

An effective weapon against pathogens and pests of cultivable plants

Combating bacterial pathogens. *Pantoea agglomerans* has been identified as an antagonist of many plant pathogens belonging to bacteria and fungi, which is associated with the production of antibiotics or other mechanisms. This enables its use as a biocontrol, and being a healthy and environmental-friendly procedure, permits the decrease in pesticide doses, or even avoid the use of chemicals [80]. Best known is its use with commercially available strains E325, P10c, C9–1 (the latter recently classified as *Pantoea vagans*) for control of the bacterial plant pathogen *Erwinia amylovora* that causes fire blight, a devastating disease of apples and pears [18, 81]. The effectiveness of *P. agglomerans* in treatment of this disease is primarily due to the above-mentioned production of antibiotics [13, 14, 15, 18, 81], some of which could target amino acid biosynthesis in the pathogen [10], and secondly to the competition in which *P. agglomerans* is more successful than the pathogen in colonizing flower stigmas of apple and pear trees [81].

Pantoea agglomerans is also effective in the protection of vegetables. Strains of this bacterium isolated from potato tubers in Canada were found to inhibit the growth of *Peptobacterium atrosepticum*, the causative agent of potato blackleg [82]. Hsieh et al. [83] proved that seed treatment with *P. agglomerans* may be an effective and practical method for the control of bean wilt caused by *Curtobacterium*

flaccumfaciens pv. *flaccumfaciens*. Sadik et al. [84] reported that the *P. agglomerans* 2066–7 strain was the most effective biocontrol agent against onion bacterial diseases caused by *Pseudomonas marginalis*, *Pantoea ananatis*, *Pseudomonas viridiflava* and *Xanthomonas retroflexus* in Morocco.

P. agglomerans is also efficient for reduction of bacterial diseases of monocotyledon crops, such as a serious blight caused by *Xanthomonas oryzae* pv. *oryzae* in rice [85]. Braun-Kiewnick et al. [86] demonstrated that strains of *P. agglomerans* suppressed the development of basal kernel blight of barley caused by *Pseudomonas syringae* pv. *syringae*, when applied to heads prior to pathogen. On the other hand, Pasichnyk et al. [87] showed that *P. agglomerans* competes effectively with *Pseudomonas syringae* pv. *atrofaciens*, the agent of basal bacteriosis of wheat, by quicker propagation and inhibition of the pathogen's growth.

Besides antibiosis and competition, the other mechanisms of the antagonistic properties of *P. agglomerans* are less well known and are often related to the induction of plant resistance. Han et al. [88] showed that *P. agglomerans* was effective in inducing systemic resistance in radish to bacterial leaf spot caused by *Xanthomonas campestris* pv. *armoraciae*. Ortmann and Moerschbacher [89] and Ortmann et al. [90] demonstrated that exopolysaccharides of this bacterium induce the disease resistance of monocotyledon plant species, such as wheat and rice, by priming their cells for potentiation of the defence response elicited by contact with the pathogen (or a corresponding substance) which could manifest by the generation of H_2O_2 defined as an 'oxidative burst'.

Combating fungal pathogens. *Pantoea agglomerans* has also shown an inhibitory activity against a number of important fungal plant pathogens, including: • *Fusarium culmorum*, a soil-borne fungal pathogen that causes seedling and head blight, and foot and root rot of wheat, and *Puccinia recondita* f. sp. *tritici* causing brown rust of wheat [11]; • phytopathogenic and mycotoxin-producing strains *Fusarium avenaceum*, *F. oxysporum,* and *F. gibbosum* (teleomorph: *Gibberella intricans*) [91]; • *Fusarium graminearum* [teleomorph: *Gibberella zeae*] reported as a cause of *Fusarium* head blight or scab, responsible for major yield and grain quality losses in wheat, maize, barley, and oats – known also as a myxotoxin producer (Fig. 2) [92]; • *Gibberella pulicaris* (anamorph: *Fusarium sambucinum*), an ascomycete causing dry rot of potatoes [93]; • *Magnaporthe grisea* (anamorph: *Pyricularia grisea*), a rice blast fungus causing a serious disease of rice, and *Cladosporium cucumerinum*, a fungal pathogen causing scab of cucurbits, a disease of cucumber, cantaloupe, honeydew, summer and winter squash, pumpkin, and gourds [94]; • *Rhizoctonia solani* (teleomorph: *Thanatephorus* spp.),
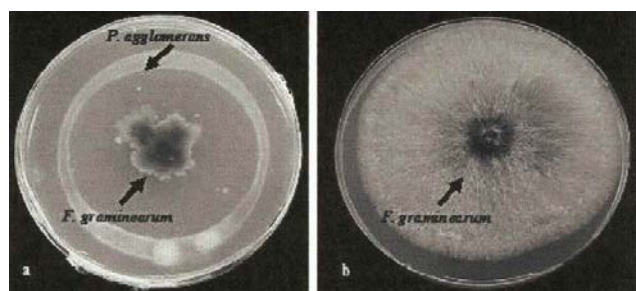

Figure 2. *Pantoea agglomerans* inhibited the growth of *Fusarium graminearum* (left) while on the medium containing only *F. graminearum* (right) the abundant growth of the fungus is seen. According to Pandolfi et al. [92], with permission

