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Risk of dissemination of *Clavibacter michiganensis* ssp. *sepedonicus* with potato waste

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Abstract To estimate the risk of dissemination of *Clavibacter michiganensis* ssp. *sepedonicus* through potato residues from processing industries, the various processes and the usage of residues from plants from different processing branches were analysed with regard to the effect they can have on the pathogen. The residues were classified into different risk categories, from category 0 (no risk of dissemination) to category 4 (high risk of dissemination). Residues not heated during processing and used in agriculture, e.g., as fertilizer, were pooled in the highest risk category 4. Residues that were sanitised before use in agriculture, e.g., by composting or pasteurisation, were still classified as probably high risk (risk category 3), as no information on these treatments concerning the inactivation of the pathogen was available so far. Therefore the effect of composting and pasteurisation under varying conditions was tested on samples (ready-made compost mould) contaminated with *Clavibacter michiganensis* ssp. *sepedonicus*. Viable bacteria could be extracted after all experiments via bioassay on eggplants, and cultivated on semi-selective media from plant sap forming characteristic colonies. The viable pathogen could be extracted after composting for 6 days at maximum temperatures at 70 °C, 13 days at 55 °C and 90 min pasteurisation at 70 °C. It can be concluded that these sanitation treatments are not sufficient to inactivate *Clavibacter michiganensis* ssp. *sepedonicus* and the previous classification of treated residues in category 3 (probably high risk) could thus be confirmed.

Keywords Bacterial ring rot · Composting · Pasteurisation · Potato residues · Quarantine pest · Sanitation

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Introduction

Clavibacter michiganensis ssp. *sepedonicus* (Spieckermann and Kotthoff 1914) Davis et al. 1984 is the causal agent for bacterial ring rot on potato. It was first reported by Appel (1906) and described in 1914 by Spieckermann and Kotthoff (1914). *C. m.* ssp. *sepedonicus* is a Gram-positive coryneform bacterium with club shaped cells (Stead and Müller 2006) which is prevalent in cool northern climates. The pathogen is listed in Annex IAI of the Council Directive 2000/29/EC as a quarantine pest for the European Union (Anonymous 2000). Control measures and diagnostic procedures are described in the Council Directive 93/85/EEC (Anonymous 1993). Even though the pest is known to occur in many countries, it is still absent in some European countries and can cause

economic damage if introduced into these areas. *C. m.* ssp. *sepedonicus* causes wilting of plants and rot of tubers in the field and in storage. The infection can be easily masked by other diseases or natural senescence of the leaves and thus remain unnoticed (van der Wolf et al. 2005). In Germany it was first detected in 1906 (Appel 1906) and is still present in single cases. Dissemination is mostly by latently infected seed potatoes. Contaminated surfaces of machineries, lorries, boxes, tools etc. are known as other relevant ways for transmission of the pathogen to healthy tubers (Abdel-Kader et al. 2004). Furthermore, it has been shown that the pathogen can also survive for some days in water and therefore be spread when just recently contaminated water is used for irrigation (van der Wolf and Beckhoven 2004). Long term survival of *C. m.* ssp. *sepedonicus* is also possible in soil and in potato volunteers (Kaemmerer et al. 2007; Pankova et al. 2007; van der Wolf et al. 2005). Bröther (2003) detected the pest in potato residues from a starch plant after processing of infected potatoes, including washing water, potato liquids, soil and pulp. If such infested residues are used on arable land, e.g., for irrigation or filling of hollows, they can also present a possibility for dissemination of *C. m.* ssp. *sepedonicus*. The technical bulletin Merkblatt DWA-M 753, Abwasser aus der Kartoffelverarbeitung (Anonymous 2005) from the German Association for Water, Wastewater and Waste (DWA) recommends not to use residues from potato processing on agricultural land even if they had been sanitised. Sanitation of organic waste is prescribed in the German Ordinance on the Utilisation of Biowastes on Land used for Agricultural,. Silvicultural and Horticultural Purposes (Biowaste Ordinance) (Anonymous 1998), prescribing composting, pasteurisation and anaerobic digestion as sufficient sanitation measures. The parameters given in the Biowaste Ordinance are a minimum of two weeks at 55 °C, six days at 60 °C or three days at 65 °C for composting and one h at 70 °C for pasteurisation. Experience with *Synchytrium endobioticum*, another pathogen of potatoes, showed that the robust resting spores were not inactivated by composting or pasteurisation under the parameters given in the Biowaste Ordinance (Steinmöller et al. 2012).

To estimate the risk that comes from potato residues from processing industries, the various processes and usage of residues from plants from different processing branches were analysed with regard to the effect they can have on the pathogen. Subsequently, the effect of composting and pasteurisation on *C. m.* ssp. *sepedonicus* was tested to verify if the parameters given in the German Biowaste Ordinance are sufficient to inactivate this persistent pest in potato residues.

