Profile of *Penicillium* species in the pear supply chain

I. Scholtz[a]

[a]*Department of PlantSciences, University of Pretoria, Private bag X20, Hatfield, Pretoria 0028, South Africa*

L. Korsten[a]*

Abstract: Postharvest fruit decay caused by *Penicillium* pathogens is considered to be one of the most important challenges in the pear industry resulting in market-end losses. Moving export fruit through different environments exposes the product to extensive handling, temperature variations and microbes. The profile of *Penicillium* spp. present in the pear export chain from South Africa to the United Kingdom was therefore studied over a four year period. Sampling was done at two packhouse facilities, controlled atmosphere and cold storage areas in South Africa and at two re-pack facilities and cold storages as well as a distribution centre and a retailer in the United Kingdom. Sampling consisted of swabbing walls and floors and using active and passive air sampling. In total 5 056 isolates were obtained, purified and grouped into a total of 282 morphological groups. Of these 350 representative isolates were selected for further identification. The five most dominant species in the pear chain were: *P. glabrum* (23.40%); *P. chrysogenum* (15.13%); *P. crustosum* (14.16%); *P. brevicompactum* (8.96%); *P. expansum* (8.39%), of which the latter three were confirmed pathogenic on pears. This study provides a framework to monitor the inoculum potential in environments that fruit move through while being exported.

Keywords: decay indicators, diversity, fruit handling, market-end losses, postharvest

---

*Corresponding author. E-mail: lise.korsten@up.ac.za

Introduction

Fresh produce supply chains involve complex distribution systems, with fruit being moved over vast distances from the farm to the point of sales. Losses at the end of the chain impact negatively on the profitability of exports. Consequently, sanitation within indoor environments has become progressively more important to prevent buildup of inoculum levels and avoid decay development (Dallaire et al. 2006). Complex fruit trade networks can potentially introduce a wider range of pathogens to the hosts that would otherwise not be encountered in shorter local chains (Louw & Korsten, 2014). Large varieties of fruit from different countries can for example be retained together in storage or holding facilities for extended periods of time. Fresh produce can also be re-packed overseas to remove decaying fruit which could potentially contribute to the microbial load in indoor environments (Louw & Korsten, 2014). In their paper they described pathogenicity trials with selected isolates (*P. expansum, P. crustosum, P. solitum, P. digitatum and P. brevicompactum*) obtained from this pear supply chain study. The environmental isolates were found to be pathogenic. This finding emphasizes the importance of effective management of postharvest pathogens in complex fruit trade distribution networks to reduce losses (Zhang et al. 2011). *Penicillium* spp. are known as one of the major causes of postharvest decay of pear fruit (Mari et al. 2002; Mari et al. 2003). *Penicillium* spp. are best known as pathogens causing green and blue mould of citrus (*P. digitatum, P. italicum*), blue mould on pears and apples (*P. crustosum*, *P. expansum* and *P. solitum*), decay of strawberries and pomegranate (*P. glabrum*) and rot of stored grapes and tomatoes (*P. chrysogenum*) (Pitt, 1991; Filtenborg et al. 1996; Amiri et al. 2005; Barkai-Golan, 2008; Varga et al. 2008; Bardas et al. 2009; Palou et al. 2010; Elhariry et al. 2011). Species of this genus produce high numbers of asexual conidia and are commonly isolated from environments in fresh produce supply chains such as dump-tank water, in flume water, in contaminated wooden bins and in the atmosphere (Amiri & Bompeix, 2005).

Detection and accurate identification of pathogens is one of the first steps in controlling plant diseases (Mansouri et al. 2013). Identification of *Penicillium* spp. has always been considered difficult due to the diversity of the genus (Visagie, 2012). However, recent technological developments in taxonomy and integrated approaches combining phylogenetic, morphological and molecular methods have enabled rapid and more accurate identification of *Penicillium* spp. (Visagie, 2012). Little is known about *Penicillium* spp. and other pathogens present within the pome fruit supply chain that may cause decay at the market-end (Magan and Aldred, 2007). The aim of this study was to identify *Penicillium* spp. present in the pear export chain environment i.e. packhouse facilities, controlled atmosphere and cold storage areas in South Africa, and re-pack facilities as well as distribution centres in the United Kingdom (UK) . The pear fruit fungal microbiota in the postharvest environment has since been described (Volschenk, 2016)

.Materials and methods

Sampling approach. . The environments from packhouses in South Africa through to the final retail destination on the export market were monitored by, following a consignment of pear fruit. Local sampling included two central packhouses (packhouse 1 and 2) in the Western Cape. Packhouse1 and 2 were respectively sampled in 2008/2009/2010 and 2010/2011. At least three sampling trips were done per year (early, mid and late season) over a period of four years (2008-2011). Local areas sampled included crates coming from the farms; walls and floors of the packhouses, various areas of the pack lines and walls, floors and inside crates in controlled atmosphere (CA) and regular atmosphere (RA) cold storage environments.

Sampling of the export chain from SA to the UK was only conducted in 2010 (packhouse1) and 2011 (packhouse2). The walls, floors and air in an experimental container that was used to export the consignment of pears from SA to the UK were also sampled before and after shipment. International sampling areas included two re-pack facilities (further referred to as re-pack1 and re-pack2), a distribution centre and a retailer. At least two RA cold storage facilities were sampled at each re-pack facility as well as various areas of the re-pack lines. Two areas where pears were unloaded and stored in the distribution center were also selected for sampling. Storage and display areas were sampled within the retail facility.

Sampling methods. Swab (Transwab®, Medical Wire and Equipment, Wiltshire, England) sampling (Legnani et al. 2004) was done by using between 10 to 30 swabs (depending on the size of the room) collected at random from walls and floors of packhouses, RA and CA cold storage facilities, experimental containers, international re-pack and cold storage facilities, distribution centres, retail storage and display areas. Crates containing or having previously contained pears were sampled (up to 30 swabs per farm), in cold storage facilities (at least 10 swabs per storage facility) and at the retail-end in the UK (at least 10 swabs). Five to ten swabs were used to sample various areas of a selected pear packline in SA and the UK. Packline areas sampled in SA included packbowls, brushes, conveyor belts, sorting tables, rebinfillers and packers' hands. Conveyor belts and packers' hands were sampled in UK re-pack facilities. In some instances (if present), waste bins were also sampled within the re-pack facilities.

Aerial environments were monitored through active and passive air sampling. Active air sampling was done by using an automated SAS Compact Surface Air System® (PBI International, Italy) collecting 100 litres of air deposited onto malt extract agar (MEA) (Merck, Biolab Diagnostics (Pty) Ltd, Johannesburg, South Africa). Passive air sampling was done using

MEA plates exposed to the environment for an optimised 20 min. Between nine and twenty (depending on the size of the room), active and passive air sampling plates were also randomly collected at selected points in all the facilities where walls and floors were sampled as described previously. No active air sampling was conducted in 2009 due to technical difficulties with the automated air-sampler. Passive air was however only included during 2010 and 2011 export chain sampling. All swab and air samples were transported in cooler-boxes to reduce the effect of temperature fluctuations during transportation.

Shipment and sampling conditions. Standard export conditions are prescribed for the fruit industry and are regulated by PPECB, the South African export inspection body (PPECB, 2009). In short: Atmospheric conditions used in CA storage are -0.5 to 1 ºC, 97% nitrogen (N), 1.5% oxygen (O_2) and 1.5% carbon dioxide (CO_2). For export purposes pears are either bagged (20 micron perforated polyethylene bags sold as 1.5 Kg packs) and then placed in single-layer display carton boxes (7 Kg) with standard ventilation holes; or can be individually wrapped in tissue paper and packed in multi-layer display carton boxes (12.5 Kg); or in bulk bins (350 Kg). Packed pear bulk bins or boxes are exported on standard size (1 000 mm x 1 200 mm x 155 mm) wooden pallets. Pallets are shipped in standard 6-foot refrigerated containers with corrugated interior walls and a T-floor to facilitate air passage. Optimal recommended temperatures during export are -1 ºC to 0 ºC and a ventilation rate of 15 to 50 CMH. All commercial fresh produce exports are monitored by PPECB for temperature compliance from pre-loading containers to the final destination end-point on the export market (PPECB, 2009). Shipment takes between 18 to 21 days. To confirm that standard prescribed export conditions were adhered to during this trial the average of at least two measurements of temperature and relative humidity within the various facilities were recorded during the second year of sampling. **Sample**

processing and *Penicillium* isolation. All air sampling plates were incubated at 25 ºC for five days directly after sampling and transportation. Swab samples were processed promptly by aseptically placing the swab in nine ml Ringer's solution (Merck). Swabs were then vortexed (Labotech, Johannesburg) for 30 s. A standard serial dilution was performed and spread plated onto MEA plates. Plates were incubated at 25 ºC for up to seven days and the number of total and morphologically distinctive *Penicillium* colonies were counted respectively and recorded. Conidia of single, representative *Penicillium* spp. were isolated and purified on MEA plates for identification through standard microbiological methods (Johnston, 2008).

***Penicillium* grouping, selection of representative isolates and preservation.** To make handling and identification more manageable, *Penicillium* isolates were grouped according to similar cultural characteristics such as: colony size, -color, -texture and –formation; mycelia coloration and –formation; reverse plate coloration and the production of exudates as described in Johnston (2008). A number was assigned to each group and representative isolates were chosen at random from each group. The number of representative isolates was dependent upon the size of the group. In total 350 representative isolates were obtained for further identification. All representative isolates were preserved in duplicate for future referencing by sub-culturing in sterile water and through cryopreservation. Cultures are maintained in the fungal culture collection of the Department of Microbiology and Plant Pathology, University of Pretoria.

***Penicillium* identification.**

Single spore isolations. Representative isolates were selected to make single spore isolations through dilution plating to obtain pure and genetically uniform isolates. A 10 ml spore suspension was made by aseptically placing approximately five to ten agar blocks in sterile water. Ten µl spore suspension were spread plated onto 90 mm 0.4% water agar (Bacteriological agar, Merck) plates. This process was done in triplicate for each representative isolate and the plates were incubated at 25 ºC for approximately three to 12 hours. After incubation the plates were examined under a stereomicroscope. Germinating single spores were inoculated onto 65 mm MEA plates by using the flat side of an inoculation needle. Inoculated MEA plates were incubated at 25 ºC for approximately seven to 10 days.

DNA extraction. The DNeasy® Plant Mini Kit from Qiagen (Southern Cross Biotechnology, Johannesburg) was used according to the manufacturer's specifications for total DNA extraction from the mycelia and conidia of representative *Penicillium* isolates. Mechanical disruption of the cells were facilitated by using 0.5 g of 0.5 mm silica beads (Biospec Products Inc., Separations, Johannesburg). Cells were lysed by a FastPrep® Instrument FP 120 (Bio 101® Systems, France) at 4 m/s for 30 s. Following electrophoresis at 100 V, a 1% agarose gel (Whitehead Scientific, Johannesburg) stained with a 0.01% ethidium bromide was used to view total DNA extracts. Extractions were viewed through ultraviolet illumination in an electrophoresis gel documentation system (VilberLourmat, OmniScience, Johannesburg).

Polymerase chain reaction. A partial beta-tubulin (β-tubulin) gene region was amplified by using the Bt2a (5'- GGT AAC CAA ATC GGT GCT GCT TTC – 3') and Bt2b (5'- ACC CTC AGT GTA GTG ACC CTT GGC – 3') primers (Glass and Donaldson, 1995). The polymerase chain reaction (PCR) mixtures contained 15-150 ng genomic DNA, 1X NH_4 reaction buffer, 2.5 mM magnesium chloride, 0.2 mM of each of the dNTPs, 4% (of the final volume) stock dimethyl sulphoxide (DMSO, Merck, Germany), 0.1 mM of each oligonucleotide primer and 1U of *Taq* DNA polymerase in a total reaction volume of 50 µl.

The PCR amplification was carried out on a Mastercycler® pro (Eppendorf International, Hamburg, Germany) with the following amplification conditions: initial denaturation at 95 ºC for three min, followed by 35 cycles of denaturation at 94 ºC for 30 s, primer annealing at 60 ºC for 30 s and primer extension at 72 ºC for two min, followed by a final extension of 10 min at 72 ºC. Agarose gel electrophoresis was performed at 100 V for 90 min followed by visualisation of amplicons on a 1% SeaKem® LE agarose gel with TBE buffer containing 0.01% ethidium bromide under an ultraviolet illuminator.