212   Annals of Agricultural and Environmental Medicine 2016, Vol 23, No 2

Jacek Dutkiewicz, Barbara Mackiewicz, Marta Kinga Lemieszek, Marcin Golec, Janusz Milanowski. *Pantoea agglomerans*: a mysterious bacterium of evil and good. Part IV…

a plant pathogenic fungus with a wide host range [95]; • *Exserohilum turcicum* (synonym: *Helminthosporium turcicum*) causing the northern leaf blight of maize [96]; • *Macrophomina phaseolina*, a causal agent of charcoal rot of soybean [97]; • *Colletotrichum musae* and *Lasiodiplodia* (*Botryodiplodia*) *theobromae*, fungal pathogens causing crown rot of bananas [98]. Three *P. agglomerans* strains isolated from the rhizosphere of grape bushes in Uzbekistan showed a strong anti-fungal activity due to production of chitinolytic enzymes [99]. The stimulation of plant resistance was also considered as a mechanism that protects against fungal pathogens. Verhagen et al. [100] demonstrated that *P. agglomerans* was capable of inducing oxidative burst and phytoalexin production in grapevine, leading to differential local and systemic resistance to a fungal pathogen *Botrytis cinerea* causing grey mould disease. Similarly, Zhang et al. [101] evidenced that an application of *Pantoea agglomerans* to cucumbers significantly reduced, by inducing systemic acquired resistance, the severity of anthracnose, a disease caused by a pathogenic fungus *Colletotrichum orbiculare*. Vanneste et al. [102] showed that *Pantoea agglomerans* enhanced the resistance in kiwi fruit and tobacco, thus protecting these plants from a disease called white mould, caused by an ascomycete, *Sclerotinia sclerotiorum*.

Very good results were obtained by the use of *P. agglomerans* for the protection of stored fruits against fungal pathogens. It has been shown that the *Pantoea agglomerans* CPA-1 and CPA-2 strains provided excellent control against fungi of the species *Botrytis cinerea*, *Penicillium expansum* that *Rhizopus stolonifer*, causing post-harvest diseases of pome fruits (pear and apple), and inhibited the accumulation of patulin, a mycotoxin produced by *P. expansum* that affects human health [80, 103, 104]. The CPA-2 strain also significantly reduced the incidence of *Penicillium digitatum* (green mould) and *Penicillium italicum* (blue mould) on stored citrus fruits (oranges, mandarins and lemons) [105, 106]. *P. agglomerans* strain EPS125 also efficiently protected stone fruits (apricot, peach and nectarine) against brown rot caused by *Monilinia laxa* and soft rot caused by *Rhizopus stolonifer* [107], while 4 *P. agglomerans* strains isolated from pome fruits in Turkey efficiently inhibited the growth of *Aspergillus flavus* on stored lemons [108]. Zhang et al. [109] recently reported that the *P. agglomerans* XM2 strain isolated from prunes in China was effective in post-harvest control of black spot disease caused by the fungus *Alternaria alternata* on pears.

Combating other pathogens and pests. Bardin et al. [110] reported the *Pantoea agglomerans* 2–2 strain as one of 6 bacterial strains which proved to be effective seed-treatment agents for the control of damping-off of canola, safflower, dry pea, and sugar beet in fields naturally infested by *Pythium* sp. (an oomycete protist with fungal-like features), a causative agent of the disease. Munif et al. [111] reported that the *P. agglomerans* MK-29 strain was among 4 strains of endophytic Gram-negative bacteria isolated from tomato which significantly reduced the infestation of root-knot nematode *Meloidogyne incognita* on tomato when applied as a seed treatment, root dipping, or as a soil drench. This suggests a potential for the efficient use of bacterial biocontrol agents instead of anti-nematode pesticides which exhibit high toxicity and negative impact on the environment.

Pantoea agglomerans has also been shown to be pathogenic to couch grass (*Elytrigia* (=*Elymus*) *repens*), which creates the possibility to use it as biocontrol agent against this widespread plant pest [112].

Growth promotion of cultivable plants

Growth promotion by *P. agglomerans* strains associated with rhizosphere. *Pantoea agglomerans* strains occurring in rhizosphere (root-soil interface) of cultivable plants may promote the growth of these plants by various mechanisms. Son et al. in Korea [113], Malboobi et al. in Iran [114, 115] and Sulbarán et al. in Venezuela [116] demonstrated that *P. agglomerans* strains isolated from soil exerted a beneficial effect for crops by solubilization of otherwise insoluble inorganic phosphates. Malboobi et al. [114, 115] demonstrated in both the greenhouse and in field trials that *P. agglomerans* was very efficient in the promotion of potato growth, mostly if applied in combination with the other phosphate-solubilizing strains belonging to the species *Microbacterium laevaniformans* and *Pseudomonas putida*. The authors expressed an opinion that these isolates are superior candidates for biofertilizers. Lakshmanan et al. [117] proved that *Pantoea* sp. strain EA106, a natural rice rhizospheric isolate, abates arsenic uptake in rice by a high siderophore (iron binding) activity, resulting in increasing Fe plaque formation on rice roots. This promotes the rice growth and prevents accumulation of toxic arsenic in the plant tissues. Khalimi et al. [118] demonstrated that treatment of rice seeds with the bacterial suspension of 2 P. *agglomerans* strains isolated from rhizosphere of a groundnut, significantly increased the plant growth and yield of rice in Bali, Indonesia. Amellal et al. [119] observed that the *P. agglomerans* NAS206 strain isolated from the rhizosphere of wheat in Morocco secreted an exopolysaccharide which had a positive effect on rhizosphere soil aggregation and plant growth. Two strains exhibiting potent useful properties have been isolated from rhizosphere in India: the *P. agglomerans* NBRISRM strain that enhanced the macronutrient uptake by maize and chickpea and promoted the growth of these plants [120], and the *Pantoea* sp. NII-186 strain, which showed multiple plant growth-promoting attributes, such as phosphate solubilization activity, as well as indole acetic acid (IAA), siderophore, and HCN production [121]. Additionally, the latter strain showed significant growth inhibitory activity against phytopathogenic fungi, and extremely wide tolerance to different environmental conditions, such as temperature, salt concentration and pH range, which makes it an ideal candidate for a bioinoculant replacing chemical fertilizers [121]. Similar properties to the NII-186 strain revealed the plant-growth-promoting *P. agglomerans* diazotrophic (N_2-fixing) lma2 strain, isolated from the wheat rhizosphere of an arid region in Algeria, recommended it as a potentially good fertilizer in arid and saline zone [122]. Earlier, the *P. agglomerans* diazotrophic strains detected in the rhizosphere of wheat and barley in Germany had been indicated as potential bio-fertilizers [123].