Methods and material

Analysis of potato industry processes and usage of residues

Plants from each potato processing branch were selected for the analysis, including the production of starch, chips, French fries and potato flakes. All steps and processes were recorded during interviews or via questionnaires, from the delivery of the potatoes to the use of the residues, including relevant technical data, e.g., possible heat treatment or the use of chemicals. The accumulating residues were registered, together with their usual treatment and disposal (Fig. 1). All processes were assessed with regard to their effect on the pathogen (possible inactivation of the bacteria cells); residues were additionally sorted according to their usage. Subsequently the residues were classed in five risk categories from category 0 (no risk of dissemination of *C. michiganensis* ssp. *sepedonicus*) to category 4 (high risk of dissemination of *C. michiganensis* ssp. *sepedonicus*).

Investigation on the effect of composting and pasteurisation on *Clavibacter michiganensis* ssp. *sepedonicus*

The classification of potato residues which were sanitised, e.g. composted or pasteurised before use in agriculture, in a higher risk category was based on the fact that no information on the effect of such treatments on *Clavibacter michiganensis* ssp. *sepedonicus* was available. Therefore composting and pasteurisation were tested to examine the survival or inactivation of *C. m.* ssp. *sepedonicus* during these processes and to verify or disprove the risk classification in the analysis.

Pathogen introduction

For composting and pasteurisation ready-made compost mould was contaminated with *C. m.* ssp. *sepedonicus* NCPPB 2140^{strep1} in a concentration of 10^6 cfu/g substrate to provide a determined initial concentration of bacteria

[1] Provided by the National Collection of Plant Pathogenic Bacteria in York

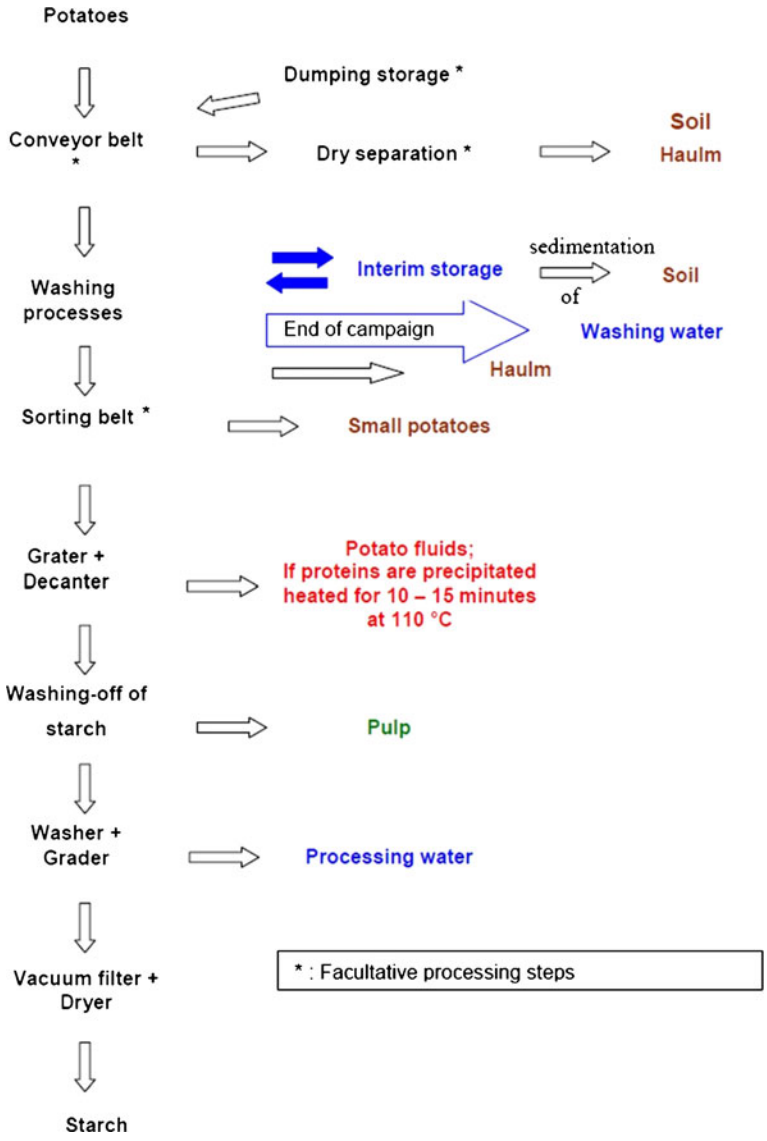

Fig. 1 Schematic process diagram of starch production with regard to accumulating main potato residues