Polymerase chain reaction – restriction fragment length polymorphism. The method used by Johnston (2008) was adopted and the β-tubulin PCR product was digested with the *BfaI* (isochitzomer – *FspBI*) restriction enzyme according to the manufacturer's specifications. A volume of 7.65 µl sterile water, 0.15 µl restriction enzyme (10U) and 2.2 µl Tango buffer (10x) was added to 20 µl of PCR product resulting in a total volume of 30 µl per reaction. The reaction mixture was incubated at the optimal temperature (37 ºC) for approximately three to four hours in a water bath. Products were left overnight at room temperature to ensure complete product digestion. To view polymorphisms between different *Penicillium* spp., 20 µl of each PCR-RFLP products was loaded on a 3% agarose gel stained with a 0.01% ethidium bromide. A 100 bp Hyperladder IV (Bioline, Celtic Molecular Diagnostics (Pty) Ltd, Cape Town, South Africa) molecular marker was included to visually distinguish between fragment sizes in the banding patterns of a species. The gel was run at 75 V between three to five hours (minimum and maximum run time) to separate the fragments and was visualised under an ultraviolet illuminator.

Sequencing. Isolates were grouped according to similar base pair sizes on PCR-RFLP gels and selected isolates were sequenced for conformation of identity. The BT gene region was then sequenced. A QIAquick® PCR purification Kit from Qiagen (Southern Cross Biotechnology, Cape Town, South Africa) was used according to the manufacturer's specifications. By using the BigDye® Terminator V3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, USA), the forward strands of the BT amplicons were sequenced. Components per sequencing reaction were 4 µl sterile water, 1µl dilution buffer, 2 µl BigDye® Reaction mix, 1 µl of a 2 µM primer (forward) and 2 µl purified PCR product which resulted in a total volume of 10 µl. Sequencing reactions were performed by using the 2700 Perkin-Elmer PCR thermocycler. Sequencing cycle conditions were 96 ºC for 10 s, 50 ºC for 5 s, 60 ºC for 4 min and samples were held at 4 ºC.

Purification of the PCR products was performed prior to sequencing. The sequencing reaction was centrifuged briefly for 30 s with 10 µl of sterile water. To facilitate DNA precipitation 2 µl of 3 M sodium acetate was mixed with the reaction. Fifty µl of chilled absolute ethanol was added to the reaction and vortexed. The mixture was incubated for 10 min on ice and centrifuged at 4 ºC (13 000 rpm) for 20 min. Thereafter the absolute ethanol was removed and 80 µl of 70% ethanol was added and centrifuged for five min at room temperature (6 000 rpm). Following centrifugation, the 70% ethanol was removed and the tubes were left exposed to allow any remaining ethanol to evaporate. Sample analysis was done by using an ABI 3130 Genetic Analyser (Applied Biosystems). Sequences were edited with CodonCode Aligner 4.2 software and consensus sequences were subjected to BLAST search analysis to clarify identification results. Sequences were deposited in Genbank and accession numbers were assigned to all isolates of the BT gene region.

Results

Environmental conditions. The temperature and relative humidity (RH), respectively, of shipment containers and sampling areas were recorded as follows: Packhouse facilities (SA)

19.6ºC, 65.00%; CA cold-storage (SA) 3.85 ºC, 79.60%; RA cold-storage (SA) 2.9 ºC, 73.75%; Container before export (SA) 13.75 ºC, 65.55%; Container after export (UK) 8.5 ºC, 79.9%; Receival area (UK) 7.8 ºC, 72.9%; Re-pack facilities (UK) 20.9 ºC, 55.3%; RA cold-storage (UK), 4.6 ºC, 71.1%; Distribution centre (UK) 11.2 ºC, 66.5%; Retail storage (UK) 19.1 ºC, 45.5%; Retail display (UK) 19.1 ºC, 68.5%.

Number of *Penicillium* colonies and isolates obtained. A total of 49954 *Penicillium* colonies were counted over the four year sampling period. Taxonomic representatives were selected from the *Penicillium* colonies with 5 056 isolates used for further studies. The highest average *Penicillium* colony count per sample was obtained from walls and the lowest from contact surfaces. The number of *Penicillium* colonies counted and isolated from each environment sampled is illustrated in Table 1.

Table 1: Summary of the total number of *Penicillium* colonies that were counted and number of isolates obtained in the pear supply chain

Environment:	**Number of samples:**	**Total *Penicillium* colonies counted:**	**Number of *Penicillium* isolates obtained:**	**Average *Penicillium* colonies per sample:**
Active air	834	6 047	1 123	7.25
Passive air	546	5 849	508	10.71
Walls	1 228	21 225	1 232	17.28
Floors	1 301	8 377	980	6.44
All other environments	1 764	8 456	1 203	4.79
Total	**5 673**	**49 954**	**5 056**	**8.81**

Grouping and identification of *Penicillium* species. *Penicillium* isolates were purified and grouped into a total of 282 morphologically similar groups over a four year period. A total of 350 representative isolates were selected for further identification purposes. After PCR-RFLP analysis using Bfa1 and HaeIII restriction enzymes, 222 groups were obtained and one isolate of each group was selected for identity verification through sequencing. The Bt2a and Bt2b primers that were used amplify a 495 bp fragment from *N. crassa* (Glass & Donaldson, 1995). An average of 320 bp reads was obtained. Shortest reads were 211 bp and longest reads were 416 bp. Of the 222 isolates, 17 could not be identified to species level and were recorded as *Penicillium* spp. According to BLAST results, only twelve isolates had a percentage identity of below 97% [96% (x5); 95% (x2); 93%; 86%; 85%; 83%; 80%]. All the other isolates had percentage identities between 97% - 100%. E-values ranged between highest 4E-93 and lowest 0.0. Results of PCR-RFLP and sequencing have been summarised in Table 2 and detailed information on PCR-RFLP grouping has been presented as Supporting Information for online publication only under the title 'Grouping and identification of *Penicillium* isolates obtained from all sampling done from 2008-2011 in various areas of the pear export chain from South Africa to the United Kingdom'.

Table 2: *Penicillium* isolates obtained from all sampling done from 2008-2011 in various areas of the pear export chain from South Africa to the United Kingdom

Sequence identification of partial β-tubulin gene:	Country sequenced isolate obtained from:	Sample sequenced isolate obtained from:	BLAST search E-value	BLAST search % ID	Genbank accession nr:
P. angulare	SA	Passive air: of RA cold storage facility in packhouse Walls: of packing area at packhouse	1.00E-159 - 5.00E-128	97%	KJ140288, KJ140289
P. bialowiezense	UK	Roof: of container after export Walls: of RA cold storage facility at re-pack facility, Waste bins: in re-pack area of re-pack facility	5.00E-168 - 5.00E-112	100%	KJ140290 - KJ140293
P. brevicompactum	UK and SA	Active air : at distribution centre, of RA cold storage facility at packhouse, of packing area of packhouse, of CA cold storage facility in packhouse Floors: of RA storage at packhouse, of distribution centre, of packing area in packhouse (2) Passive air: in retail display area, of CA cold storage facility at packhouse, of RA cold storage facility at Re-pack, at receival area of re-pack facility, Plastic crate/bins: in RA storage of packhouse, plastic display bins pear inside at retailer, Walls: of re-pack area at re-pack facility, of RA cold storage facility at Re-pack, of CA cold storage facility at packhouse, of retailer storage area, of receival area at re-pack facility, of packing area at packhouse, Waste bins (small): in re-pack facility,	0 - 1.00E-107	98% - 100%	KJ140294 - KJ140319,
P. chermesinum	UK	Walls: RA cold storage facility of Re-pack,	7.00E-147	96%	KJ140320
P. chrysogenum	SA and UK	Active air: of distribution centre, of CA cold storage of packhouse, Conveyor belt: of packline in packhouse, Floors: packing area of packhouse, of retailer display area, of RA cold storage at packhouse, of container sampled before packing for export, of packing area of packhouse, of RA cold storage at packhouse, of packing area in packhouse, Packbowl: on packline of packhouse, Packers' hands: of packhouse, Passive air: at distribution centre, of CA cold storage at packhouse, Plastic crate: on farm supplying to packhouse, crate of CA cold storage of packhouse Walls: of container after export, of retailer storage area (2), of receival area at re-pack facility, of RA	0 - 1.00E-70	80% - 100%	KJ140321 - KJ140342

Sequence identification of partial β-tubulin gene:	Country sequenced isolate obtained from:	Sample sequenced isolate obtained from:	BLAST search E-value	BLAST search % ID	Genbank accession nr:
		cold storage facility at packhouse			
P. citreonigrum	SA	Plastic crate: on farm supplying to packhouse1	2.00E-167	100%	KJ140343
P. citrinum	UK	Roof : of container after export Walls: of container after export	9.00E-176 - 5.00E-168	99% - 100%	KJ140344, KJ140345
P. commune	UK	Walls: of RA cold storage facility at re-pack facility 2	1.00E-144	100%	KJ140346
P. corylophilum	SA	Active air: of CA cold storage of packhouse, of RA cold storage facility at packhouse, Drench: packhouse, Floor: of RA cold storage facility at packhouse, of CA cold storage facility at packhouse, Plastic crate: on farm supplying to packhouse,	0 - 2.00E-137	95% - 99%	KJ140347 - KJ140356
P. crustosum	SA and UK	Active air: of CA cold storage of packhouse, of RA cold storage facility at packhouse, of packing area of packhouse, of container before packing for export, Floors: of re-pack area at re-pack facility, of CA cold storage facility in packhouse, Plastic crate: on farm supplying to packhouse, Walls: of holding area at packhouse, of CA cold storage facility at packhouse, of packing area in packhouse,	0 - 2.00E-94	95% - 100%	KJ140357 - KJ140368
P. decaturnese	UK	Active air: of container after export Passive air: of CA cold storage facility at re-pack facility	2e-151 - 4.00E-118	99% - 100%	KJ140369, KJ140370
P. digitatum	UK	Floors: of RA cold storage facility at Re-pack, Passive air: of receival area at Re-pack, Small waste bins: of re-pack facility, Waste bin: of Re-pack	9.00E-151 - 2.00E-110	99% - 100%	KJ140371 - KJ140374
P. echinulatum	UK	Passive air: of RA cold storage facility at Re-pack	1.00E-163	99%	KJ140375
P. expansum	UK and SA	Active air: of RA cold storage facility in packhouse, of RA cold storage facility in Re-pack, of CA cold storage facility in packhouse, Crate: of RA cold storage of packhouse Display crates: in retailer, Floors: of RA cold storage facility in packhouse Passive air: of retailer storage, in retail display area, of CA cold storage facility in packhouse,	0 - 1.00E-113	98% - 100%	KJ140376 - KJ140386
P. fellutanum	UK	Passive air: in retail display area	3.00E-51	83%	KJ140387
P. glabrum	UK and SA	Active air: in RA cold storage facility Re-pack, of container after export, air of RA cold storage facility in packhouse, Conveyor belt: of packline in Re-pack, Floors: of CA cold storage facility in packhouse, of packing area in packhouse, of RA cold storage facility in packhouse, Passive air: of container after export, of RA cold storage facility at Re-pack, of packing area in packhouse, Plastic crates: of RA cold storage facility in packhouse, farm crate supplying to packhouse Walls: of CA cold storage facility at packhouse, of container after export, of RA cold storage facility at re-pack facility, of re-pack area at re-pack facility, of packhouse area in packhouse,	0 - 1.00E-97	95% - 100%	KJ140388 - KJ140430
P. griseofulvum	SA and UK	Active air: of RA cold storage facility in packhouse Packers' hands: packing in Re-pack	3.00E-170 - 5.00E-163	99%	KJ140431, KJ140432
P. italicum	UK and SA	Active air: of container after export, of distribution centre, of RA cold storage facility in Re-pack, of receival area in Re-pack, Brushes: of packline in packhouse, Passive air: of RA cold storage facility in Re-pack, of receival area at Re-pack, Walls: of RA cold storage facility in packhouse, of holding area at packhouse,	5.00E-168 - 4.00E-113	98% - 100%	KJ140433 - KJ140444
P. nordicum	SA	Floors: of CA cold storage facility at packhouse Walls: of CA cold storage facility at packhouse	5.00E-57 - 2.00E-82	85% - 86%	KJ140445, KJ140446
P. olsonii	UK	Walls: of distribution centre	6.00E-157	100%	KJ140447
P. palitans	SA and UK	Active air: of CA cold storage facility at packhouse and Passive air: of RA cold storage facility at Re-pack	3.00E-150	100%	KJ140448, no accession nr
P. paneum	SA	Drench: at packhouse Floors: of RA cold storage facility at packhouse and	0 - 1.00E-159	100%	KJ140449, KJ140450
P. polonicum	SA and UK	Active air: of CA cold storage at packhouse, Floors: of retailer display area, of packing area in packhouse, of CA cold storage facility at packhouse Plastic crates: in CA cold storage facility of packhouse, Walls: of re-pack area at re-pack facility,	0 - 4.00E-107	99% - 100%	KJ140451 - KJ140459
P. roquefortii	UK and SA	Passive air: at distribution centre, Active air: of RA cold storage facility of packhouse, of container before export, Drench: at packhouse, Plastic crates: of farm supplying to packhouse, in CA cold storage facility of packhouse Walls: of RA cold storage facility in packhouse and	0 - 1.00E-10	97% - 100%	KJ140460 - KJ140466
P. sclerotiorum	UK	Active air: of container after packing	7.00E-132	93%	KJ140467
P. sizovae	SA	Plastic crates: of CA cold storage facility in packhouse	2.00E-151	99%	KJ140468
P. skrjabinii	SA	Floors: of RA cold storage facility at packhouse1	0	99%	KJ140469
P. solitum	UK and SA	Active air: of RA cold storage facility in packhouse, of CA cold storage facility in packhouse, Drench: of packhouse, Floors: of CA cold storage facility in packhouse, of RA cold storage facility in packhouse, Passive air: of CA cold storage facility at packhouse, Plastic crates: in RA cold storage facility of packhouse, farm supplying to packhouse, of CA cold storage of packhouse, Walls: of distribution centre, of retailer storage area, of RA cold storage facility in packhouse, of CA cold storage facility at packhouse,	0 - 2.00E-116	99% - 100%	KJ140470 - KJ140489
P. spinulosum	SA and UK	Active air: of RA cold storage facility of packhouse, of RA cold storage facility in Re-pack, in CA cold storage of packhouse, of packing area of packhouse Drench: of packhouse, Floors: of RA cold storage facility of packhouse, of RA cold storage facility of Re-pack, Passive air: of distribution centre, in retailer display area, Plastic crates: of CA cold storage facility of packhouse, on farm supplying to packhouse, Waste bins (small): in Re-pack,	0 - 2.00E-63	84% - 100%	KJ140490 - KJ140507
P. waksmanii	UK	Passive air: of distribution centre	6.00E-173	99%	KJ140508