Pantoea agglomerans and closely related *Pantoea* species produce phytase, an enzyme degrading phytate [124, 125, 126, 127]. Phytate, the salt form of a saturated cyclic acid called phytic acid, occurs commonly in plants and soil, making up to 90% of the natural phosphorus in the world [126]. However, only ruminant animals decompose phytate, whereas other animals and plants do not. Thus, phytase

Annals of Agricultural and Environmental Medicine 2016, Vol 23, No 2

213

Jacek Dutkiewicz, Barbara Mackiewicz, Marta Kinga Lemieszek, Marcin Golec, Janusz Milanowski. *Pantoea agglomerans*: a mysterious bacterium of evil and good. Part IV…

enzymes produced by *P. agglomerans* and other bacterial species occurring in rhizosphere and other soil layers are crucial for making this rich phosphorus source available to plants and the majority of animals. In this context, the studies by Suleimanova et al. [126, 127] are very significant. They isolated from a sample of forest soil collected in the Republic of Tatarstan, Russia, a *Pantoea* sp. 3.5.1. strain exhibiting a very high activity of phytase encoded by the *agpP* gene [127]. The strain is closely related to *P. agglomerans* but probably represents a new species [126]. The authors sequenced the full genome of this strain [126] and proposed its potential use as an environmentally friendly biofertilizer, as a feed supplement for livestock production, or as a raw material for the industrial production of microbial phytase, which by now is mostly produced from the *Aspergillus* strains [127].

Growth promotion by the endophytic *P. agglomerans* strains. The endophytic *P. agglomerans* strains developing inside plant tissues may promote plant growth mainly by the atmospheric nitrogen (N_2) fixation ability, production of phytohormones, and disease control [128]. Scholz-Seidel and Ruppel [129] demonstrated that the *P. agglomerans* strain isolated from phyllosphere (air-surface interface of plant leaves) of wheat in Germany showed the presence of nitrogenase (N_2-fixing enzyme) and phytohormone activities that promote plant growth and increase the root length and yield of winter wheat. Inoculation experiments with diazotrophic *P. agglomerans* strains on wheat and barley in temperate regions have demonstrated the possibility of increasing yield up to 500 kg/ha [130]. A growth-promoting effect of inoculation with *P. agglomerans* on wheat was confirmed by Remus et al. [131], who evidenced that inoculating seeds and shoots with this bacterium increased the yield of wheat grain up to 23.5%, and by Egamberdiyeva and Höflich [132], who noted the growth promotion of winter wheat.

Verma et al. [133] demonstrated that *P. agglomerans* strains isolated from deepwater rice in India appear to be very competent plant growth-promoting endophytes, which are equipped with the ability to fix atmospheric nitrogen, produce indole acetic acid (IAA) and mineralize insoluble phosphates. They produce pectinases and cellulases enabling penetration and spread inside rice and providing biologically fixed nitrogen to the distant parts of plant. Similarly, Feng et al. [128] evidenced that the *P. agglomerans* YS19 diazotrophic strain identified in China as rice endophyte, produced phytohormones, enhanced transportation of the photosynthetic assimilation products and promoted host plant growth showing a potential for increasing rice production in field application. Miao et al. [134] demonstrated a strong adsorption of the *P. agglomerans* YS19 cells by rice root hairs indicating a close interrelation between *P. agglomerans* and host plant. Further studies on the properties of the YS19 strain showed the presence of the defined in preceding article [3, 7] quorum sensing (QS) system. In this case, QS was proved to regulate, by means of acyl-homoserine lactone (AHL) diffusible signal molecule (identified as N-3-oxooctanoyl-L-homoserine lactone (OOHL)), such life functions of bacteria as forming of cell aggregates, called symplasmata, and interrelations between bacterium and host, including the colonization of plant tissues and promotion of rice growth [9]. The potential beneficial effects of *P. agglomerans* on rice growth has been confirmed recently by Banik et al.

[135], who identified the presence of 2 *Pantoea* spp. strains among 11 diazotrophic bacterial isolates from rice plants in India, which possessed the *nifH* (nitrogen fixation) gene and promoted the growth of rice seedlings. All these findings are very significant because rice is the staple food of 50% of world's population and 80% the population of Asia [135].