cells in the sample material prior to the treatment. In addition for some pasteurisation experiments, pulp (a by-product from starch industries) was contaminated with *C. m.* ssp. *sepedonicus* NCPPB 2140^{strep1} in a concentration of 10^6 cfu/g substrate. The mutant NCPPB 2140^{strep} is similar to the wild type but has a higher tolerance to the antibiotic streptomycin, which allows the use of streptomycin in the agar to inhibit the growth of saprophytic bacteria on the agar plate. For contamination, bacteria from a pure culture NCPPB 2140^{strep} were plated on yeast dextrose agar and cultivated for seven days. Colonies were then ablated from the agar and suspended in 0.01 M phosphate buffer. The suspension was adjusted to a dilution of 10^9 cfu/ml buffer with a photometer and then further diluted with 0,01 M phosphate buffer to a concentration of 10^7 cfu/ml buffer. The garden mould or the pulp were filled in 2-l beakers and contaminated with the bacterial suspension in a ratio of 10:1 to attain a bacterial concentration of the substrate of 10^6 cfu/g substrate. The contaminated substrate was shaken for 30 min at 280 rpm on a shaker, and then left resting for a day at room temperature. The substrate was again stirred by hand prior to use.

As *C. m.* ssp. *sepedonicus* is a quarantine pest which is subject to specific safety precautions, a carrier system was developed consisting of plastic tubes (10 ml) with bottoms and tops closed with poly-tetrafluor-ethylene membrane with a pore size of 0.2 μm (Fig. 2). The carriers allowed easy introduction into the process and a secure extraction

Fig. 2 Carriers for introduction of composting substrate contaminated with *C. m.* ssp. *sepedonicus* into sanitation processes

of the pathogens from the substrate while allowing for percolation of fluids and the warming of the contents to the same temperatures as the surrounding substrate. Temperature probes were placed in and around carriers during a composting and a pasteurisation process in advance of the sanitation experiments to confirm that the substrate in the carriers warmed up to the same temperatures as the surrounding substrate with a maximum delay of a few minutes. After the composting process, the substrate in the carriers was analysed and compared to the surrounding substrate by the Brandenburg Technical University Cottbus,[2] to prove that the consistence of the resulting compost in and outside the carriers was comparable.

Sanitation experiments

Composting was conducted in 60-l composters under conditions comparable to a commercial composting facility. Fresh garden compost was mixed with potato residues and other organic waste in a ratio 1:1 (pH 7.0) as a composting substrate. Composting process was optimised in advance by the Brandenburg Technical University Cottbus[2], preparation and mixture of the substrate, oxygen capacity and moisture of substrate during the process were adjusted to ensure high temperatures for a sufficient time and to ensure that the end product was meeting the standard on compost material.

For the sanitisation experiments, 27 carriers with contaminated sample material were used in each experiment and placed radically around the middle in three layers (bottom, middle, top) in the composting substrate. Composting durations were 12 (Experiment 1a and 2a) and 21 days (Experiment 1b and 2b). The experiments were set up to test, if the conditions described in the German Biowaste Ordinance of composting with maximum temperatures at 55 °C for 14 days, 60 °C for six days and 65 °C for three days were sufficient to inactivate *C. m.* ssp. *sepedonicus*. To control the temperature development, temperature probes were placed in any layer in the middle of the composter and in three positions near the carriers. Temperatures were measured every 15 min during the whole process.

The effect of pasteurisation was tested in a water bath. Pulp was used as conductor for the heat in the beakers, as pre-tests showed that it reaches the temperatures of the water bath with a delay of 15–20 min. For 18 beakers, sample material was filled in carriers which were placed in the pulp. Another nine beakers were filled directly with contaminated pulp in a concentration of 10^6 cfu/ml as a control, to ensure that carriers do not modify the effect of pasteurisation. Nine beakers each (six with carriers, three filled with contaminated pulp) were treated at 70 °C for 60, 90 and 120 min. Considering the delay in warming-up of the pulp and the samples, the real pasteurisation time correlated to 30, 60 and 90 min at 70 °C.

Pathogen extraction and test of viability

A bioassay with the eggplant 'Black Beauty' was performed to extract the pathogen from the substrate. Compost is a complex substrate that does not allow for direct dilution plating of the samples after composting without risking overgrowing of *C. m.* ssp. *sepedonicus* by fungi or saprophytic bacteria. The bioassay with eggplants is an approved method for extraction of the pathogen from complex organic substrates according to directive 2006/56/EC (Anonymous 2006). The suitability of this method for potato residues and other compost was tested in advance. Compost was inoculated with a defined dilution series of *C. m.* ssp. *sepedonicus* NCPPB 2140^{strep} in the concentrations 10^6, 10^4, 10^3 and 10^2 cfu/g substrate and inoculated in eggplant 'Black Beauty'. The bioassay and the following examinations were conducted as described below. Even though bioassay plants showed no typical symptoms, *C. m.* ssp. *sepedonicus* could be isolated from the bioassay plants on semi-selective media and confirmed with immunofluorescence test at all dilution factors. Hence, the detection limit was defined at 10^2 cfu/g substrate.