Thirty-one known *Penicillium* spp. were confirmed. Isolates that could not be identified to species level were grouped as *Penicillium* spp. Species identified in order of decreasing incidence are as follow: *P. glabrum* (Wehmer) Westling (23.40%); *P. chrysogenum* Thom (15.13%); *P. crustosum* Thom (14.16%); *P. brevicompactum* Dierckx (8.96%); *P. expansum* Link (8.39%); *P. solitum* Westling (6.70%); *P. polonicum* K. M. Zalessky (5.43%); *P. italicum* Wehmer (4.47%); *P. bialowiezense* K. M. Zalessky (3.12%); *P. commune* Thom (1.61%); *P. digitatum* (Pers.: Fr.) Sacc (1.55%); *P. sizovae* Baghd (1.43%); *P. roquefortii* Thom (1.37%); *P. skrjabinii* Schmotina and Golovleva (0.67%); *P. nordicum* Dragoni and Cantoni (0.45%); *P. echinulatum* Raper and Thom (0.39%); *P. decaturense* Peterson, Bayer and Wicklow (0.32%); *P. palitans* Westling (0.21%); *P. waksmanni* K. M. Zalessky (0.18%); *P. citrinum* Thom (0.16%); *P. chermesinum* Biourge (0.16%); *P. angulare* Peterson, Bayer and Wicklow (0.14%); *P. spinulosum* Thom (0.14%); *P. corylophilum* Dierckx (0.12%); *P. minioluteum* Dierckx (0.04%); *P. sclerotiorum* J. F. H. Beyma (0.02%); *P. griseofulvum* Dierckx (0.02%); *P. paneum* Frisvad (0.01%); *P. fellutanum* Biourge (0.01%); *P. citreonigrum* Dierckx (0.01%); *P. olsonii* Bainier and Sartory (0.00%) and *Penicillium* spp. (1.22%).

Penicillium spp. not isolated in SA but unique to the UK were *P. citrinum*, *P. fellutanum* and *P. olsonii*. *Penicillium* spp. not isolated in the UK but found unique to SA were *P. angulare, P. citreonigrum, P. corylophilum*, *P. paneum* and *P. skrjabinii*.

Discussion

This is the first study of its kind that provides an overview of the *Penicillium* population dynamics in the pear export chain environments. This study therefore provides an indication of high *Penicillium* inoculum loads in the pear export chain environment that have the potential to compromise the quality of the final product due to the prevalence of pathogenic species. A rich diversity of *Penicillium* spp. exists in the pear supply chain, similar to that reported for the litchi fruit export environments (Johnston, 2008). However, more species were identified in the pear fruit chain (31) compared to a similar litchi chain (17). Similar species were also reported except for *P. steckii* and *P. sumatrense* which were not isolated from the pear chain. *Penicillium* spp. identified in the pear chain that were not isolated from the litchi export chain are *P. digitatum, P. sizovae, P. roquefortii*, *P. skrjabinii*, *P. nordicum, P. decaturense*, *P. palitans, P. waksmanni P. chermesinum*, *P. angulare*, *P. spinulosum*, *P. minioluteum*, *P. sclerotiorum*, *P. griseofulvum*, *P. fellutanum* and *P. olsonii*. No other similar study could be found for comparative purposes.

Five most dominant *Penicillium* species isolated in this study from the pear chain were *P. glabrum* (23.40%), *P. chrysogenum* (15.13%), *P. crustosum* (14.16%), *P. brevicompactum* (8.96%) and *P. expansum* (8.39%). These five *Penicillium* spp. accounted for approximately 70% of the total species isolated in the pear chain environment. The five dominant *Penicillium* species are therefore considered important postharvest organisms in the pear chain and will be discussed further. Other *Penicillium* species identified in this study accounted for 30% of the total *Penicillium* population.

In this study *P. glabrum* was the most frequently isolated species in the pear supply chain over a four year sampling period. Johnston (2008) indicated that *P. glabrum* was the second most dominant isolate in the litchi export chain which supports the fact that *P. glabrum* is common in fresh produce indoor environments. *Penicillium glabrum* and *P. chrysogenum* are reported to be associated with the saprophytic colonisation of wounded or decaying fruit and therefore are important in the context of rot (Filtenborg et al. 1996; Bardas et al. 2009). Even though *Penicillium* spp. are not often associated with fresh vegetables, *P. glabrum* has been known to cause spoilage and disease of onions (Moss, 2008), and is a common pathogen of stored grapes and pomegranates (Barkai-Golan, 2008; Bardas et al. 2009). The high *P. glabrum* prevalence detected in this study could therefore pose secondary infection risks for pears.

Penicillium chrysogenum was the second most dominant species found in the pear chain. This species is known as one of the most common representatives of its genus in indoor environments because of its ability to grow at low water activities (Filtenborg et al. 1996; Dao et al. 2008). A study done by Beguin & Nolard (1994), showed that *P. chrysogenum* was one of the most frequently isolated species obtained from walls and horizontal surfaces.

Penicillium crustosum was the third most frequently isolated *Penicillium* spp. in the pear supply chain. Its prominence is in contrast with the litchi fruit chain where *P. crustosum* was the most frequently isolated species of its genus (Johnston, 2008). These species have been described as robust, being able to tolerate osmotic imbalances and to easily adjust to nutritionally deprived habitats (Gunde-Cimerman et al. 2003). Several authors (Pitt, 1991; Frisvad and Samson, 2004) reported the ease of dislodging high numbers of spores from *P. crustosum* fruiting structures, which could explain the prevalence of this species in the pome fruit chain. Conidia of *P. crustosum* are small-sized and therefore able to rapidly spread and colonise indoor surfaces such as walls, floors, ceilings, fruit bins, etc. (Sonjak et al. 2006). Small-sized conidia also add to the success of this species and its ability to readily occupy micro-environments and metabolise nutrients more efficiently, all contributing to its "fitness" (Sonjak et al. 2006). Even though *P. crustosum* can cause moulds on almonds, hazelnuts, pistachios and walnuts (Varga et al. 2008), it is a more important pathogen of pome fruit (Sanderson & Spotts, 1995; Barkai-Golan, 2008). An isolate of *P. crustosum* from the pear chain that is the subject of the current study was confirmed pathogenic to pears and apples (Louw & Korsten, 2014). High inoculum loads increase the risk of postharvest infection of pear fruit under favorable environmental conditions.

The fourth most dominant *Penicillium* spp. isolated in the pear chain was *P. brevicompactum*. This species is commonly isolated from house dust and is known as a degrader of fine cellulose fibres (Scott et al. 2008). Under optimal moisture conditions house dust and various other cellulose-rich indoor materials are primary habitats for *P. brevicompactum*. Some indoor materials that *P. brevicompactum* has previously been isolated from included broadloom, painted plaster walls, gypsum wallboard paper, potted plants, and urea formaldehyde foam insulation (Scott et al. 2008). In this study walls were often made of gypsum boards that potentially contributed to the cellulose fibre nutritional source. Louw & Korsten (2014) showed that *P. brevicompactum* including an isolate from the currently studied pear chain can be a weak pathogen of pears but non-pathogenic to apples. The presence of *P. brevicompactum* in the pome fruit environment and its potential to cause market-end decay can impact on fruit quality at the retail level. To determine its actual importance, future studies should therefore focus on determining reasons for actual losses at the market- and retail-end and the causal agents of decay and indoor inoculum levels.

Considering the total *Penicillium* profile in the pear chain, 8.39% of the species identified were *P. expansum*. This pathogen is the most important pome fruit decay organism and is in the top five most frequently isolated *Penicillium* spp. in the pear chain. Its prevalence in the export chain is therefore of great importance and can directly be linked to market-end losses reported by industry. The aggressiveness of this pathogen including isolates from the currently studied pear chain was shown by Louw & Korsten (2014). Optimally, *P. expansum* grows at temperatures close to 25 ºC, but can also grow at -3 ºC (Pitt, 2002; Jackson & Al-Taher, 2008). *P. expansum* can furthermore grow at low oxygen levels, atmospheric levels below 2% and is growth-stimulated by carbon dioxide concentrations of up to 15% (Pitt, 2002; Jackson & Al-Taher, 2008). These environmental characteristics enable the pathogen to survive under controlled atmosphere storage conditions, hence, it is likely that a build-up of inoculum could occur over time. Louw & Korsten (2014) confirmed the pathogenicity of several *Penicillium* spp. including *P. expansum* under cold storage conditions. The ability of *P. expansum* to cause disease symptoms under different environmental conditions makes it an important pathogen to consider in the postharvest environment of the pear chain.

According to the literature *Penicillium aurantiogriseum*, *Penicillium brevicompactum, Penicillium commune, Penicillium crustosum*, *Penicillium expansum*, *Penicillium griseofulvum*, *Penicillium solitum* and *Penicillium* spp. can be responsible for postharvest decay of apple and pear fruit (Moslem et al. 2010). However, it has commonly been found that *P. expansum*, *P. crustosum* and *P. solitum* in order of severity cause the greatest extent of pome fruit losses (Sanderson & Spotts, 1995; Kim et al. 2005; Barkai-Golan, 2008). Of all the pear pathogens described before, only *P. aurantiogriseum* was not isolated from the pear export chain. The three pathogens, *P. crustosum*, *P. expansum* and *P. solitum*, were isolated from all environments sampled in this study (active air, passive air, walls and floors) and were confirmed pathogenic (Louw & Korsten, 2014). The presence of these pathogens indicates the disease causing potential in the pear export chain. Vigilant fruit handling, packhouse sanitation, well-timed fruit cooling, and correct temperature management during storage and transportation to the market are therefore essential in reducing market-end losses (Kupferman, 2003).