A similar *Pantoea* sp. 9C diazotrophic strain with an extremely high nitrogenase activity was identified in Cuba as an endophyte of sugarcane [136]. Similar to an Indian NII-186 strain [121], the sugarcane strain revealed an exceptionally wide tolerance to environmental conditions and has been proposed as a candidate for an environment-friendly biofertilizer [136]. Gond et al. [137] recently demonstrated that *P. agglomerans* isolated from teosinte (an ancestor of corn) induces salt tolerance in tropical corn, which creates the chance to increase its cultivation area and yield on saline soils (Fig. 3). Quecine et al. [138] showed that the *P. agglomerans* endophytic 33.1 strain, originally isolated from *Eucalyptus grandis*, was able to cross-colonize and promote the growth of sugarcane. It also induced the synthesis of chitinase and endoglucanase enzymes, which are associated with plant protection against pathogens. These results suggest that this strain could be used as an inoculant to improve sugarcane productivity. Andreote et al. [139], Ferreira et al. [140] and De Lima Procopio et al. [141], have proposed using the endophytic, plasmid-harbouring *P. agglomerans* strains abundantly colonizing seedlings of citrus plants and eucalyptuses, as vehicles for the introduction of exogenous genes promoting growth of these cultivars.
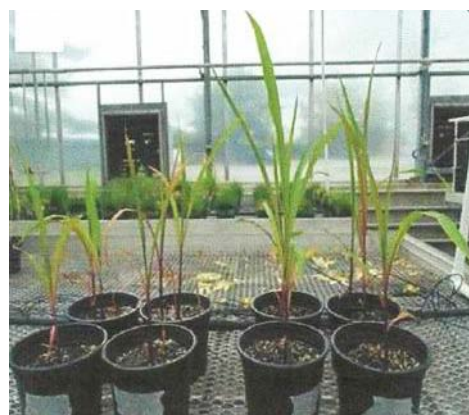

Figure 3. Induction of salt tolerance by *Pantoea agglomerans*. The tropical corn seedlings treated with *P. agglomerans* suspension and grown in saline soil in a greenhouse (right) showed a significant growth enhancement compared to untreated plants (left). According to Gond et al. [137], with permission

Asis and Adachi [142] isolated from the stem of sweet potato in Japan, a diazotrophic *P. agglomerans* strain, with a potential to increase nitrogen uptake by this important Japanese crop plant. Jasim et al. [143] have recently demonstrated that the strain determined as *Pantoea* sp., isolated from the cardamom (*Elettaria cardamomum*) fruit tissue in India, showed the ability to produce ACC (1-aminocyclopropane-1-carboxylate) deaminase which regulates the ethylene level in plant tissues and thereby promotes plant growth. De Almeida Lopes et al. [144] have evidenced that *Pantoea agglomerans* and *Variovorax paradoxus* strains showed the highest efficiency in IAA production and phosphate solubilization among all bacterial endophytic strains isolated from soybean

214   Annals of Agricultural and Environmental Medicine 2016, Vol 23, No 2

Jacek Dutkiewicz, Barbara Mackiewicz, Marta Kinga Lemieszek, Marcin Golec, Janusz Milanowski. *Pantoea agglomerans*: a mysterious bacterium of evil and good. Part IV…

in Brazil, thus revealing the potential to promote the growth of this important cultivable plant. Dursun et al. [145] stated that a foliar spray with a suspension of *P. agglomerans* FF strain significantly increased the yield, growth and mineral contents of tomato and cucumber fruits in Turkey.

Efficient bioremediation

Strong biodegradation activity. Recent reports indicate that the bacteria of the *Pantoea* genus reveal a great potential for bioremediation, e.g. a waste management technique that involves the use of organisms to remove or neutralize chemical pollutants from the natural environment. It has been documented that the *Pantoea* strains show biodegradation activity on various substrates with aromatic rings, such as phenol [146], alkylbenzosulfonate and sodium dodecyl sulphate surfactants [147], 2,4,6-trinitrotoluene [148], tannic acid [149], and the herbicide mesotrione [150, 151]. Jacobucci et al. [152] found that a biosurfactant produced by *P. agglomerans* was efficient in the bioremediation of the oily effluent released by the margarine and soap industry, as shown by the reduction of the COD (Chemical Oxygen Demand) index by 70%.

Biodegradation of rice straw. The most interesting approach to this issue has been presented by Xiong et al. [151], who found that the rice endophytic strain *Pantoea* sp. Sd-1, closely related to *P. agglomerans*, showed an exceptional ability to degrade rice straw and lignin (Fig. 4). This creates an
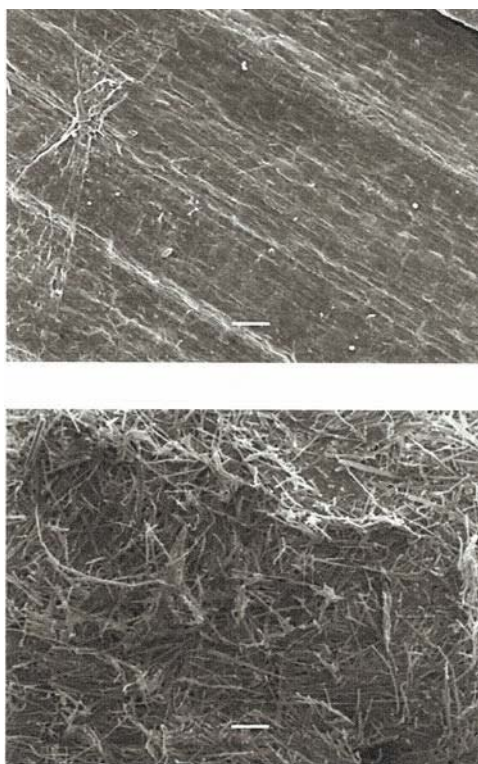