[2] Chair in Waste Management

After all experiments, three carriers each from a layer were combined in a composite sample. Ten grams of the substrate were mixed with 5 g gravel, 40 ml 0.1 % sodium phosphate buffer and 10 μl Tween 80 and shaken at room temperature for 30 min at 250 rpm. After suspending for 30 min, the samples were centrifuged at 350 × g and 8 °C for 10 min in order to remove soil and other organic particles. The supernatant was decanted in a fresh tube and centrifuged at 10,000 × g and 8 °C for another 10 min. The pellet was resuspended with 1,000 μl 0.01 M phosphate buffer and used for further tests.

Ten eggplants per sample were inoculated directly above the cotyledons shortly before three leaf stages. As positive control, five plants were inoculated with a pure culture of *C. m.* ssp. *sepedonicus* NCPPB 2140^{strep} in the concentration of 10^8 cfu/ml 0.01 M phosphate buffer and two plants were inoculated with 0.01 M phosphate buffer as negative control. Plants were cultivated at 21 °C and 16 h daylight for four weeks. After seven days all plants were controlled visually twice a week for characteristic symptoms on the plants induced by the multiplication of *C. m.* ssp. *sepedonicus*.

After cultivation, 1 cm of the stems was cut off shortly above the inoculation point and further used in composite samples. Stem pieces were disinfected for 1 min in 70 % alcohol and subsequently washed twice with sterile distilled water. Pieces were prepared according to the protocol given in 2006/56/EC (Anonymous 2006). A dilution series was plated on the semi selective medium NCP88^{strep}. Plates were incubated at 21 °C for five days and controlled for morphologically characteristic colonies with a stereo microscope on a regular basis. Simultaneously, an immunofluorescence test (IF-test) was performed with the extracted samples, according to Janse and Van Varenbergh (1987) and the protocol given in 2006/56/EC (Anonymous 2006). A polyclonal antibody from goat against *C. m.* ssp. *sepedonicus* was used for the IF-test [Loewe], diluted 1:10.000 in phosphate buffered saline (PBS) and the conjugate was Anti-Goat lgG FITC Conjugate [Sigma] diluted 1:200 in PBS. Test slides were examined on an epifluorescence microscope with filters suitable for excitation of FITC under oil immersion at a magnification of 1000.

Presumptive *C. m.* ssp. *sepedonicus* colonies isolated from the semi-selective medium were identified using IF-test and simultaneously checked with a polymerase chain reaction according to the protocol given in 2006/56/EC (Anonymous 2006). A pathogenicity test on eggplants was performed to confirm the pathogenicity and to fulfill the whole diagnostic according to the Koch's postulates.

Results

Analysis of potato industry processes and usage of residues

Data from different potato processing plants were recorded for all steps of the process, from arrival of the potatoes at the plant, processing procedures and residues/waste management. Type and usage of the residues varied between the different processing branches, but particular residues accumulated after most processes. This includes soil, roots, haulms and small potatoes as well as potato liquids (liquids from potatoes that are left over after lacerating the potato cells for starch extraction), washing and processing water. Other residues were specific, as pulp (potato remnants after starch extraction) which is a by-product of the starch industry, or as distiller's wash (organic residues of distillation) that occurs only in breweries. Potato peelings and potato parts (potatoes that are too small or are damaged) result from the production of non-perishable potato products as potato flakes for mashed potatoes and frozen products such as French fries. Some plants disposed of all residues outside agriculture, e.g., by waste combustion, but some residues were generally used in agriculture. A distinction could be made between direct utilisation, e.g., as fertilizer, for filling-in of dips or as cattle food, and indirect utilisation, e. g. after composting, pasteurisation or anaerobe fermentation or as sewage sludge from in-house wastewater systems.

For the estimation of the dissemination risk of *C. m.* ssp. *sepedonicus*, the residues were divided in five categories (Table 1). Category 0 (no risk) was only given to residues which were not utilised in agriculture but on building sites or similar or were destroyed completely. Category 1 (presumably no risk) and 2 (low risk) were only given to residues which were treated with heat during the production process (steam peeling, boiling or cooking) or which were utilised on permanent grassland or in a similar way where the chance of contact with potatoes is low. The indirect utilisation of unheated residues on arable land (e.g. as compost) was still categorised with probable high risk (category 3), as no information on the effects of treatments such as composting or fertilisation on the quarantine pests of

Table 1 Division of residues accumulating after potato processing in categories according to the risk of dissemination of *C. m.* ssp. *sepedonicus*