Penicillium digitatum was found at less than 5% of sample prevalence in the pear chain. This pathogen has commonly been known as the causal agent of green mould on citrus. A recent study by Louw & Korsten (2014), however, showed *P. digitatum* isolates including from the current pear supply chain were highly aggressive on selected pear cultivars. Studies further indicated that *P. digitatum* infections were more prominent in over-mature fruit (Louw & Korsten, 2014). Therefore even low *P. digitatum* inoculum levels in a fruit re-packing environment can contribute to market-end losses. The link between inoculum load, pathogen presence and susceptible hosts being handled should be investigated. Infection is also more likely to occur at the end of the export chain since opportunities for wounding increase the more the fruit is handled and in cases of temperature abuse and when fruit is physiologically less "fit". *Penicillium digitatum* is not well adapted to survive under cold temperatures and therefore effective cold chain management is essential to contain the disease (Vilanova et al. 2012).

Some *Penicillium* spp. that have been isolated at a frequency of more than one percent include *P. polonicum, P. italicum, P. bialowiezense, P. commune, P. sizovae* and *P. roquefortii*. Most of these species are ubiquitous in the food environment and are typical indoor environmental organisms. *Penicillium polonicum* can cause diseases of peanuts, corn, wheat or spoilage of dried meat such as salami (Frisvad and Samson, 2004; Varga et al. 2008). This pathogen can also produce the mycotoxin verrucosidin (Frisvad & Samson, 2004). Varga et al. (2008) have reported that *P. biolowiezense* can cause decay of brussel sprouts.

Inoculation studies by Louw & Korsten (2014) indicated that different *P. expansum, P. crustosum, P. solitum* and *P. digitatum* isolates from both citrus and pear (the current study) chain environments did not differ significantly in terms of the lesion sizes produced when they were inoculated into pears. This finding leads to the conclusion that environmental isolates that originate from different areas (e.g. surfaces, air, other fruit sources) may cross-infect different fruit types. The risk of cross-contamination therefore exists in areas of the pear chain where various fruit types from diverse origins are received, stored and sometimes re-packed.

Conclusion

This *Penicillium* supply chain study provided information regarding species diversity in pear handling environments over a four year period. *Penicillium glabrum*, *P. chrysogenum*, *P. crustosum*, *P. brevicompactum* and *P. expansum* were dominant species in the postharvest pear environment. Even though a high diversity of *Penicillium* spp. were found in the pear export chain, only a few species, namely *P. crustosum, P. digitatum, P. expansum, P. solitum,* and *P. brevicompactum,* were confirmed to cause extensive losses as postharvest pathogens (Louw and Korsten, 2014). Future studies should therefore focus on the link between inoculum load in the fruit handling environment, fruit surface microbiota and market-end product loss.

Acknowledgments

Financial support was provided by the Technology and Human Resources for Industry Programme (TP 2011071100006)., the Department of Science and Technology, The South African Apple and Pear Producers Association (administered by Fruitgro Science), the Fresh Produce Exporters Forum, and the Postharvest Innovation Programme Phi, while the University of Pretoria and the National Reasearch Foundation supported in the form of bursaries. We thank Ms Candice Johnston-Eksteen and René Jacobs for assistance and guidance and all the people involved and assisting at farms, packhouses, exporters, importers, re-packers, distribution centres and retailers visited.

Literature cited

Amiri A, Cholodowski D, Bompeix G. 2005. Adhesion and germination of waterborne and airborne conidia of *Penicillium expansum* to apple and inert surfaces. *Physiological and Molecular Plant Pathology* **67**: 40-48.

Amiri A, Bompeix G. 2005. Diversity and population dynamics of *Penicillium* spp. on apples in pre- and postharvest environments: Consequences for decay development. *Plant Pathology* **54**: 74-81.

Bardas GA, Tzelepis GD, Lotos L, Karaoglanidis GS. 2009. First Report of *Penicillium glabrum* causing fruit rot of pomegranate (*Punica granatum*) in Greece. *Plant Disease* **93**: 1347.

Barkai-Golan R. 2008. *Penicillium* mycotoxins, p 154-155. *In* Barkai-Golan R and Paster N (eds), Mycotoxins in fruits and vegetables, Academic Press, London.

Beguin H, Nolard N. 1994. Mould biodiversity in homes. Air and surface analysis of 130 dwellings. *Aerobiologia* **10**: 157-66.

Dallaire R, Le Blanc DI, Tranchant CC, Vasseur L, Delaquis P, Beaulieu C. 2006. Monitoring the microbial populations and temperatures of fresh broccoli from harvest to retail display. *Journal of Food Protection* **69**: 1118-25.

Dao T, Bensoussan M, Gervais P, Dantigny P. 2008. Inactivation of conidia of *Penicillium chrysogenum, P. digitatum* and *P. italicum* by ethanol solutions and vapours. *International Journal of Food Microbiology* **122**: 68-73.

Elhariry H, Bahobial AA, Gherbawy Y. 2011. Genotypic identification of *Penicillium expansum* and the role of processing on patulin presence in juice. *Food and Chemical Toxicology* **49**: 941-46.

Filtenborg O, Frisvald JC, Thrane U. 1996. Moulds in food spoilage. *International Journal of Food Microbiology* **33**: 85-102.

Frisvad JC, Samson RA. 2004. Polyphasic taxonomy of *Penicillium* subgenus *Penicillium* A guide to identification of food and airborne terverticillate penicillia and their mycotoxins. *Studies in Mycology* **49**: 1-174.

Frisvad JC, Smedsgaard J, Larsen TO, Samson RA. 2004. Mycotoxins, drugs and other extrolites produced by species in *Penicillium* subgenus *Penicillium*. *Studies in Mycology* **49**: 201-41.

Glass NL, Donaldson GC. 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology* **61**:1323-30.

Gunde-Cimerman N, Sonjak S, Zalar P, Frisvad JC, Diderichsen B, Plemenitaš A. 2003. Extremophilic fungi in arctic ice: a relationship between adaptation to low temperature and water activity. *Physics and Chemistry of the Earth* **28**: 1273-78.

Jackson LS, Al-Taher F. 2008. Factors affecting mycotoxin production in fruits, p *75-104. In* Barkai-Golan R and Paster N (eds), Mycotoxins in fruits and vegetables, Academic Press, London.

Johnston CL. 2008. Identification of *Penicillium* species in South African litchi export chain. M.Sc. dissertation, University of Pretoria, South Africa.

Kim JH, Lee WH, Cheong SS, Choi JS, Ryu J, Choi YG. 2005. Identification and characteristics of *Penicillium* spp. isolated from postharvest decay of pear. *Plant Disease* **8**: 107-12.

Kupferman E. 2003. Controlled atmosphere storage of apples and pears. *Acta Horticulturae* **600**: 729-34.

Legnani P, Leoni E, Berveglieri M, Mirolo G, Alvaro N. 2004. Hygienic control of mass catering establishments, microbiological monitoring of food and equipment. *Food Control* **3**: 205-11.

Louw JP, Korsten L. 2014. Pathogenic *Penicillium* spp. on apples and pears. *Plant Disease* **98**: 590-98.

Magan N, Aldred D. 2007. Postharvest control strategies: Minimizing mycotoxins in the food chain. *International Journal of Food Microbiology* **119**: 131-39.

Mansouri S, Houbraken J, Samson RA, Frisvad JC, Christensen M, Tuthill DE, Koutaniemi S, Hatakka A, Lankinen P. 2013. *Penicillium subrubescens*, a new species efficiently producing inulinase. *Antonie van Leeuwenhoek* **103***:* 1343-57.

Mari M, Bertolini P, Pratella GC. 2003. A review: Non-conventional methods for the control of postharvest pear diseases. *Journal of Applied Microbiology* **94**: 761-66.

Mari M, Leoni O, Lori R, Cembali T. 2002. Antifungal vapour-phase activity of allyl-isothicyanate against *Penicillium expansum* on pears. *Plant Pathology* **51**: 231-36.

Moslem M, Abd-Elsalam K, Yassin M, Bahkali A. 2010. First morphomolecular identification of *Penicillium griseofulvum* and *Penicillium aurantiogriseum* toxicogenic isolates associated with blue mould on apple. *Food-Borne Pathogens and Disease* **7**: 857-61.

Moss MO. 2008. Fungi, quality and safety issues in fresh fruits and vegetables. *Journal of Applied Microbiology* **104**: 1239-43.

Palou L, Guardado A, Montesinos-Herrero C. 2010. First report of *Penicillium* spp. and *Pilidiella granati* causing postharvest fruit rot of pomegranate in Spain. *New Disease Reports* **22**: 21-22.

Pitt JI. 1991. A laboratory guide to common *Penicillium* species, p 1-5. Commonwealth Scientific and Industrial Research Organization, Food Research Laboratory, New South Wales, Australia.

Pitt JI. 2002. Biology and ecology of toxigenic *Penicillium* species, p 29–41. *In* deVries JW, Trucksess MW and Jackson LS (eds), Mycotoxins and food safety, Kluwer Academic and Plenum Publishers, New York.

Perishable products export control board (PPECB). 2009. Handling procedure and optimum temperature requirements for sea export of pome fruit. URL: http://ppecb.com/wp-content/uploads/2015/03/HP28-HANDLING-PROCEDURE-AND-OPTIMUM-TEMPERATURE-REQUIREMENTS-FOR-SEA-EXPORT-OF-POME-FRUIT.pdf. Accessed 27 October 2015.

Sanderson PG, Spotts RA. 1995. Postharvest decay of winter pear and apple fruit caused by species of *Penicillium*. *Phytopathology* **85**: 103-110.

Scott JA, Wong B, Summerbell RC, Untereiner WA. 2008. A survey of *Penicillium brevicompactum* and *P. bialowiezense* from indoor environments, with commentary on the taxonomy of the *P. brevicompactum* group. *Botany* **86**: 732-41.

Sonjak S, Frisvad JC, Gunde-Cimerman N. 2006. *Penicillium* mycobiota in arctic sub-glacial ice. *Microbial Ecology* **52**: 207-16.

Varga J, Houbraken J, Samson RA, Frisvad JC. 2008. Molecular diversity of *Aspergillus* and *Penicillium* species on fruits and vegetables. *In* Barkai-Golan R and Paster N (eds), Mycotoxins in fruits and vegetables, Academic Press, London.

Vilanova L, Teixidó N, Torres R, Usall J, Viñas I. 2012. The infection capacity of *P. expansum* and *P. digitatum* on apples and histochemical analysis of host response. *International Journal of Food Microbiology* **157**: 360-67.

Visagie CM. 2012. The polyphasic taxonomy of *Penicillium* and *Talaromyces* spp. isolated from the diverse Fynbos biome. PhD thesis, Stellenbosch University, South Africa.

Volschenk Q. 2016. Microflora associated with *Pyrus communis* focusing on yeast ecology and the antagonistic potential within the population. MSc dissertation, University of Pretoria, South Africa.

Zhang H, Wang Y, Zhang C, Wang X, Li H. 2011. Scientia horticulturae isolation, characterisation and expression analysis of resistance gene candidates in pear (*Pyrus* spp.). *Scientia Horticulturae* **127**: 282-89.

