LOW TEMPERATURE GROWTH AND ENZYME PRODUCTION IN *PENICILLIUM* SER. *CORYMBIFERA* SPECIES, CASUAL AGENTS OF BLUE MOLD STORAGE ROT IN BULBS

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SUMMARY

Taxa from the *Penicillium* ser. *Corymbifera* are reported as causing a damaging storage rot upon commercially grown vegetables and flower bulbs; however many reports of blue mould rot in the literature are based on outdated taxonomy. *Pencillium* ser. *Corymbifera* taxa are unique from other *Penicillia* because of their ability to grow at 0°C and proliferate at 5°C, their hemi-cellulase and cellulase activity at 5 and 15°C, and lack of protease production. *Penicillium hordei* differs slightly from this characterization by the production of proteases and *Penicillium allii* differs by the lack of cellulase and hemicellulase production below 15°C. With the exception of *P. allii*, all six of the remaining ser. *Corymbifera* taxa have a potential to proliferate at low temperatures and would therefore cause disease under storage conditions.

Key words: storage rot, *Corymbifera*, blue mould, enzymatic profiling, bulbs.

INTRODUCTION

The genus *Penicillium* encompasses a large number of species that are capable of growing on a diverse range of substrates. Several *Penicillium* species are known to cause a storage rot upon commercially grown agricultural products, commonly referred to as blue mould or wet rot. *Penicillium italicum* Wehmer and *Penicillium digitatum* (Pers.: Fr) are known to rot citrus fruit (Birkinshaw *et al.*, 1931; Filtenborg *et al.*, 1996); *Penicillium expansum* Link, *Penicillium crustosum* Thom and *Penicillium solitum* Wesling to rot pomaceous (especially *Malus* spp.) and drupaceous fruit (Raper and Thom, 1949; Samson *et al.*, 1976; Pitt *et al.*, 1991); *Penicillium crustosum* Thom and *Penicillium discolor* Frisvad and Samson have been reported to rot various nuts (El-Banna and Leistner, 1988; Frisvad *et al.*, 1997; Overy *et al.*, 2003); *Penicillium brevicompactum* Dierckx to rot ginger (Overy and Frisvad, 2004) and *Penicillium sclerotigenum* Yamamoto to rot yams (Yamamoto *et al.*, 1955). These fungi are capable of producing a range of extracellular enzymes to support growth and species-specific variations in enzyme production have been used as a tool in the past to aid in the taxonomic separation of species (Cruickshank and Pitt, 1987).

Taxa from the *Penicillium* ser. *Corymbifera* have the ability to cause a storage rot of commercially grown flower and vegetable bulbs. Blue mould rot has previously been reported from various *Allium* spp. (Vincent and Pitt, 1989; Bertolini and Tian, 1996; Filtenborg *et al.*, 1996), *Hyacinthus orientalis* (Chauhan and Saaltink, 1969), *Iris hollandica* (Doss *et al.*, 1989; Hill, 1977), *Narcissus* sp. (Nicholson and Ingram, 1989) and *Tulipa gesneriana* (Prince *et al.*, 1988; Smid *et al.*, 1995); however many of these reports are based upon outdated fungal taxonomy, mostly attributing bulb losses to a fungus identified as *Penicillium corymbiferum* Westling [currently accepted as a synonym of *Penicillium hirsutum* Dierkx (Frisvad *et al.*, 2000)]. To date, the ser. *Corymbifera* is currently comprised of 7 accepted taxa (Table 1) (Frisvad *et al.*, 2000; Overy and Frisvad, 2003). Frisvad *et al.* (2000) based their series description upon commonalities in secondary metabolite production, micro morphology and habitat commonalities associated with the rhizosphere of vegetables. All of the ser. *Corymbifera* fungi produce the neurotoxin roquefortine C (Frisvad and Filtenborg, 1989). Additionally *Penicillium radicicola* Overy and Frisvad produces the nephrotoxin citrinin and *Penicillium tulipae* Overy and Frisvad produces the potent neurotoxin penitrem A (Overy and Frisvad, 2003).