Risk category	Risk description	Residues
0—No risk	Residues are used on building sites or similar or were destroyed (e.g., waste combustion) ⇒ no contact with agricultural land	• Stones • Roots, haulms, small potatoes • Soil
1—Presumably no risk	Pathogen presumably inactivated through treatment of process, e.g., due to high temperatures, e.g., Steam peeling Blanching/Boiling Fermentation/Distillation	• Potato peelings • Potato parts • Distiller's wash
2—Low risk	Pathogen dissemination not likely due to utilisation, e.g., use on permanent grassland	• Pulp • Potato peelings • Potato parts • Distiller's wash • Potato liquids • Processing water • Washing water
3—Probably high risk	Use of residues in agriculture after sanitation treatment, but pathogen inactivation through treatment not verified, e.g., composting, pasteurisation or anaerobe fermentation	• Roots, haulms, small potatoes • Soil • Potato peelings • Potato parts • Potato liquids[a] • Processing water[a] • Washing water
4—High risk	Residues were not heated or sanitised or elsewhere treated in a way that could inactive the pathogen and used in agriculture, e.g., as fertilizer, for irrigation or filling-in of dips	• Roots, haulms, small potatoes • Soil • Potato peelings • Potato parts • Potato liquids[a] • Processing water[a] • Washing water

[a]If no heat treatment occurred during processing as protein precipitation or boiling etc.

potato were available. Residues from processes without any heating that were used untreated on arable land were categorised as highest risk for dissemination of *C. m.* ssp. *sepedonicus* (category 4). This includes mainly adherent soil, roots, small or damaged potatoes, pulp (from starch production) and washing waters. Potato liquids were only categorised with high risk, if no protein denaturation (heating for 10–15 min at 110 °C) was executed.

Sanitation experiments

During the composting experiments, significant variations in temperature progression could be observed not only between the experiments, but also between the three experimental layers in each experiment (see Fig. 3). In all experiments the highest temperatures could be observed in the middle layer. In experiment 1a (composting for

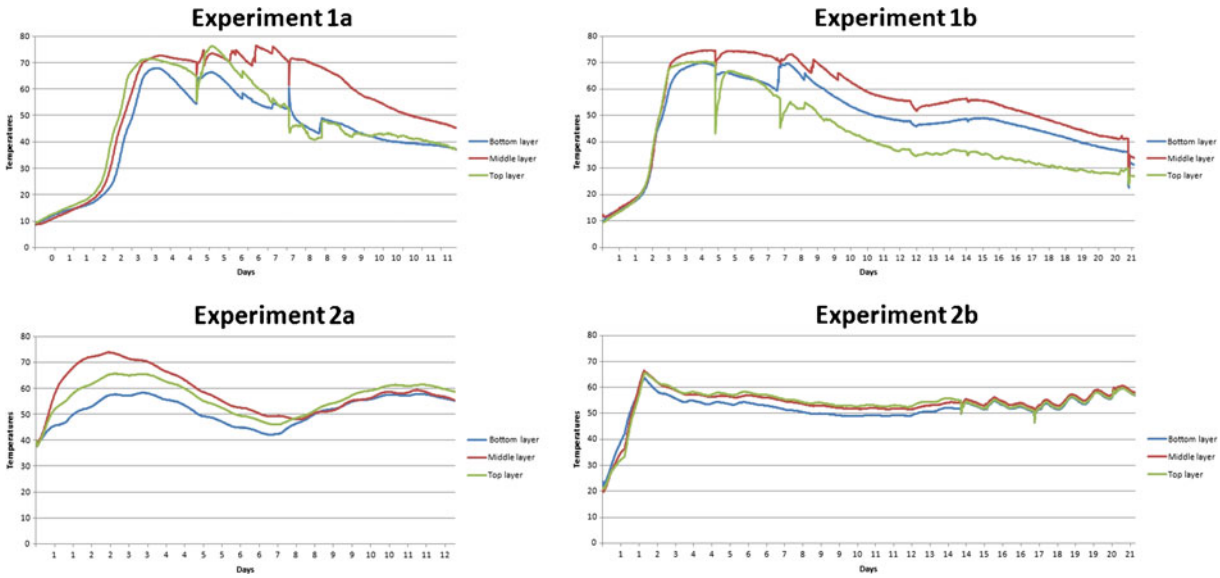

Fig. 3 Temperature progression during the composting experiments 1a (12 d), 1b (21 d), 2a (12 d) and 2b (21 d) with regard to the three experimental layers where carriers with *C. m.* ssp. *sepedonicus* were placed in the composter

12 days) temperatures went up steadily in the first two days and then alternated between 60 and 70 °C for three to four days. In the bottom and the top layers, temperatures then decreased to under 50 °C for the rest of the composting process. Temperatures in the middle layer stayed above 70 °C for two more days, before decreasing slowly. In experiment 1b (composting for 21 days) temperatures went above 60 °C for four (top layer), six bottom layer) and seven days (middle layer) before decreasing slowly. In experiment 2a (composting for 12 days) temperatures went up to 60 and 70 °C only for 2 days before decreasing under 50 °C, but increased again near the end of the composting process to 60 °C for 2 days. In experiment 2b (composting for 21 days) temperatures were most similar in the three layers, so temperatures peaked at 60 °C only for a day, then decreased to temperatures between 50 and 55 °C for nearly 20 days, before rising to 60 °C at day 21.