Figure 4. Degradation of rice straw with *P. agglomerans* strain Sd-1, causing changes in its surface morphology recorded by scanning electron microscopy (SEM). The untreated rice straw (upper photo) has a smooth surface and its fragments closely connected, while the surface structure of rice straw treated with *P. agglomerans* (lower photo) is rough, and the fragments broken into very loose, small pieces. According to Xiong et al. [151], with permission

encouraging perspective for utilizing rice straw that could be used in industry for the production of bioethanol and paper, or as a renewable energy source. At present, however, the durability of lignin is the major obstacle to the utilization of rice straw and the reason for large-scale deposition of this abundant agricultural by-product which poses a considerable threat to the natural environment. The application of *Pantoea* for biodegradation of rice straw and other materials containing lignocellulose biomass may efficiently solve this important problem.

Degradation of hydrocarbons. *Pantoea* has also been identified as a member of microbial communities used for the biological degradation of polycyclic aromatic hydrocarbons, produced from the incomplete combustion of organic materials and fossil fuels in various industrial activities, and regarded as environmental pollutants of particular concern [153]. Vasileva-Tonkova and Gesheva [154] found that the *Pantoea* sp. A-13 strain isolated from soil in Antarctica produced a potent glycolipid biosurfactant which could be used for the degradation of petroleum hydrocarbons and environmental bioremediation. The research performed by a group of scientists from Pakistan and Austria has documented the outstanding potential of *Pantoea agglomerans* strains for the degradation of petroleum hydrocarbons in spilled soil [155, 156, 157, 158]. Yousaf et al. [155] proved a very high efficiency of hydrocarbon degradation by the *P. agglomerans* BTRH79 and ITSI10 plant isolates in combination with plants: Italian ryegrass (*Lolium multiflorum* var. Taurus) and bird's-foot trefoil (*Lotus corniculatus* var. Leo). The high performance of the *P. agglomerans* BTRH79 strain in hydrocarbon removal was related to the presence of alkane hydroxylase CYP153 gene with a substrate range between C_4–C_{16} [155]. Further studies by the Pakistani-Austrian group [156, 157, 158] confirmed this performance, either for the BTRH79 strain alone or in combination with the other *P. agglomerans*, *Pseudomonas* sp., and *Burkholderia* sp. inoculant strains. Based on these studies, the group proposed an efficient phytoremediation system for the reduction of hydrocarbon contamination in which the inoculation of soil with pollutant-degrading bacteria was combined with the planting of proper vegetation, and amendment of soil with nutrients, such as compost or fertilizers containing nitrogen, phosphorus and potassium. In this system of a plant-bacterium partnership, inoculated bacteria promote plant growth by both detoxification of the environment and the ACC deaminase activity, receiving in turn habitat and nutrients [157]. *P. agglomerans* is also an important member of the microbiota found in most biological degreasing systems used for degradation and removal of oil, grease, or lubricants from the metal parts of machines or installations, as an environment-friendly alternative to hazardous chemical solvents [159].

Reduction of toxic metals and metalloids. The facultative anaerobic *Pantoea agglomerans* strains show a potential to detoxify the environment through the oxidation of acetate or H_2 to dissimilatory reduction of metals, such as Fe(III), Mn(IV) and the highly toxic Cr(VI) [160, 161]. These results correspond with a study performed by Ozdemir et al. [162], who demonstrated that the *Pantoea* sp. TEM18 copper tolerant strain, isolated from activated sludge of a petrochemical industry wastewater treatment plant, showed

Annals of Agricultural and Environmental Medicine 2016, Vol 23, No 2

Jacek Dutkiewicz, Barbara Mackiewicz, Marta Kinga Lemieszek, Marcin Golec, Janusz Milanowski. *Pantoea agglomerans*: a mysterious bacterium of evil and good. Part IV…

215

a high adsorption capacity for heavy metal ions Cr(VI), Cd(II) and Cu(II), creating a promising perspective for the effective treatment of wastewater polluted with toxic metals. *P. agglomerans* was also among 7 strains which were proved by Zaets et al. [163] to be effective in the protection of soybean plants against accumulation of toxic cadmium present in soil. Similarly, Pishchik et al. [164] demonstrated that inoculation of *P. agglomerans* into soil highly contaminated with toxic cadmium and lead resulted in a decrease of their content by 2–3 times and 4–5 times, respectively, and furthermore, caused a significant increase of the oats yield which exceeded even the yield noted in soils not contaminated with heavy metals. According to Tian et al. [165], the *Pantoea* sp. IMH strain carrying the *ars*C gene and highly resistant to heavy metals, was effective in the aerobic reduction of arsenic(V) present in the solid waste released by a copper-smelting plant.

Prevention of penetration of toxic contaminants into soil. Besides significant chemical effects, *Pantoea agglomerans* may prevent penetration of harmful industrial contaminants into deeper parts of soil on a physicochemical basis. Kurlanda-Witek et al. [166] have experimentally demonstrated that even a thin layer of a biofilm formed by *P. agglomerans* can hinder the migration of zinc oxide nanoparticles downwards porous media. The authors postulated that such biofilms could be used as a potential remediation strategy against the migration of nanoparticle contaminants in heterogeneous aquifers [166].