Supplementary Information

Table S1 *Penicillium* isolates obtained from all sampling carried out from 2008 to 2011 in various areas of the pear export chain from South Africa to the UK

Species	Country of origin	Source
P. angulare	SA	Passive air: of RA cold storage facility in packhouse
		Walls: of packing area at packhouse
P. bialowiezense	UK	Roof: of container after export
		Walls: of RA cold storage facility at repack facility
		Waste bins: in repack area of repack facility
P. brevicompactum	SA, UK	Active air: at distribution centre, of RA cold storage facility at packhouse, of packing area of packhouse, of CA cold storage facility in packhouse
		Floors: of RA storage at packhouse, of distribution centre, of packing area in packhouse (2)
		Passive air: in retail display area, of CA cold storage facility at packhouse, of RA cold storage facility at repack, at receival area of repack facility
		Plastic crate/bins: in RA storage of packhouse, plastic display bins inside at retailer
		Walls: of repack area at repack facility, of RA cold storage facility at repack, of CA cold storage facility at packhouse, of retailer storage area, of receival area at repack facility, of packing area at packhouse
		Waste bins (small): in repack facility
P. chermesinum	UK	Walls: RA cold storage facility of repack
P. chrysogenum	SA, UK	Active air: of distribution centre, of CA cold storage of packhouse
		Conveyor belt: of packline in packhouse
		Floors: packing area of packhouse, of retailer display area, of RA cold storage at packhouse, of container sampled before packing for export, of packing area of packhouse, of RA cold storage at packhouse
		Packbowl: on packline of packhouse
		Packers' hands: of packhouse
		Passive air: at distribution centre, of CA cold storage at packhouse
		Plastic crate: on farm supplying to packhouse, crate of CA cold storage of packhouse
		Walls: of container after export, of retailer storage area (2), of receival area at repack facility, of RA cold storage facility at packhouse
P. citreonigrum	SA	Plastic crate: on farm supplying to packhouse 1
P. citrinum	UK	Roof: of container after export
		Walls: of container after export
P. commune	UK	Walls: of RA cold storage facility at repack facility 2
P. corylophilum	SA	Active air: of CA cold storage of packhouse, of RA cold storage facility at packhouse
		Drench: packhouse
		Floor: of RA cold storage facility at packhouse, of CA cold storage facility at packhouse
		Plastic crate: on farm supplying to packhouse

P. crustosum	SA, UK	Active air: of CA cold storage of packhouse, of RA cold storage facility at packhouse, of packing area of packhouse, of container before packing for export Floors: of repack area at repack facility, of CA cold storage facility in packhouse Plastic crate: on farm supplying to packhouse Walls: of holding area at packhouse, of CA cold storage facility at packhouse, of packing area in packhouse
P. decaturnese	UK	Active air: of container after export Passive air: of CA cold storage facility at repack facility
P. digitatum	UK	Floors: of RA cold storage facility at repack Passive air: of receival area at repack Small waste bins: of repack facility Waste bin: of repack
P. echinulatum	UK	Passive air: of RA cold storage facility at repack
P. expansum	SA, UK	Active air: of RA cold storage facility in packhouse, of RA cold storage facility in repack, of CA cold storage facility in packhouse Crate: of RA cold storage of packhouse Display crates: in retailer Floors: of RA cold storage facility in packhouse Passive air: of retailer storage, in retail display area, of CA cold storage facility in packhouse
P. fellutanum	UK	Passive air: in retail display area
P. glabrum	SA, UK	Active air: in RA cold storage facility repack, of container after export, air of RA cold storage facility in packhouse Conveyor belt: of packline in repack Floors: of CA cold storage facility in packhouse, of packing area in packhouse, of RA cold storage facility in packhouse Passive air: of container after export, of RA cold storage facility at repack, of packing area in packhouse Plastic crates: of RA cold storage facility in packhouse, farm crate supplying to packhouse Walls: of CA cold storage facility at packhouse, of container after export, of RA cold storage facility at repack facility, of repack area at repack facility, of packhouse area in packhouse
P. griseofulvum	SA, UK	Active air: of RA cold storage facility in packhouse Packers' hands: packing in repack
P. italicum	SA, UK	Active air: of container after export, of distribution centre, of RA cold storage facility in repack, of receival area in repack Brushes: of packline in packhouse Passive air: of RA cold storage facility in repack, of receival area at repack Walls: of RA cold storage facility in packhouse, of holding area at packhouse
P. nordicum	SA	Floors: of CA cold storage facility at packhouse Walls: of CA cold storage facility at packhouse
P. olsonii	UK	Walls: of distribution centre
P. palitans	SA, UK	Active air: of CA cold storage facility at packhouse Passive air: of RA cold storage facility at repack
P. paneum	SA	Drench: at packhouse Floors: of RA cold storage facility at packhouse

P. polonicum	SA, UK	Active air: of CA cold storage at packhouse Floors: of retailer display area, of packing area in packhouse, of CA cold storage facility at packhouse Plastic crates: in CA cold storage facility of packhouse Walls: of repack area at repack facility
P. roquefortii	SA, UK	Passive air: at distribution centre Active air: of RA cold storage facility of packhouse, of container before export Drench: at packhouse Plastic crates: of farm supplying to packhouse, in CA cold storage facility of packhouse Walls: of RA cold storage facility in packhouse
P. sclerotiorum	UK	Active air: of container after packing
P. sizovae	SA	Plastic crates: of CA cold storage facility in packhouse
P. skrjabinii	SA	Floors: of RA cold storage facility at packhouse 1
P. solitum	SA, UK	Active air: of RA cold storage facility in packhouse, of CA cold storage facility in packhouse Drench: of packhouse Floors: of CA cold storage facility in packhouse, of RA cold storage facility in packhouse Passive air: of CA cold storage facility at packhouse Plastic crates: in RA cold storage facility of packhouse, farm supplying to packhouse, of CA cold storage of packhouse Walls: of distribution centre, of retailer storage area, of RA cold storage facility in packhouse, of CA cold storage facility in packhouse
P. spinulosum	SA, UK	Active air: of RA cold storage facility of packhouse, of RA cold storage facility in repack, of CA cold storage of packhouse, of packing area of packhouse Drench: of packhouse Floors: of RA cold storage facility of packhouse, of RA cold storage facility of repack Passive air: of distribution centre, in retailer display area Plastic crates: of CA cold storage facility of packhouse, on farm supplying to packhouse Waste bins (small): in repack
P. waksmanii	UK	Passive air: of distribution centre

Table S2 Grouping and identification of *Penicillium* isolates obtained from all sampling done from 2008 to 2011 in various areas of the pear export chain from South Africa to the UK

Sequence nr:	**Sequence identification of partial β-tubulin gene:**	**Year sampled:**	**Country sequenced isolate obtained from:**	**Sequenced isolate obtained from:**	**BLAST search E-value**	**BLAST search % ID**	**Genbank accession nr:**	**Groups:**	**UP culture collection nr of representative isolates:**	**β-tubulin PCR-RFLP fragment size ranges**	
										BfaI	**HpaII**
1	*P. angulare*	2011	SA	Passive air of RA cold storage facility in packhouse2	5.00E-128	97%	KJ140288	Group 28	CZ28R	N/A	N/A
2	*P. angulare*	2009	SA	Walls of packing area at packhouse1	1.00E-159	97%	KJ140289	Group 26	PC126R	N/A	N/A
3	*P. biolowienzense*	2011	UK	Roof of container after export	5.00E-112	100%	KJ140290	Group 25 Group 26	ISA25.1R ISA26.1R	–	182 110-120 51 30 - 40 24
4	*P. biolowienzense*	2011	UK	Wall of RA cold storage facility at re-pack facility 2	5.00E-168	100%	KJ140291	Group 31 Group 32 Group 33 Group 34 Group 38 Group 39 Group 40 Group 43	ISA31R ISA32R ISA33R ISA34R ISA38R ISA39R ISA40R ISA43R	–	182 110-120 51 30 - 40 24
5	*P. biolowienzense*	2011	UK	Wastebin in re-pack area of re-pack facility 2	1.00E-149	100%	KJ140292	Group 49	ISA50R	N/A	N/A
6	*P. bialowiezense*	2011	UK	Roof of container after export	4.00E-164	100%	KJ140293	Group 12	ISA12.1R	N/A	N/A
7	*P. brevicompactum*	2010	UK	Wall of re-pack area at re-pack facility 2	7.00E-172	100%	KJ140294	Group 33 Group 34 Group 35 Group 38 Group 40 Group 41 Group 45 Group 46 Group 47 Group 60 Group 61 Group 69 Group 70 Group 67	IS37.1R IS38.24R IS39.5R IS42.7R IS44.1R IS46.1R IS50.10R IS51.6R IS52.12R IS71.4R IS73.2R IS87.1R IS88.1R IS84.2R	–	255 110 - 120 51 30 - 40 24
8	*P. brevicompactum*	2010	SA	Plastic crate in RA storage of packhouse2	7.00E-172	99%	KJ140295	Group 14	IS16.6R	N/A	N/A
9	*P. brevicompactum*	2010	SA	Floor of RA storage at packhouse 2	2.00E-172	100%	KJ140296	Group 72	IS90.1R	N/A	N/A
10	*P. brevicompactum*	2011	UK	Passive air in retail display area	2.00E-171	99%	KJ140297	Group 21	ISA21.1R	N/A	N/A
11	*P. brevicompactum*	2011	UK	Wall of RA cold storage facility at Re-pack2	9.00E-166	99%	KJ140298	Group 59	ISA60R	N/A	N/A
12	*P. brevicompactum*	2011	SA	Wall of CA cold storage facility at packhouse 2	4.00E-159	98%	KJ140299	Group 64 Group 65	ISA67R ISA68R	–	255 110 - 120 51 30 - 40 24
13	*P. brevicompactum*	2011	SA	Wall of retailer storage area	1.00E-169	99%	KJ140300	Group 79	ISA84R	N/A	N/A
14	*P. brevicompactum*	2011	SA	Wall of retailer storage area	8.00E-156	100%	KJ140301	Group 83	ISA88R	N/A	N/A
15	*P. brevicompactum*	2011	UK	Wall of re-pack area at re-pack facility 2	3.00E-165	99%	KJ140302	Group 88	ISA94R	N/A	N/A
16	*P. brevicompactum*	2011	UK	Wall of receival area at re-pack facility 2	1.00E-154	100%	KJ140303	Group 92 Group 97	ISA99.1R ISA106R	–	255 110 - 120 51 30 - 40 24
17	*P. brevicompactum*	2011	UK	Small waste bins of re-pack facility 2	0	100%	KJ140304	Group 94	ISA101R	N/A	N/A
18	*P. brevicompactum*	2011	UK	Active air at distribution centre	6.00E-111	100%	KJ140305	Group 98 Group 99 Group 101	ISA107R ISA108R ISA110R	–	255 110 - 120 51 30 - 40