The slightly cooler temperatures in the bottom layer and the top layer in each experiment may be due to oxygen supply at the bottom of the composters and to stacking of the substrate at the top. The temperature variation between the experiments might be due to variation in surrounding climate, as all experiments were set up identically. Concerning the parameters of the German Biowaste Ordinance that were tested here, temperatures of 55 °C for 14 days were reached in experiment 2b and temperatures of 65 °C for 3 days were reached in all other experiments. Temperatures of 60 °C for 6 days could only be reached in experiment 1b and in the top layer of experiment 1a.

C. m. ssp. *sepedonicus* could not be inactivated by composting, independent of composting duration and maximum temperatures. Eggplants inoculated with composted substrates containing *C. m.* ssp. *sepedonicus* displayed no typical symptoms of an infection with *C. m.* ssp. *sepedonicus* during the testing duration of 4 weeks, while eggplants in the positive control inoculated with a pure culture of *C. m.* ssp. *sepedonicus* displayed typical symptoms. However, after dilution plating of plant sap from bioassay plants on semi-selective media, presumptive colonies were identified as *C. m.* ssp. *sepedonicus* with the immunofluorescence test and polymerase chain reaction. During the pathogenicity test, these colonies induced the typical symptoms of yellowing of intercostals and wilting of leaf edges on the eggplants in about two weeks after inoculation.

Although no inactivation of *C. m.* ssp. *sepedonicus* cells was achieved by the composting experiments, an influence of higher composting temperatures could be observed in experiment 1b. An immunofluorescence test on plant sap from bioassay after composting for

21 days displayed a noticeable reduction in the number of bacteria cells in the middle layer, where temperatures exceeded 70 °C for 6 days. In the bottom and in the top layer, where temperatures stayed below 70 °C during that period, considerably more bacteria cells could be observed in the immunofluorescence test after the bioassay (see Fig. 4). This effect was even more demonstrable after dilution plating of *C. m.* ssp. *sepedonicus* cells from plant sap from bioassay after the same experiment (see Fig. 5). Again, the amount of bacteria grown on the NCP88^{strep} medium was less in samples from the middle layer (temperatures above 70 °C for six days) compared to the samples from the bottom and the top levels (temperatures below 70 °C). This result points to an increasing disintegration of the bacteria cells during the composting process with increasing temperatures. The exact reduction rate of *C. m.* ssp. *sepedonicus* cells through the composting process could not be quantified, as the multiplication rate of the bacteria in the bioassay plants was not known.

Pasteurisation experiments gave similar results to composting and viable *C. m.* ssp. *sepedonicus* cells were extracted from the samples via bioassay on eggplants, plated on semi-selective media and identified with immunofluorescence tests and the polymerase chain reaction. In the immunofluorescence test and after dilution plating on NCP88^{strep} medium, no difference in the quantity of bacteria cells or colonies could be observed, independently of whether contaminated garden-mould was introduced via carriers or contaminated pulp was filled directly in the beakers for the experiments. Again bioassay plants did not express typical symptoms in all experiments, but colonies cultivated from plant sap on semi-selective media induced typical symptoms in the pathogenicity test of yellowing of intercostals and wilting of leaf edges.

The experimental parameters and the results from all sanitation experiments are summarised in Table 2. A statistical analysis of the data was not made as no quantitative determination of viable or inactivated bacteria cells was possible due to the uncontrolled multiplication of viable bacteria cells in the bioassay plants. Experiments were only rated on a clear binary basis (yes/no reaction).

Discussion

The experiments described here clearly assist the classification of potato residues that were sanitised by composting or pasteurisation in risk category 3 (probably high risk) in the analysis, as *C. m.* ssp. *sepedonicus* was not inactivated by composting or pasteurisation under the presented conditions. The given parameters in the German Biowaste Ordinance for the sanitation of organic waste (pasteurisation for 60 min at 70 °C and composting either at 55 °C for 14 days, 60 °C for six days or 65 °C for three days), were tested during the experiments and it can be concluded that these given parameters for sanitation of organic waste are not sufficient to eradicate *C. m.* ssp. *sepedonicus*, the causal agent of bacterial ring rot of potato.