Waste recycling associated with energy acquisition. The strains of *P. agglomerans* could also be useful in the acquisition of energy from various alternative sources, such as waste recycling. An example is the salt-tolerant *P. agglomerans* BH-18 strain isolated by Zhu et al. [167] from mangrove sludge, which possesses the ability to produce hydrogen. The authors proposed using this strain for the biological treatment of marine aquaculture wastewater and marine organic waste, associated with the production of biohydrogen that represents a promising alternative source of energy due to its reproducibility, non-polluting nature, and high energy yield. In a subsequent study, the authors demonstrated that the yield of hydrogen could be significantly greater (by 36.94%) if the *P. agglomerans* BH-18 strain is used in a mixed culture with a *Candida tropicalis* BH-6 salt-tolerant strain, which had been isolated from the same mangrove ecosystem [168].

Conclusion. In the light of the above-cited reports, *P. agglomerans* appears to be a valuable bioremediator that could be efficiently used for the removal of different pollutants from the living environment, in some cases even for the acquisition of an alternative source of cheap energy.

Production of the cryoprotective protein, other potential applications

Production of the cryoprotective protein. In the preceding article [3] there was described the ice nucleation activity of *P. agglomerans* causing damage to plants, which also has some positive implications. The bacterium protects itself against self-induced decrease of temperature by the production of an anti-freeze cryoprotective protein. Koda et al. [169] have

isolated from the cell mass of *P. agglomerans* IFO12686 ice nucleating strain a protein exhibiting extremely high cryoprotective activity that could be used for the protection of plants and/or food products. This was a novel monomer, heat-stable protein with a molecular mass of 29,000, protecting the freeze-labile enzymes against freezing-thawing denaturation.
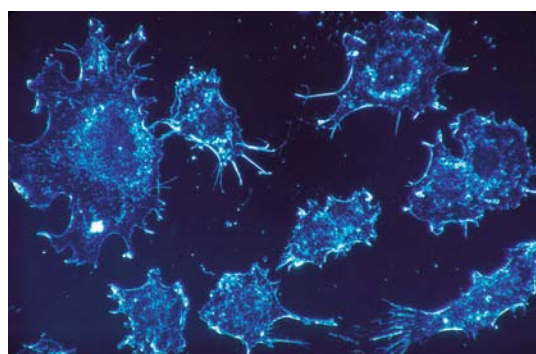
Production of a potential bio-indicator. Another potential application of *Pantoea agglomerans* was indicated by Fujikawa and Akimoto [170], who isolated a unique strain of this bacterium producing a deep blue pigment. The production of the pigment was temperature-dependant and observed between 10–30ºC. Most probably it was regulated by the above-described quorum sensing system. The authors determined the optimal conditions for the pigment production and suggested a potential use of the strain as a biological time-temperature indicator (TTI) for the management of foods and storage of clinical materials at low temperatures [170].

## Genetic variability and problems related to the European Union Directive

Phylogenetic analysis performed by Delétoile et al. [171] revealed that the *Pantoea* genus is highly diverse, and this versatility was recently confirmed by Walterson and Stavrinides [6]. According to De Maayer et al. [172], this is associated with the acquisition and maintenance of plasmids, in particular the Large *Pantoea* Plasmid (LPP-1), described as 'an important driver of the biological, ecological and lifestyle diversification observed among the *Pantoea* species'. Because of the large variability of *Pantoea* isolates, it is assumed that they cannot be accurately assigned to species based on metabolic profiling [173, 174]. Nevertheless, the results obtained by Delétoile et al. [171], Völksch et al. [4], and Nadarasah and Stavrinides [5], suggested that clinical and plant-associated *P. agglomerans* strains do not form distinct populations, and might possess indistinguishable virulence potential. Rezzonico et al. [173, 174] expressed an opinion that the use of *P. agglomerans* to combat fire blight should not be hampered by the European Union ban that was imposed because of the classification of this bacterium as a biosafety level 2 organism. This classification was based on reports of cases of opportunistic infections which, according to the cited authors, are doubtful because of the misidentification of causative strains. Although the authors are absolutely right concerning the nonsense of this ban, the essentials of the matter seem rather different.

Firstly, the strains of *P. agglomerans* and related species should, without any doubt, be classified as biosafety level 2 organisms, essentially not because of accidental infections, but because of the important occupational hazard due to potential inhalation exposure to strong endotoxins and allergens produced by *Pantoea* strains (most probably also by those used as biocontrol agents), particularly in agricultural occupations. Although the current European Directive 2000/54/EC [175] on work-related risks addresses not 'infectious' but 'biological agents at work' (which must be understood as infectious and/or allergenic and/or toxic agents of biological origin), nevertheless, the content of this Directive does not adhere to the promise implicit in title. Regrettably, the content and attached list of harmful biological agents are outdated and confined only to infectious agents, and do not meet the requirements of contemporary science [176].