Sequence nr:	Sequence identification of partial β-tubulin gene:	Year sampled:	Country sequenced isolate obtained from:	Sequenced isolate obtained from:	BLAST search E-value	BLAST search % ID	Genbank accession nr:	Groups:	UP culture collection nr of representative isolates:	β-tubulin PCR-RFLP fragment size ranges	
										BfaI	HpaII
											24
19	*P. brevicompactum*	2011	SA	Passive air of CA cold storage facility at packhouse 2	6.00E-111	100%	KJ140306	Group 35 Group 36 Group 42 Group 44	ISA35R ISA36R ISA42.1R ISA44R	–	255 110 - 120 51 30 - 40 24
20	*P. brevicompactum*	2011	UK	Floors of distribution centre	8.00E-146	98%	KJ140307	Group 45	ISA46R	N/A	N/A
21	*P. brevicompactum*	2011	UK	Walls of retailer storage area	1.00E-107	100%	KJ140308	Group 46 Group 47 Group 51 Group 52 Group 53	ISA47R ISA48R ISA52R ISA53.1R ISA54R	–	255 110 - 120 51 30 - 40 24
22	*P. brevicompactum*	2011	UK	Plastic display bins pear inside at retailer	5.00E-153	100%	KJ140309	Group 56	ISA57R	N/A	N/A
23	*P. brevicompactum*	2011	UK	Walls of re-pack area at re-pack facility 2	8.00E-115	100%	KJ140310	Group 75	ISA79.1R	N/A	N/A
24	*P. brevicompactum*	2011	UK	Passive air of RA cold storage facility at Re-pack2	2.00E-162	98%	KJ140311	Group 73	ISA77R	N/A	N/A
25	*P. brevicompactum*	2011	UK	Passive air at receival area of re-pack facility 2	2.00E-171	100%	KJ140312	Group 76	ISA80R	N/A	N/A
26	*P. brevicompactum*	2011	UK	Active air of distribution centre	1.00E-169	99%	KJ140313	Group 107	ISA116R	N/A	N/A
27	*P. brevicompactum*	2008	SA	Active air of RA cold storage facility at packhouse1	3.00E-165	100%	KJ140314	Group 46 Group 8	PP73R PP13R	–	255 110 - 120 51 30 - 40 24
28	*P. brevicompactum*	2008	SA	Active air of packing area of packhouse1	0	100%	KJ140315	Group 40	PP63R	N/A	N/A
29	*P. brevicompactum*	2009	SA	Floor of packing area in packhouse1	0	100%	KJ140316	Group 39	PC143R	N/A	N/A
30	*P. brevicompactum*	2009	SA	Walls of packing area at packhouse1	0	100%	KJ140317	Group 23	PC123R	N/A	N/A
31	*P. brevicompactum*	2009	SA	Floor of packing area in packhouse1	0	99%	KJ140318	Group 40	PC146R	N/A	N/A
32	*P. brevicompactum*	2008	SA	Active air of CA cold storage facility in packhouse1	0	99%	KJ140319	Group 27 Group 21 Group 9	PP39R PP31R PP14R	–	255 110 - 120 51 30 - 40 24
33	*P. chermesinum*	2011	UK	Walls RA cold storage facility of Re-pack2	7.00E-147	96%	KJ140320	Group 106	ISA115R	N/A	N/A
34	*P. chrysogenum*	2010	SA	Packers' hands of packhouse1	1.00E-113	100%	KJ140321	Group 23	IS25.9R	N/A	N/A
35	*P. chrysogenum*	2010	SA	Floor packing area of packhouse1	2.00E-157	100%	KJ140322	Group 24	IS26.60R	N/A	N/A
36	*P. chrysogenum*	2010	UK	Wall of container after export	8.00E-161	100%	KJ140323	Group 36	IS40.4R	N/A	N/A
37	*P. chrysogenum*	2010	UK	Wall of retailer storage area	1.00E-102	98%	KJ140324	Group 39	IS43.11R	N/A	N/A
38	*P. chrysogenum*	2010	UK	Wall of retailer storage area	1.00E-165	98%	KJ140325	Group 50 Group 58	IS55.1R IS68.8R	170-175 145 - 155 122-124	–
39	*P. chrysogenum*	2011	UK	Wall of receival area at re-pack facility 2	4.00E-93	96%	KJ140326	Group 17 Group 18	ISA17R ISA18R	170-175 145 - 155 122-124	–
40	*P. chrysogenum*	2011	UK	Floor of retailer display area	2.00E-142	98%	KJ140327	Group 22 Group 23 Group 27	ISA22R ISA23.1R ISA27R	170-175 145 - 155 122-124	–
41	*P. chrysogenum*	2011	UK	Passive air at distribution centre	4.00E-159	98%	KJ140328	Group 80	ISA85R	N/A	N/A
42	*P. chrysogenum*	2011	SA	Floor of RA cold storage at packhouse2	1.00E-159	99%	KJ140329	Group 90	ISA97R	N/A	N/A
43	*P. chrysogenum*	2011	UK	Active air of distribution centre	1.00E-144	99%	KJ140330	Group 41 Group 102 Group 111	ISA41R ISA111R ISA120R	170-175 145 - 155 122-124	–
44	*P. chrysogenum*	2011	SA	Passive air of CA cold storage at packhouse2	7.00E-162	99%	KJ140331	Group 22 Group 28 Group 29 Group 30 Group 37 Group 77 Group 78	CZ22R ISA28R ISA29R ISA30.1R ISA37R ISA82R ISA83R	170-175 145 - 155 122-124	–
45	*P. chrysogenum*	2011	SA	Floors of container sampled before packing for export	2.00E-161	100%	KJ140332	Group 26 Group 26 Group 15 Group 85 Group 108	CZ26R ISA26.2R CZ15R ISA90R ISA117R	170-175 145 - 155 122-124	–
46	*P. chrysogenum*	2009	SA	Conveyor belt of packline of	5.00E-158	100%	KJ140333	Group 1	PC10.1R	170-175	–

Sequence nr:	Sequence identification of partial β-tubulin gene:	Year sampled:	Country sequenced isolate obtained from:	Sequenced isolate obtained from:	BLAST search E-value	BLAST search % ID	Genbank accession nr:	Groups:	UP culture collection nr of representative isolates:	β-tubulin PCR-RFLP fragment size ranges	
										BfaI	HpaII
				packhouse1				Group 1	PC10.2R	145 - 155 122-124	
								Group 2	PC19R		
47	*P. chrysogenum*	2011	SA	Floors of packing area of packhouse2	2.00E-127	96%	KJ140334	Group 20	CZA20R	N/A	N/A
48	*P. chrysogenum*	2008	SA	Plastic crate on farm supplying to packhouse1	1.00E-70	80%	KJ140335	Group 17	PP24.1R	N/A	N/A
49	*P. chrysogenum*	2009	SA	Floor of RA cold storage at packhouse1	5.00E-158	100%	KJ140336	Group 8	PC105R	N/A	N/A
50	*P. chrysogenum*	2008	SA	Active air	0	100%	KJ140337	Group 14	PP21.1R	N/A	N/A
51	*P. chrysogenum*	2009	SA	Wall of RA cold storage facility at packhouse1	5.00E-163	100%	KJ140338	Group 20	PC120R	N/A	N/A
52	*P. chrysogenum*	2008	SA	Active air of CA cold storage of packhouse1	1.00E-158	100%	KJ140339	Group 14	PP21.2R	170-175 145 - 155 122-124	–
								Group 55	PP105R		
53	*P. chrysogenum*	2009	SA	Packbowl on packline of packhouse1	0	100%	KJ140340	Group 10	PC107R	N/A	N/A
54	*P. chrysogenum*	2009	SA	Floor of packing area in packhouse1	3.00E-161	96%	KJ140341	Group 33	PC136R	N/A	N/A
55	*P. chrysogenum*	2009	SA	Crate of CA cold storage of packhouse1	0	99%	KJ140342	Group 38	PC142R	N/A	N/A
56	*P. citreonigrum*	2009	SA	Plastic crate on farm supplying to packhouse1	2.00E-167	100%	KJ140343	Group 28	PC128R	N/A	N/A
57	*P. citrinum*	2011	UK	Roof of containerafter export	9.00E-176	100%	KJ140344	Group 63	ISA65R	N/A	N/A
58	*P. citrinum*	2011	UK	Wall of container after export	5.00E-168	99%	KJ140345	Group 95	ISA104R	N/A	N/A
59	*P. commune*	2011	UK	Wall of RA cold storage facility at re-pack facility 2	1.00E-144	100%	KJ140346	Group 89	ISA96R	–	227 160 40-50 25-35
								Group 91	ISA98R		
								Group 93	ISA100R		
								Group 104	ISA113R		
								Group 105	ISA114R		
								Group 13	ISA13.1R		
								Group 21	ISA21.1R		
								Group 4	ISA4.1R		
								Group 92	ISA99.2R		
								Group 92	ISA99.3R		
								Group 109	ISA118R		
								Group 112	ISA133R		
								Group 113	ISA134R		
								Group 114	ISA140R		
60	*P. corylophilum*	2008	SA	Plastic crate on farm supplying to packhouse1	6.00E-152	98%	KJ140347	Group 45	PP71R	N/A	N/A
61	*P. corylophilum*	2008	SA	Active air of CA cold storage of packhouse1	2.00E-137	95%	KJ140348	Group 10	PP16R	N/A	N/A
62	*P. corylophilum*	2008	SA	Active air of CA cold storage of packhouse1	5.00E-163	99%	KJ140349	Group 38	PP60R	N/A	N/A
63	*P. corylophilum*	2008	SA	Plastic crate on farm supplying to packhouse1	1.00E-174	99%	KJ140350	Group 18	PP26R	N/A	N/A
64	*P. corylophilum*	2008	SA	Active air of RA cold storage facility at packhouse1	5.00E-178	99%	KJ140351	Group 4	PP8R	N/A	N/A
65	*P. corylophilum*	2008	SA	Floor of RA cold storage facility at packhouse1	2.00E-168	99%	KJ140352	Group 37	PP59R	N/A	N/A
66	*P. corylophilum*	2008	SA	Drench packhouse1	3.00E-176	99%	KJ140353	Group 29	PP41R	297 169	–
								Group 30	PP46R		
								Group 48	PP80R		
								Group 49	PP82R		
								Group 50	PP85R		
67	*P. corylophilum*	2008	SA	Drench packhouse1	0	99%	KJ140354	Group 26	PP38R	N/A	N/A
68	*P. corylophilum*	2008	SA	Drench packhouse1	0	99%	KJ140355	Group 36	PP57R	N/A	N/A
69	*P. corylophylum*	2010	SA	Floor of CA cold storage facility at packhouse2	7.00E-167	99%	KJ140356	Group 14	CZ16R	N/A	N/A
70	*P. crustosum*	2010	SA	Active air of CA cold storage of packhouse2	3.00E-104	100%	KJ140357	Group 1	IS1.2R	–	227 160 40-50 25-35
								Group 17	IS19.2R		
								Group 18	IS20.7R		
								Group 19	IS21.13R		
								Group 19	IS21.18R		
								Group 66	IS83.1R		
								Group 68	IS86.1R		
								Group 4	IS4.2R		
								Group 5	IS6.5R		
								Group 6	IS6.16R		
71	*P. crustosum*	2010	SA	Active air of CA cold storage facility at packhouse2	1.00E-113	100%	KJ140358	Group 1	IS1.23R	–	227 160
								Group 7	IS7.3R		

Sequence nr:	Sequence identification of partial β-tubulin gene:	Year sampled:	Country sequenced isolate obtained from:	Sequenced isolate obtained from:	BLAST search E-value	BLAST search % ID	Genbank accession nr:	Groups:	UP culture collection nr of representative isolates:	β-tubulin PCR-RFLP fragment size ranges	
										BfaI	HpaII
											40-50 25-35
72	*P. crustosum*	2010	SA	Active air of RA cold storage facility at packhouse2	2.00E-94	100%	KJ140359	Group 16	IS18.9R	N/A	N/A
73	*P. crustosum*	2010	SA	Active air of packing area of packhouse1	0	98%	KJ140360	Group 73	IS91.1R	N/A	N/A
74	*P. crustosum*	2010	SA	Active air of RA cold storage facility at packhouse1	8.00E-172	96%	KJ140361	Group 1	IS1.3R	N/A	N/A
75	*P. crustosum*	2011	SA	Wall of holding area at packhouse2	1.00E-158	98%	KJ140362	Group 1 Group 2 Group 5 Group 6 Group 7 Group 30	ISA1R ISA2R ISA5.1R ISA6.1R ISA7R ISA30.2R	–	227 160 40-50 25-35
76	*P. crustosum*	2011	UK	Floor of re-pack area at re-pack facility 2	1.00E-118	100%	KJ140363	Group 13	ISA13.2R	N/A	N/A
77	*P. crustosum*	2011	SA	Wall of CA cold storage facility at packhouse 2	7.00E-157	95%	KJ140364	Group 14	ISA14.1R	N/A	N/A
78	*P. crustosum*	2011	SA	Floor of CA cold storage facility in packhouse2	2.00E-157	98%	KJ140365	Group 9 Group 10 Group 12 Group 14 Group 23	CZ9R ISA10.1R ISA12.2R ISA14.2R ISA23.2R	–	227 160 40-50 25-35
79	*P. crustosum*	2011	SA	Wall of packing area in packhouse2	4.00E-154	98%	KJ140366	Group 11	CZ11R	N/A	N/A
80	*P. crustosum*	2008	SA	Plastic crate on farm supplying to packhouse1	2.00E-146	99%	KJ140367	Group 23	PP33R	N/A	N/A
81	*P. crustosum*	2011	SA	Active air of container before packing for export	7.00E-167	99%	KJ140368	Group 19	CZ19R	N/A	N/A
82	*P. decaturnese*	2010	UK	Passive air of CA cold storage facility at re-pack facility 1	?2e-151	99%	KJ140369	Group 42	IS47.14R	N/A	N/A
83	*P. decaturense*	2011	UK	Active air of container after export	4.00E-118	100%	KJ140370	Group 87 Group 42	ISA92R ISA42.2R		
84	*P. digitatum*	2010	UK	Floor of RA cold storage facility at Re-pack1	9.00E-151	99%	KJ140371	Group 32	IS36.22R	N/A	N/A
85	*P. digitatum*	2011	UK	Small waste bins of re-pack facility 2	1.00E-159	99%	KJ140372	Group 66	ISA69R	N/A	N/A
86	*P. digitatum*	2011	UK	Passive air of receival area at Re-pack2	2.00E-110	100%	KJ140373	Group 72	ISA75R	N/A	N/A
87	*P. digitatum*	2011	UK	Waste bin of Re-pack2	1.00E-159	99%	KJ140374	Group 67 Group 68 Group 69 Group 60 Group 81	ISA70R ISA71R ISA72R ISA62.1R ISA86R	37 121 319	–
88	*P. echinulatum*	2010	UK	Passive air of RA cold storage facility at Re-pack2	1.00E-163	99%	KJ140375	Group 52 Group 53 Group 54	IS59.6R IS60.2R IS63.4R	–	370-390 50-55 40-45
89	*P. expansum*	2010	UK	Display crates in retailer	1.00E-113	99%	KJ140376	Group 15 Group 22	IS17.1R IS24.4R	155-160 135-140 124 27	–
90	*P. expansum*	2010	SA	Active air of RA cold storage facility in packhouse1	1.00E-169	100%	KJ140377	Group 17	IS19.7R	N/A	N/A
91	*P. expansum*	2010	UK	Active air of RA cold storage facility in Re-pack1	0	100%	KJ140378	Group 29 Group 29	IS33.15R IS33.22R	155-160 135-140 124 27	–
92	*P. expansum*	2010	UK	Active air of RA cold storage facility in Re-pack1	4.00E-169	100%	KJ140379	Group 43 Group 50 Group 4	IS48.13R IS56.4R IS5.21R	155-160 135-140 124 27	–
93	*P. expansum*	2011	UK	Passive air of retailer storage	5.00E-163	99%	KJ140380	Group 3	ISA3R	N/A	N/A
94	*P. expansum*	2011	UK	Passive air in retail display area	5.00E-143	98%	KJ140381	Group 11 Group 25	ISA11R ISA25.2R	155-160 135-140 124 27	
95	*P. expansum*	2011	SA	Passive air of CA coldstorage facility in packhouse2	3.00E-155	100%	KJ140382	Group 24	CZ24R	N/A	N/A
96	*P. expansum*	2008	SA	Active air of CA cold storage facility in packhouse1	1.00E-154	100%	KJ140383	Group 15	PP22.1R	N/A	N/A
97	*P. expansum*	2008	SA	Crate of RA cold storage of packhouse1	6.00E-157	100%	KJ140384	Group 52	PP89R	N/A	N/A
98	*P. expansum*	2009	SA	Active air of CA cold storage facility in packhouse1	0	99%	KJ140385	Group 3	PC27R	N/A	N/A
99	*P. expansum*	2008	SA	Floor of RA cold storage facility in packhouse1	0	100%	KJ140386	Group 15	PP22.2R	N/A	N/A
100	*P. fellutanum*	2011	UK	Passive air in retail display area	3.00E-51	83%	KJ140387	Group 86	ISA91R	N/A	N/A
101	*P. glabrum*	2010	UK	Active air in RA cold storage facility Re-pack2	3.00E-155	99%	KJ140388	Group 7 Group 13	IS7.16R IS15.5R	370-380 80-90	–