By comparing samples from the three experimental layers in experiment 1b where temperatures in the middle layer exceeded 70 °C for 7 days, a clear decrease in the quantity of viable bacteria cells was observed in the immunofluorescence test and with dilution plating on semi-selective media after the bioassay. Samples from the top or the bottom layer where temperatures did not

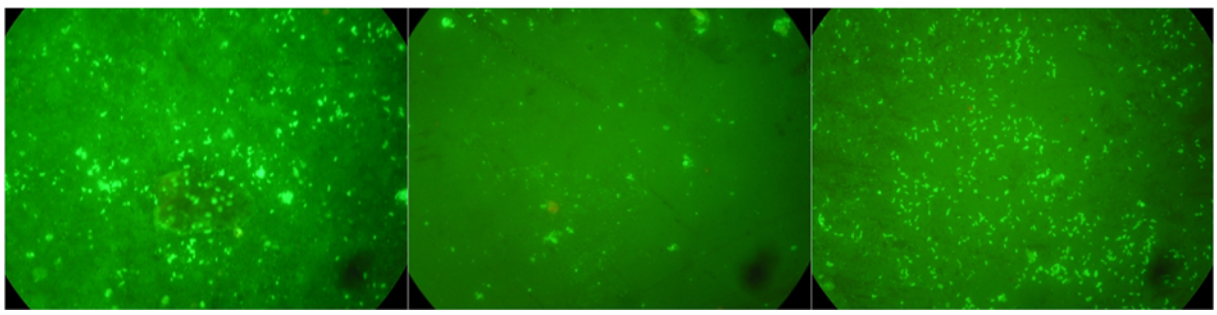

Fig. 4 Immunofluorescence test on samples from the three experimental levels composted for 21 days (experiment 1b) after bioassay displaying only few fluorescent *C. m.* ssp. *sepedonicus* cells in samples from the middle layer (middle picture), pointing to an increased disintegration of bacteria cells after exposure to temperatures above 70 °C for 6 days compared to the bottom layer on the left (65 °C for 6 days) and the top layer on the right (55 °C for 6 days)

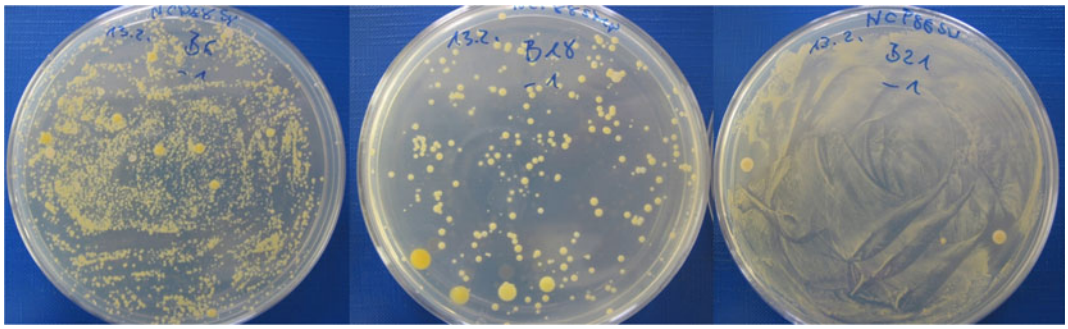

Fig. 5 *C. m.* ssp. *sepedonicus* colonies growing on NCP88^{strep} after a bioassay with samples from the three experimental levels composted for 21 days (experiment 1b) displaying only few colonies in samples from the middle layer (middle picture), pointing to an increased disintegration of bacteria cells after exposure to temperatures above 70 °C for 6 days compared to the bottom layer on the left (65 °C for 6 days) and the top layer on the right (55 °C for 6 days)

exceed 70 °C for the same period showed a much higher density of colonies on the incubated plates and more cells in the immunofluorescence test. Possibly, temperatures around 70 °C for a longer period or higher temperatures above 80 °C might be sufficient to inactivate *C. m.* ssp. *sepedonicus* in potato residues, but this was not investigated here. However, composting is a process that relies on microorganism activity and if temperatures get too high, this can slow down the composting process (de Bertoldi et al. 1983).

Some robust pathogens are known to be difficult to eradicate by composting (Christensen et al. 2002; Downer et al. 2008; Hermann et al. 1994; Hoitink and Fahy 1986; Mikkelsen et al. 2006; Noble and Roberts 2004; Noble et al. 2004), but these results refer mostly to fungi or viral pathogens and not to bacteria. Sometimes pathogens seem to be inactivated after only a few days, while for other experiments a considerably longer time for the eradication has been noted (Noble and Roberts 2004). As well as differences in the inoculated material used for the experiments, this might also be due to difficulties to extract the pathogens successfully from the treated substrates after the experiments. For the validation of eradication measures it is therefore relevant to ensure that each pathogen that had been entered into the sanitation process can be properly extracted again from the treated substrate and that the method used for determining viability of the organisms is sufficiently sensitive and reliable. The introduction of the pathogen via carriers in the experiments described here, not only ensured that quarantine regulations were maintained, but also allowed for the reliable extraction of the pathogens after composting. At the same time, the bacteria were completely exposed to all temperature progressions and other chemical processes which are part of composting. Even so Bollen et al. (1989) stated heat to be the most crucial factor for pathogen inactivation. Also, no difference could be observed between pasteurised samples in carriers and contaminated pulp that was pasteurised without carriers, therefore it can be precluded that the carriers are not the reason why *C. m.* ssp. *sepedonicus* could survive composting under the presented conditions as well as pasteurisation.