Commercially grown *Allium cepa* (onion) is also susceptible to losses from blue mold rot both during storage and while grown in the field. The cell wall of *A. cepa* have been reported to be composed of approximately 42% pectin, 20% cellulose (ranging up to 30%) and 37% hemicellulose (with xyloglucan as the principle hemicellulose); while having a protein content of less than 1% of the cell wall dry weight (Mankarios *et al.*, 1980; Séné *et al.*, 1994). In dicotyledonous and non-graminaceous monocotyledonous plants, xyloglucan is

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Table 1. List of subgenus *Penicillium* species surveyed from the IBT culture collection, including series and species name and isolation source (categorized as either originating from a food product, a soil sample, plant or animal tissue).

Series name	Isolate name	IBT culture collection numbers [a]	Isolation source
Corymbifera	*P. albocoremium*	10682, 16884, 21071*, 21502, 22521*	Food, plant
Corymbifera	*P. allii*	3772*, 4112, 21503	Plant
Corymbifera	*P. hirsutum*	12398, 18739*, 19340, 21531*, 22221	Animal, plant, soil
Corymbifera	*P. hordei*	4154, 21532, 23024*	Plant
Corymbifera	*P. radicicola*	10693*, 10696*, 22520, 22526*, 22536	Plant, soil
Corymbifera	*P. tulipae*	3458, 10676*, 10681	Plant
Corymbifera	*P. venetum*	16215, 22111*, 23039, 23040*	Plant, soil
Camemberti	*P. commune*	21513	Plant
Camemberti	*P. crustosum*	21518	Food
Camemberti	*P. palitans*	21540, 23372	Plant, soil
Chrysogena	*P. chrysogenum*	21511, 23350	Plant, soil
Chrysogena	*P. nalgiovense*	23346	Soil
Claviformia	*P. coprophilum*	12724	Plant
Expansa	*P. expansum*	15658, 21525	Food, plant
Solita	*P. echinulatum*	21524	Food
Solita	*P. solitum*	23307	Soil
Urticicolae	*P. dipodomyicola*	19341	Plant
Verrucosa	*P. nordicum*	15295, 23327	Plant, soil
Viridicata	*P. aurantiocandidum*	14124, 21506	Plant, soil
sg. *Furcatum*	*P. lanosum*	17537, 20686	Soil
sg. *Furcatum*	*P. raistrickii*	18057, 21398	Soil
sg. *Furcatum*	*P. soppii*	18221	Soil

[a] * indicates isolates not used for enzyme screening.

thought to form a matrix acting to interlock cellulotic micro fibrils to form a supportive network within the cell wall (Carpita and Gibeaut, 1993).

In the following study we profiled 38 species representing 9 *Penicillium* series, plus strains from the subgenus *Furcatum*, by growth rate and extracellular enzyme production at varying temperatures using principle component analysis in an attempt to: i) substantiate the ser. *Corymbifera* definition as being distinct within the subgenus *Penicillium* and ii) determine which of the ser. *Corymbifera* taxa have a potential to proliferate under storage conditions and thus most likely to be problematic in a storage environment.

MATERIALS AND METHODS

A list of isolates representing a variety of *Penicillium* species available in the IBT culture collection, BioCentrum-DTU was compiled (Table 1) and cultures were revived by streak plating on Czapek yeast extract autolysate agar (CYA; for recipe see Samson *et al.*, 2000). Spore suspensions were prepared and all isolates were 3-point inoculated on CYA and incubated in the dark at 0, 5, 10, 15, 20, 25 and 30°C. All inoculations were initially kept at room temperature for 12 h to facilitate germination. Colony diameters were measured at 7 days for cultures incubated at 10, 15, 20, 25 and 30°C; 14 days