Sequence nr:	Sequence identification of partial β-tubulin gene:	Year sampled:	Country sequenced isolate obtained from:	Sequenced isolate obtained from:	BLAST search E-value	BLAST search % ID	Genbank accession nr:	Groups:	UP culture collection nr of representative isolates:	β-tubulin PCR-RFLP fragment size ranges	
										BfaI	HpaII
								Group 30	IS34.5R		
102	*P. glabrum*	2010	UK	Active air in RA cold storage facility Re-pack1	6.00E-157	100%	KJ140389	Group 7	IS7.25R	N/A	N/A
103	*P. glabrum*	2010	UK	Conveyor belt of packline in Re-pack1	0	99%	KJ140390	Group 7	IS7.40R	N/A	N/A
104	*P. glabrum*	2010	UK	Active air in RA cold storage facility of Re-pack1	0	100%	KJ140391	Group 7	IS9.22R	N/A	N/A
105	*P. glabrum*	2010	SA	Wall of CA cold storage facility at packhouse 2	2.00E-167	98%	KJ140392	Group 8	IS8.15R	N/A	N/A
106	*P. glabrum*	2010	UK	Wall of container after export	1.00E-169	97%	KJ140393	Group 8	IS8.24R	N/A	N/A
107	*P. glabrum*	2010	UK	Wall of RA cold storage facility at re-pack facility1	8.00E-151	99%	KJ140394	Group 11	IS13.1R	N/A	N/A
108	*P. glabrum*	2010	UK	Wall of re-pack area at re-pack facility 2	3.00E-180	100%	KJ140395	Group 11	IS13.2R	N/A	N/A
109	*P. glabrum*	2010	UK	Active air in RA cold storage facility of Re-pack1	1.00E-179	99%	KJ140396	Group 12	IS14.2R	N/A	N/A
110	*P. glabrum*	2010	UK	Passive air of container after export	3.00E-180	99%	KJ140397	Group 12	IS14.3R	N/A	N/A
111	*P. glabrum*	2010	UK	Active air of RA cold storage facility of Re-pack1	4.00E-179	99%	KJ140398	Group 12	IS14.56R	N/A	N/A
112	*P. glabrum*	2010	UK	Active air of RA cold storage facility of Re-pack2	3.00E-180	99%	KJ140399	Group 2	IS2.11R	N/A	N/A
113	*P. glabrum*	2010		Wall of RA cold storage facility at Re-pack1	1.00E-149	99%	KJ140400	Group 9	IS11.6R	N/A	N/A
114	*P. glabrum*	2010	UK	Passive air of RA cold storage facility at Re-pack2	2.00E-162	97%	KJ140401	Group 21 Group 20	IS23.8R IS22.8R	370-380 80-90	–
115	*P. glabrum*	2010	UK	Active air of RA cold storage facility in Re-pack2	6.00E-157	99%	KJ140402	Group 21 Group 26 Group 10	IS23.14R IS30.5R IS12.7R	370-380 80-90	–
116	*P. glabrum*	2010	UK	Wall of RA coldstorage facility of Re-pack2	1.00E-163	100%	KJ140403	Group 20	IS22.14R	N/A	N/A
117	*P. glabrum*	2010	UK	Wall of re-pack area at re-pack facility 2	2.00E-156	100%	KJ140404	Group 25	IS29.9R	N/A	N/A
118	*P. glabrum*	2010	UK	Active air of RA cold storage facility in Re-pack1	3.00E-160	100%	KJ140405	Group 28 Group 51	IS32.21R IS57.3R	370-380 80-90	–
119	*P. glabrum*	2010	UK	Passive air in container after export	0	100%	KJ140406	Group 8 Group 3	IS8.2R IS3.5R	370-380 80-90	–
120	*P. glabrum*	2010	UK	Wall of re-pack area at re-pack facility 2	9.00E-146	97%	KJ140407	Group 37	IS41.1R	N/A	N/A
121	*P. glabrum*	2010	UK	Passive air in RA cold storage facility in Re-pack1	9.00E-104	99%	KJ140408	Group 48	IS53.1R	N/A	N/A
122	*P. glabrum*	2010	UK	Passive air of RA cold storage facility at Re-pack1	8.00E-161	100%	KJ140409	Group 55	IS65.6R	N/A	N/A
123	*P. glabrum*	2010	SA	Wall of packhouse area in packhouse1	1.00E-97	97%	KJ140410	Group 63	IS79.1R	370-380 80-90	–
124	*P. glabrum*	2011	UK	Active air of container after export	9.00E-156	99%	KJ140411	Group 16	ISA16R	N/A	N/A
125	*P. glabrum*	2011	UK	Active air of container after export	2.00E-161	100%	KJ140412	Group 8 Group 5 Group 6	ISA8R ISA5.2R ISA6.2R	370-380 80-90	–
126	*P. glabrum*	2011	UK	Passive air of RA cold storage facility in Re-pack2	6.00E-127	95%	KJ140413	Group 9 Group 9 Group 9 Group 10 Group 12 Group 12 Group 6	ISA9.2R ISA9.3R ISA9.1R ISA10.2R ISA12.3R ISA12.4R ISA6.3R	370-380 80-90	–
127	*P. glabrum*	2011	UK	Active air of RA cold storage facility in Re-pack2	3.00E-155	100%	KJ140414	Group 55 Group 75	ISA56R ISA79.2R	370-380 80-90	–
128	*P. glabrum*	2011	SA	Floor of CA cold storage facility in packhouse2	1.00E-149	99%	KJ140415	Group 2	CZ2R	N/A	N/A
129	*P. glabrum*	2011	SA	Passive air of packing area in packhouse2	6.00E-157	99%	KJ140416	Group 31	CZ31R	N/A	N/A
130	*P. glabrum*	2008	SA	Active air of RA cold storage facility in packhouse1	6.00E-152	100%	KJ140417	Group 5	PP9R	N/A	N/A
131	*P. glabrum*	2008	SA	Floor of CA cold storage facility at packhouse1	2.00E-152	100%	KJ140418	Group 17	PP24.2R	N/A	N/A
132	*P. glabrum*	2009	SA	Wall of CA cold storage facility at packhouse 1	6.00E-157	99%	KJ140419	Group 27	PC127R	N/A	N/A
133	*P. glabrum*	2009	SA	Floor of pacing area in packhouse1	6.00E-147	98%	KJ140420	Group 25	PC125R	N/A	N/A
134	*P. glabrum*	2008	SA	Plastic crate of RA cold storage facility in packhouse1	2.00E-156	98%	KJ140421	Group 43	PP68R	N/A	N/A
135	*P. glabrum*	2009	SA	Floor of RA cold storage facility in packhouse1	9.00E-176	100%	KJ140422	Group 15	PC112R	N/A	N/A
136	*P. glabrum*	2008	SA	Active air of RA cold storage facility in packhouse1	7.00E-177	100%	KJ140423	Group 6	PP11R	N/A	N/A
137	*P. glabrum*	2009	SA	Wall of RA cold storage facility in packhouse1	4.00E-179	100%	KJ140424	Group 21	PC121R	N/A	N/A