Hardly any information on the eradication of *C. m.* ssp. *sepedonicus* by sanitation treatments is available. Fatmi et al. (1991) describe the inactivation of the related pathogen *C. m.* ssp. *michiganensis*, the causal agent of bacterial canker of tomato from naturally infected tomato seeds soaked in water at 52 °C for 20 min. Secor et al. (1988) describe eradication of *C. m.* ssp. *sepedonicus* after 5 min at 82 °C. However, both pathogens have been used as suspensions in these experiments. Secor et al. (1988) observed that bacteria could survive the treatment in hot water for a longer time if organic substances were added. This might be an explanation for the ability of *C. m.* ssp. *sepedonicus* to survive composting and pasteurisation at temperatures of 70 °C in organic substances as garden-mould or pulp. However, the ability of *C. m.* ssp. *sepedonicus* to survive high temperatures of 70 °C and more for a few days in organic material is noticeable. However, investigations on the survival of the bacteria on transport boxes or in soil (Abdel-Kader et al. 2004; van der Wolf et al. 2005) already referred to the hardiness of *C. m.* ssp. *sepedonicus*. Similar results to the ones reported here were achieved recently during experiments on anaerobic digestion of *C. m.* ssp. *sepedonicus* (Liebe et al. 2012).

Table 2 Parameters of sanitation treatments and results from dilution plating, immunofluorescence test from bioassay plant sap on the detection of living cells of *C. m.* ssp. *sepedonicus* and the expression of symptoms in the bioassay and pathogenicity test

Number of experiment	Treatment	Number of introduced samples	Bioassay eggplants displaying typical symptoms	Dilution plating from bioassay plant sap	IF-test from bioassay plant sap	Pathogenicity test from identified Cms-Isolates extracted from bioassay plants, displaying typical symptoms
1a	Composting 12 d Maximum temperatures above 70 °C for 5 days	27	No	Positive	Positive	Yes
1b	Composting 21 d Maximum temperatures above 70 °C for 6 days	27	No	Positive	Positive	Yes
2a	Composting 12 d Maximum temperatures above 65 °C for 4 days	27	No	Positive	Positive	Yes
2b	Composting 21 d Maximum temperatures above 55 °C for 13 days	27	No	Positive	Positive	Yes
–	Pasteurisation 30 min at 70 °C	6 in carriers 3 without carriers	No	Positive	Positive	Yes
–	Pasteurisation 60 min at 70 °C	6 in carriers 3 without carriers	No	Positive	Positive	Yes
–	Pasteurisation 90 min at 70 °C	6 in carriers 3 without carriers	No	Positive	Positive	Yes

It was demonstrated that composting and pasteurisation with the tested parameters given in the German Biowaste Ordinance for the sanitisation of organic waste, were not sufficient to inactivate *C. m.* ssp. *sepedonicus* in an organic matrix. *C. m.* ssp. *sepedonicus* is also known to survive for some time in soil (van der Wolf et al. 2005), in volunteer potatoes (Pankova et al. 2007), and in surface water (van der Wolf and Beckhoven 2004). These aspects confirm the risk of possible dissemination of *C. m.* ssp. *sepedonicus* as they support the survival of the bacteria if composted or pasteurised residues from infested potato lots were used in agriculture. Then again, no soil-borne infection were detected in any potato plant grown on infested plots in experiments in Bavaria (Kaemmerer et al. 2007). However, considering that *C. m.* ssp. *sepedonicus* is a quarantine pest, for which introduction and also spread in the European Union is forbidden according to the directive 2000/29/EC (Anonymous 2000), it has to be stated, that composting and pasteurisation according to the parameters of the German Biowaste Ordinance are not adequate sanitation treatments for residues from potato processing industries, if infested lots were processed. These results underpin the recommendation of the technical bulletin Merkblatt DWA-M 753, Abwasser aus der Kartoffelverarbeitung (Anonymous 2005) from the German Association for Water, Wastewater and Waste (DWA) not to use residues from potato processing on agricultural land even if they had been sanitised according to the German Biowaste Ordinance. Measures for an economic and usable sanitation of potato residues that securely inactivate robust quarantine pests of potato such as *C. m.* ssp. *sepedonicus* still need to be explored.

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