216   Annals of Agricultural and Environmental Medicine 2016, Vol 23, No 2

Jacek Dutkiewicz, Barbara Mackiewicz, Marta Kinga Lemieszek, Marcin Golec, Janusz Milanowski. *Pantoea agglomerans*: a mysterious bacterium of evil and good. Part IV…

Pantoea agglomerans: benefits for humans, animals and plants

Curing tumors and other diseases
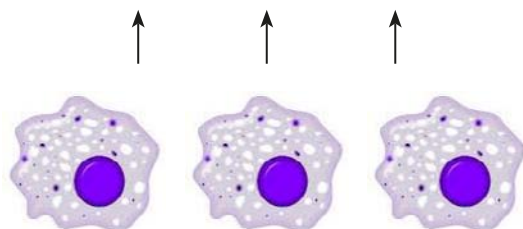
Macrophage activation by
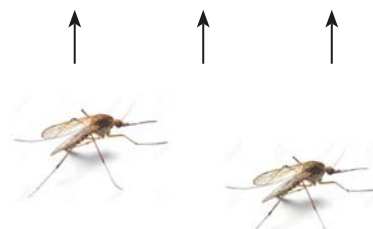*P. agglomerans* LPS
Maintenance of homeostasis

Killing malaria parasites in mosquito gut

Paratransgenesis: introduction
of transformed *P. agglomerans*
bacteria into wild populations
of *Anopheles* mosquitoes

Combating plant pathogens

Promotion of the growth of wheat, rice
and other crops

Production of antibiotics, successful
competition, stimulation of systemic
resistance of plants. As a result:
reduction of the use of agrochemicals.

Promotion of plant growth by: fixation of
atmospheric nitrogen, making available
soil phosphorus by phytate degradation
and phosphate solubilization, production
of phytohormones, indole acetic acid and
enzymes, induction of salt tolerance.

Figure 5. *Pantoea agglomerans* benefits for humans, animals and plants. Photo depicting malaria oocysts on stomach of mosquito according to London School of Hygiene/Science Photo Library, www.malariasite.com.

Annals of Agricultural and Environmental Medicine 2016, Vol 23, No 2

217

Jacek Dutkiewicz, Barbara Mackiewicz, Marta Kinga Lemieszek, Marcin Golec, Janusz Milanowski. *Pantoea agglomerans*: a mysterious bacterium of evil and good. Part IV…

Pantoea agglomerans: benefits for environment – bioremediation

Degradation of various substances with aromatic rings: polycyclic petroleum hydrocarbons, phenol, 2,4,6-trinitrotoluen
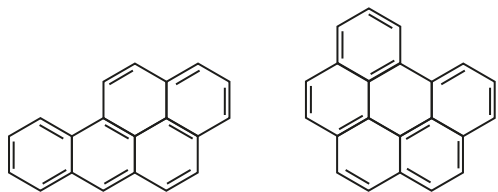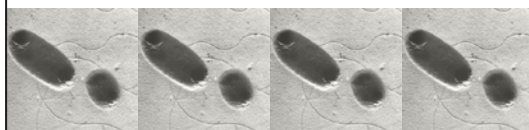
Reduction of toxic metals and metalloids

Cr(VI)	Fe(III)	Mn(IV)
Cd(II)	Cu(II)	Pb(II)
Co(II)	Ni(II)	Pd(II)
Zn(II)	Hg(II)	As(V)

Biofilm formed by *P. agglomerans* can hinder migration of toxic metal and metalloids nanoparticles downwards soil.

Cr(VI)   Cd(II)   Zn (II)   As(V)

Degradation of waste of plant origin: exceptional ability for the degradation of rice straw and lignin with a potential for use in industry or as a renewable energy source.
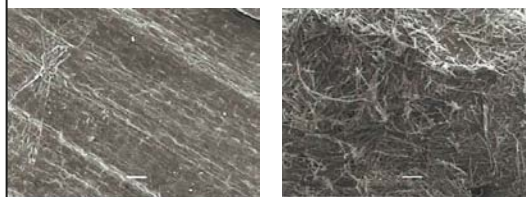
Figure 6. *Pantoea agglomerans* benefits for the environment. Photo of degradation of rice straw created on the basis of Figure 4, according to Xiong et al. (151).
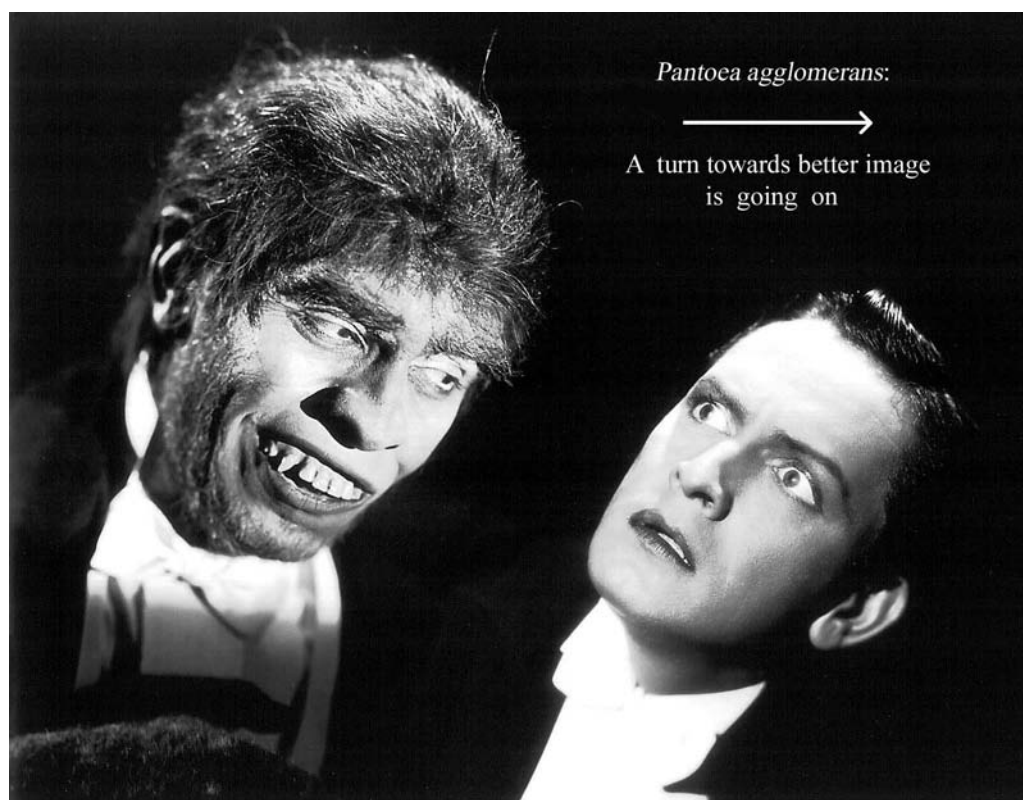