Sequence nr:	Sequence identification of partial β-tubulin gene:	Year sampled:	Country sequenced isolate obtained from:	Sequenced isolate obtained from:	BLAST search E-value	BLAST search % ID	Genbank accession nr:	Groups:	UP culture collection nr of representative isolates:	β-tubulin PCR-RFLP fragment size ranges	
										BfaI	HpaII
138	*P. glabrum*	2008	SA	Plastic farm crate supplying to packhouse1	1.00E-179	100%	KJ140425	Group 41 Group 42 Group 54 Group 44	PP66R PP67R PP97R PP70R	370-380 80-90	–
139	*P. glabrum*	2009	SA	Crate of RA cold storage of packhouse1	3.00E-180	100%	KJ140426	Group 22	PC122R	N/A	N/A
140	*P. glabrum*	2009	SA	Wall of packhouse area in packhouse1	0	97%	KJ140427	Group 37	PC141R	N/A	N/A
141	*P. glabrum*	2009	SA	Plastic crate of RA cold storage facility in packhouse1	0	100%	KJ140428	Group 14	PC111R	N/A	N/A
142	*P. glabrum*	2009	SA	Wall of packhouse area in packhouse1	0	100%	KJ140429	Group 13	PC110R	N/A	N/A
143	*P. glabrum*	2009	SA	Wall of packhouse area in packhouse1	0	100%	KJ140430	Group 24	PC124R	N/A	N/A
144	*P. griseofulvum*	2008	SA	Active air of RA cold storage facility in packhouse1	3.00E-170	99%	KJ140431	Group 2	PP3R	N/A	N/A
145	*P. griseovulfum*	2010	UK	Packers' hands packing in Re-pack1	5.00E-163	99%	KJ140432	Group 64	IS80.1R	N/A	N/A
146	*P. italicum*	2011	UK	Active air of container after export	1.00E-154	100%	KJ140433	Group 74	ISA78R	N/A	N/A
147	*P. italicum*	2010	UK	Active air of distribution centre	4.00E-159	98%	KJ140434	Group 59	IS70.19R	N/A	N/A
148	*P. italicum*	2010	UK	Passive air of RA cold storage facility in Re-pack2	4.00E-113	99%	KJ140435	Group 56 Group 65	IS66.23R IS81.1R	172 170 123	–
149	*P. italicum*	2010	UK	Active air of container after export	1.00E-168	100%	KJ140436	Group 56	IS66.32R	N/A	N/A
150	*P. italicum*	2010	UK	Active air of RA cold storage facility in Re-pack1	5.00E-168	99%	KJ140437	Group 56 Group 57	IS66.42R IS67.4R	172 170 123	–
151	*P. italicum*	2011	UK	Active air of receival area in Re-pack2	6.00E-121	100%	KJ140438	Group 24	ISA24R	N/A	N/A
152	*P. italicum*	2011	SA	Wall of RA cold storage facility in packhouse2	3.00E-155	100%	KJ140439	Group 70	ISA73R	N/A	N/A
153	*P. italicum*	2011	UK	Active air of RA cold storage facility in Re-pack2	2.00E-166	99%	KJ140440	Group 96 Group 71	ISA105R ISA74R	172 170 123	–
154	*P. italicum*	2011	SA	Wall of holding area at packhouse2	5.00E-148	99%	KJ140441	Group 100	ISA109R	N/A	N/A
155	*P. italicum*	2011	UK	Passive air of receival area at Re-pack2	4.00E-154	99%	KJ140442	Group 61	ISA63R	N/A	N/A
156	*P. italicum*	2011	SA	Brushes of packline in packhouse2	6.00E-152	99%	KJ140443	Group 29	CZ29R	N/A	N/A
157	*P. italicum*	2009	SA	Wall of RA cold storage facility at packhouse1	6.00E-152	99%	KJ140444	Group 34	PC137R	N/A	N/A
158	*P. nordicum*	2010	SA	Floors	5.00E-57	86%	KJ140445	Group 49	IS54.8R	N/A	N/A
159	*P. nordicum*	2009	SA	Wall of CA cold storage facility at packhouse1	2.00E-82	85%	KJ140446	Group 12	PC109R	N/A	N/A
160	*P. olsonii*	2011	UK	Wall of distribution centre	6.00E-157	100%	KJ140447	Group 110	ISA119R	N/A	N/A
161	*P. palitans*	2010	SA	Active air of CA cold storage facility at packhouse1	3.00E-150	100%	KJ140448	Group 62 Group 62	IS76.3R IS76.5R	5 123 164	
162	*P. palitans*	2011	UK	Passive air of RA cold storage facility at Re-pack2	3.00E-150	100%	no accession nr	Group 48	ISA49R	N/A	N/A
163	*P. paneum*	2009	SA	Floor of RA cold storage facility at packhouse1	1.00E-159	100%	KJ140449	Group 30	PC130R	N/A	N/A
164	*P. paneum*	2008	SA	Drench at packhouse1	0	100%	KJ140450	Group 24	PP35R	N/A	N/A
165	*P. polonicum*	2010	SA	Active air of CA cold storage at packhouse2	4.00E-107	100%	KJ140451	Group 23	IS25.20R	N/A	N/A
166	*P. polonicum*	2010	UK	Wall of re-pack area at re-pack facility 1	0	100%	KJ140452	Group 27	IS31.1R	N/A	N/A
167	*P. polonicum*	2010	UK	Wall of re-pack area at re-pack facility1	9.00E-171	100%	KJ140453	Group 30 Group 30	IS34.18R IS35.1R	190-195 165-170 123	–
168	*P. polonicum*	2010	UK	Wall of re-pack area at re-pack facility2	9.00E-171	100%	KJ140454	Group 40 Group 71	IS45.5R IS89.1R	190-195 165-170 123	–
169	*P. polonicum*	2010	SA	Plastic crate in CA cold storage facility of packhous2	7.00E-167	100%	KJ140455	Group 56	IS69.12R	N/A	
170	*P. polonicum*	2011	UK	Floor of retailer display area	3.00E-165	99%	KJ140456	Group 57	ISA58R	N/A	
171	*P. polonicum*	2011	SA	Floor of packing area in packhouse2	5.00E-148	100%	KJ140457	Group 27	CZ27R	N/A	
172	*P. polonicum*	2008	SA	Active air of CA cold storage facility of packhouse1	1.00E-158	100%	KJ140458	Group 13	PP20R	N/A	
173	*P. polonicum*	2009	SA	Floor of CA cold storage facility at packhouse1	0	100%	KJ140459	Group 9	PC106R	N/A	
174	*P. roquefortii*	2011	UK	Passive air at distribution centre	2.00E-152	97%	KJ140460	Group 30	ISA30.3R	N/A	
175	*P. roquefortii*	2010	SA	Active air of RA cold storage facility of packhouse1	2.00E-161	99%	KJ140461	Group 31	IS36.12R	N/A	
176	*P. roquefortii*	2011	SA	Active air of container before export	1.00E-10	100%	KJ140462	Group 20	ISA20R	N/A	
177	*P. roquefortii*	2008	SA	Drench at packhouse1	1.00E-154	100%	KJ140463	Group 20	PP30R	N/A	
178	*P. roquefortii*	2008	SA	Plastic crates of farm supplying	4.00E-179	100%	KJ140464	Group 31	PP47R	N/A	

Sequence nr:	Sequence identification of partial β-tubulin gene:	Year sampled:	Country sequenced isolate obtained from:	Sequenced isolate obtained from:	BLAST search E-value	BLAST search % ID	Genbank accession nr:	Groups:	UP culture collection nr of representative isolates:	β-tubulin PCR-RFLP fragment size ranges	
										BfaI	**HpaII**
				to packhouse1							
179	*P. roquefortii*	2009	SA	Wall of RA cold storage facility in packhouse1	0	100%	KJ140465	Group 16	PC114R	N/A	
180	*P. roquefortii*	2009	SA	Plastic crate in CA cold storage facility of packhous1	0	100%	KJ140466	Group 17	PC115R	N/A	
181	*P. sclerotiorum*	2011	UK	Active air of container after packing	7.00E-132	93%	KJ140467	Group 82 Group 84	ISA87R ISA89R	152 173	44 281
182	*P. sizovae*	2010	SA	Plastic crate of CA cold storage facility in packhouse2	2.00E-151	99%	KJ140468	Group 44	IS49.10R	N/A	N/A
183	*P. skrjabinii*	2009	SA	Floor of RA cold storage facility at packhouse1	0	99%	KJ140469	Group 31	PC131R	N/A	N/A
184	*P. solitum*	2011	UK	Wall of distribution centre	1.00E-154	100%	KJ140470	Group 52	ISA53.2R	N/A	N/A
185	*P. solitum*	2011	SA	Passive air of CA cold storage facility at packhouse2	3.00E-134	99%	KJ140471	Group 58	ISA59R	N/A	N/A
186	*P. solitum*	2011	UK	Wall of retailer storage area	2.00E-116	100%	KJ140472	Group 62	ISA64R	N/A	N/A
187	*P. solitum*	2008	SA	Drench of packhouse1	8.00E-151	100%	KJ140473	Group 28	PP40R	N/A	N/A
188	*P. solitum*	2009	SA	Floor of CA cold storage facility in packhouse1	1.00E-158	100%	KJ140474	Group 6	PC102R	N/A	N/A
189	*P. solitum*	2009	SA	Floor of CA cold storage facility in packhouse1	1.00E-158	100%	KJ140475	Group 7	PC103R	N/A	N/A
190	*P. solitum*	2009	SA	Floor of RA cold storage facility in packhouse1	1.00E-159	100%	KJ140476	Group 4	PC100R	N/A	N/A
191	*P. solitum*	2008	SA	Active air of RA cold storage facility in packhouse1	3.00E-160	100%	KJ140477	Group 33	PP52R	N/A	N/A
192	*P. solitum*	2008	SA	Plastic crates inRA cold storage facility of packhouse1	3.00E-160	100%	KJ140478	Group 51	PP87R	N/A	N/A
193	*P. solitum*	2009	SA	Floor of CA coldstorage facility in packhouse1	8.00E-161	100%	KJ140479	Group 5	PC101R	N/A	N/A
194	*P. solitum*	2009	SA	Wall of RA cold storage facility in packhouse1	8.00E-161	100%	KJ140480	Group 29	PC129R	N/A	N/A
195	*P. solitum*	2008	SA	Active air of RA cold storage facility in packhouse1	6.00E-162	100%	KJ140481	Group 1	PP2R	N/A	N/A
196	*P. solitum*	2009	SA	Wall of CA cold storage facility at packhouse1	5.00E-163	100%	KJ140482	Group 19	PC117R	N/A	N/A
197	*P. solitum*	2009	SA	Plastic crate from supplying to packhouse1	1.00E-163	100%	KJ140483	Group 35	PC138R	N/A	N/A
198	*P. solitum*	2009	SA	Crate of CA cold storage of packhouse1	0	100%	KJ140484	Group 36	PC139R	N/A	N/A
199	*P. solitum*	2009	SA	Crate of CA cold storage of packhouse1	0	100%	KJ140485	Group 18	PC116R	N/A	N/A
200	*P. solitum*	2008	SA	Plastic crate from supplying to packhouse1	0	100%	KJ140486	Group 35	PP55R	N/A	N/A
201	*P. solitum*	2008	SA	Active air of CA cold storage facility in packhouse1	0	100%	KJ140487	Group 32	PP49R	N/A	N/A
202	*P. solitum*	2008	SA	Plastic crates of RA cold storage facility of packhouse1	0	100%	KJ140488	Group 34	PP53R	N/A	N/A
203	*P. solitum*	2008	SA	Plastic crates of RA cold storage facility of packhouse1	0	100%	KJ140489	Group 47	PP74R	N/A	N/A
204	*P. spinulosum*	2010	SA	Plastic crates of CA cold storage facility of packhouse2	0	99%	KJ140490	Group 12	IS14.67R	N/A	N/A
205	*Penicillium spp.*	2008	SA	Active air of RA cold storage facility of packhouse1	1.00E-119	100%	KJ140491	Group 11	PP17R	N/A	N/A
206	*Penicillium spp.*	2008	SA	Drench of packhouse1	3.00E-69	99%	KJ140492	Group 12	PP18R	N/A	N/A
207	*Penicillium spp.*	2008	SA	Floor of RA cold storage facility of packhouse1	1.00E-119	100%	KJ140493	Group 16	PP23R	N/A	N/A
208	*Penicillium spp.*	2008	SA	Plastic crate on farm supplying to packhouse1	3.00E-69	99%	KJ140494	Group 19	PP29R	N/A	N/A
209	*Penicillium spp.*	2011	UK	Passive air of distribution centre	4.00E-139	100%	KJ140495	Group 50	ISA51R	N/A	N/A
210	*Penicillium spp.*	2011	UK	Active air of RA cold storage facility in Re-pack2	4.00E-93	97%	KJ140496	Group 54	ISA55R	N/A	N/A
211	*Penicillium spp.*	2011	UK	Floors of RA cold storage facility of Re-pack2	2.00E-121	100%	KJ140497	Group 60	ISA62.2R	N/A	N/A
212	*Penicillium spp.*	2011	UK	Small waste bins in Re-pack2	1.00E-113	98%	KJ140498	Group 103	ISA112R	N/A	N/A
213	*Penicillium spp.*	2011	UK	Passive air in retailer display area	1.00E-123	97%	KJ140499	Group 15	ISA15R	N/A	N/A
214	*Penicillium spp.*	2009	SA	Plastic crate on farm supplying to packhouse1	3.00E-115	97%	KJ140500	Group 32	PC135R	N/A	N/A
215	*Penicillium spp.*	2008	SA	Drench of packhouse1	4.00E-144	100%	KJ140501	Group 3	PP5R	N/A	N/A
216	*Penicillium spp.*	2008	SA	Active air of RA cold storage facility in packhouse1	9.00E-146	99%	KJ140502	Group 7	PP12R	N/A	N/A
217	*Penicillium spp.*	2008	SA	Plastic crate in CA cold storage facility of packhouse1	4.00E-170	100%	KJ140503	Group 22	PP32R	N/A	N/A
218	*Penicillium spp.*	2008	SA	Active air in CA cold storage of packhouse1	2.00E-63	84%	KJ140504	Group 25	PP37R	N/A	N/A
219	*Penicillium spp.*	2008	SA	Active air of packing area of packhouse1	6.00E-173	100%	KJ140505	Group 53	PP92R	N/A	N/A
220	*Penicillium spp.*	2008	SA	Floor of RA cold storage facility of packhouse1	2.00E-63	84%	KJ140506	Group 39	PP61R	N/A	N/A
221	*Penicillium spp.*	2009	SA	Floor of RA cold storage facility of packhouse1	0	100%	KJ140507	Group 11	PC108R	N/A	N/A
222	*P. waksmanii*	2011	UK	Passive air of distribution centre	6.00E-173	99%	KJ140508	Group 4	ISA4.2R	N/A	N/A