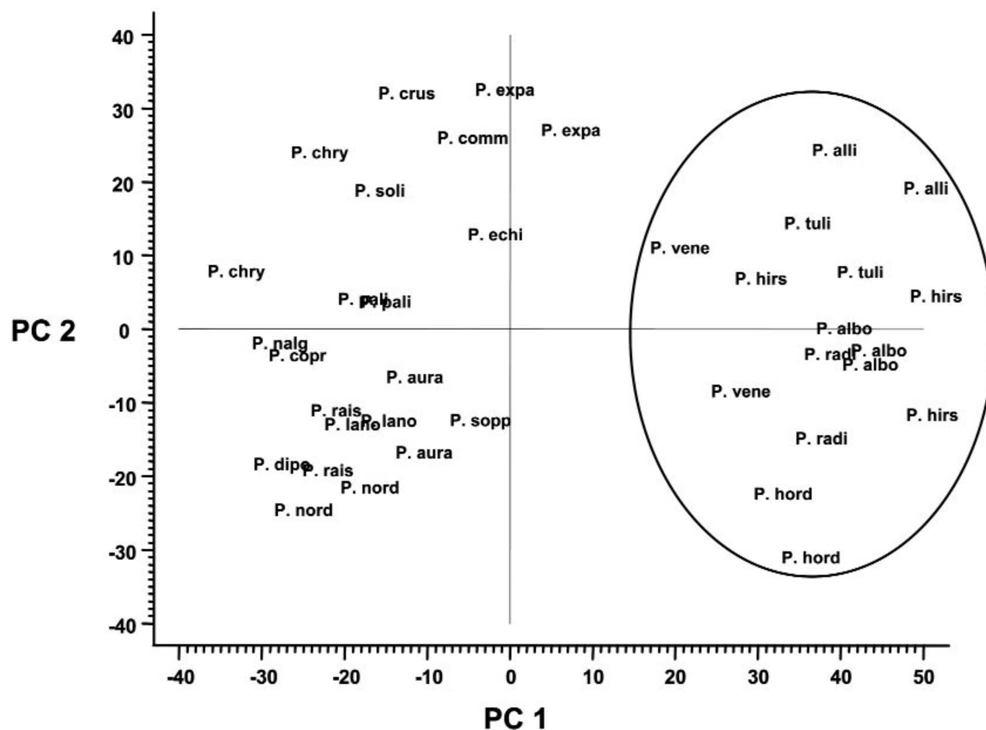

Fig. 1. PCA score plot for PC 1 (50% explained variance) and PC 2 (20% explained variance) based on growth rate and enzyme activity data collected from *Penicillium* series *Corymbifera* taxa (circled) and other Penicillia. Abreviations represented as follows: P. albo: *P. albocoremium*; P. alli: *P. allii*; P. aura: *P. aurantiocandidum*; P. chry: *P. chrysogenum*; P. comm: *P. commune*; P. copr: *P. coprophilum*; P. crus: *P. crustosum*; P. dipo: *P. dipodomyicola*; P. echin: *P. echinulatum*; P. expa: *P. expansum*; P. hirs: *P. hirsutum*; P. hord: *P. hordei*; P. lano: *P. lanosum*; P. nalg: *P. nalgiovense*; P. nord: *P. nordicum*; P. pali: *P. palitans*; P. radi: *P. radicicola*; P. rais: *P. raistrickii*; P. soli: *P. solitum*; P. sopp: *P. soppii*; P. tuli: *P. tulipae*; and P. vene: *P. venetum*.

for 5°C; 30 days for 0°C.