Figure 7. Dr. Jekyll & Mr. Hyde. Photo from the 1920 silent film classic, as an analogy to the *Pantoea agglomerans* story. A turn towards a better image of this bacterium is progressing.

218

Annals of Agricultural and Environmental Medicine 2016, Vol 23, No 2

Jacek Dutkiewicz, Barbara Mackiewicz, Marta Kinga Lemieszek, Marcin Golec, Janusz Milanowski. *Pantoea agglomerans*: a mysterious bacterium of evil and good. Part IV…

Secondly, the classification of *Pantoea* as a biosafety level 2 organism (incidentally, the obsolete classification using 1–4 levels should be replaced by one that is more accurate and logical, using 0–5 levels) should not lead to restrictions on its use, not only as a biocontrol anti-fire blight agent, but also as an anti-cancer supportive drug, an efficient plant growth promoter, a biofertilizer or bioremediator. Quite simply, the outdated regulations should be changed. The new legislation should authorize the use of *Pantoea* biopreparations under the following conditions:

1) If possible, the *Pantoea* biopreparations should not be used in the form of an aerosol; however, if so used, the workers handling these preparations must be protected by efficient respirators or masks preventing the respiration of toxic and/or allergenic nanoparticles.
2) The workers using the biopreparations must use gloves as protection from accidental skin perforation and infection.
3) The immunocompromised persons must not be engaged in the application of the *Pantoea* biopreparations.

   In conclusion:
1. *Pantoea agglomerans* should be classified as a biosafety level 2 organism.
2. Its use for biocontrol and other purposes should be permitted if safety precautions are provided.

Concluding remarks: The Strange Case of Dr. Jekyll and Mr. Hyde in the Microcosmos

The novel by the Scottish writer Robert Louis Stevenson (1850–1894) entitled *The Strange Case of Dr. Jekyll and Mr. Hyde* [177] in which Dr. Jekyll, a kind and intelligent physician, turns into Mr. Hyde, an abominable monster, is a one of the best known stories about dual-personality. Although in the world of microorganisms one cannot speak about 'personality' the story of *Pantoea agglomerans* has some analogies with the Jekyll/Hyde case. In our story, an abominable bacterium causing occupational diseases due to the inhalation of organic dusts and opportunistic infections, appears as a beneficial agent protecting human health by the efficient treatment of tumours and other diseases, as well as by combating vector-borne diseases, increasing food supply by plant growth promotion and protection from pest and pathogens, and assuring a clean, human-friendly environment, by effective biodegradation (Figs. 5–6). Who – Jekyll or Hyde – will be a winner in the fascinating *Pantoea agglomerans* story? The problem is not an easy one to solve, but in our understanding the answer will be: Jekyll! This turn of *Pantoea agglomerans* towards a better image (Fig. 7) is not a result of the positive evolution of this bacterium which cannot proceed in such a short time. Instead, it could be possible due, on one hand, to the fast progress in biotechnology, and on the other, on the increase in occupational safety. Although the strains of many other bacterial species could be more efficient in some positive traits exhibited by *P. agglomerans* (for example, nitrogen fixation or metal reduction), the unique, truly exceptional ubiquitous occurrence of this bacterium associated with an extremely strong metabolism and easy adaptation to changing environmental conditions, speaks for its broad use in biotechnology, which may contribute to the improvement of our health and quality of life, hopefully in the not too distant future.

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Annals of Agricultural and Environmental Medicine 2016, Vol 23, No 2

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219

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220 Annals of Agricultural and Environmental Medicine 2016, Vol 23, No 2

Jacek Dutkiewicz, Barbara Mackiewicz, Marta Kinga Lemieszek, Marcin Golec, Janusz Milanowski. *Pantoea agglomerans*: a mysterious bacterium of evil and good. Part IV…

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Annals of Agricultural and Environmental Medicine 2016, Vol 23, No 2

Jacek Dutkiewicz, Barbara Mackiewicz, Marta Kinga Lemieszek, Marcin Golec, Janusz Milanowski. *Pantoea agglomerans*: a mysterious bacterium of evil and good. Part IV…

221

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222   Annals of Agricultural and Environmental Medicine 2016, Vol 23, No 2

Jacek Dutkiewicz, Barbara Mackiewicz, Marta Kinga Lemieszek, Marcin Golec, Janusz Milanowski. *Pantoea agglomerans*: a mysterious bacterium of evil and good. Part IV…

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