To examine extracellular enzyme production, protein-based and cellulose-based media broths were prepared for fungal cultivation. The protein-based broth was 4 g l^{-1} casein (technical from bovine milk, C-7078; Sigma, Saint Louis, USA), while the cellulose-based broth consisted of 10 g l^{-1} cellulose (fibrous C-6288; Sigma, Saint Louis, USA), 1 g l^{-1} yeast extract (212750; Difco, Sparks, MD, USA) and 4 g l^{-1} oat flour (obtained from Michael's Bageri, Kyringevej 50, 4100 Ringsted, Denmark). To both broth media, 500 ml l^{-1} of trace metal solution [consisting of $(NH_4)H_2PO_4$ 6 g l^{-1}, K_2HPO_4 3 g l^{-1}, $MgSO_4{\cdot}7H_2O$ 2 g l^{-1}, $CaCl_2{\cdot}2H_2O$ 1 g l^{-1}, $Fe_2(SO_4)_2{\cdot}7H_2O$ 25 mg l^{-1}, $CuSO_4$ 25 mg l^{-1}, $MnCl_2{\cdot}4H_2O$ 15 mg l^{-1}, and $ZnSO_4{\cdot}7H_2O$ 15 mg l^{-1}] was added and the broths were autoclaved at 121°C for 20 min. Selected isolates (Table 1) were inoculated into 3 ml of both the cellulose-based and protein-based broths and incubated at 15 and 25°C for 8 days and 5°C for 14 days. The media was then removed with a Pasteur pipette and centrifuged to remove mycelia. The broth was tested for the presence of extracellular enzymes on 5 different substrates: Azurine-crosslinked (AZCL)-β-glucan, AZCL-xylan (oat), AZCL-arabinoxylan, AZCL-hemi-cellulose, and AZCL-casein (all Megazyme, International Ireland Ltd., Wicklow, Ireland). Each AZCL medium type was imbedded as 1% solution in 15% agar into which 4 mm diameter wells were cut and 30 ml of broth was individually added and incubated for 12 h at a corresponding temperature of 5, 15 and 25°C. The enzymatic activity of each isolate was then evaluated based on the quantity of dye released using a scale from 0 to 3 (as follows: 0 representing no dye visualized; 1 indicating weak colour development; 2 indicating average colour development; 3 to indicate strong colour development).

A matrix was constructed consisting of 38 objects (fungal isolates) and 22 variables (isolate colony diameter on CYA at 0, 5, 10, 15, 20, 25 and 37°C and extracellular enzymatic activity at 5, 15 and 25°C for 5 different AZCL media types). All data were standardized by multiplying with a weight of the inverse of the standard deviation. Principle component analysis was performed using The Unscrambler version 7.6 (CAMO, Oslo, Norway) (Esbensen *et al.*, 1998) to ascertain relationships and relatedness of the various sampled *Penicillium* species as represented by the data matrix.

RESULTS

Relatedness between the various *Penicillium* species as determined by PCA, based on their ability to grow at various temperatures and production of extracellular enzymes of the selected isolates, are displayed in Fig. 1. A total of 70% of the variance in the data could be explained after 2 principal components (PCs). Isolates representing the ser. *Corymbifera* aggregate, separated from the remaining *Pencillium* isolates by a strong positive loading along PC 1. PC 1 described 50% of the variation contained within the data matrix and was predominantly represented by growth rate on CYA at 5°C after 14 days growth and to a lesser extent by growth rates at 0, 10 and 15°C (data not shown). PC 2 described a further

20% variation in the data matrix, separating members of the series *Camemberti*, *Expansa*, *Solita* and part of *Chrysogena* (having a positive loading along PC 2 represented by growth rates at 20 and 25°C and hemicellulase activity at 25°C) from members of the series *Urticicolae*, *Verrucosa* and *Viridicata* and taxa from *subgenus Furcatum* (having a negative loading represented by arabinoxylanase, xylanase and protease activity at 5, 15 and 25°C) (data not shown). To investigate further the role of extracellular enzyme activity in the separation of Penicillia, the growth rate data were removed from the data matrix and a second PCA was run (data not shown). Explained variance of the data matrix was rather low in comparison with PC 1 and PC 2 explaining 29 and 20% of the variance respectively. In this PCA, ser. *Corymbifera* species isolates [*Penicillium albocoremium* (Frisvad) Frisvad, *P. hirsutum*, *P. radicicola*, *P. tulipae* and *Penicillium venetum* (Frisvad) Frisvad] had slightly positive loadings along PC 1 and separated from the remaining *Penicillia* as a result of a negative loading along PC 2 (represented by β-glucanse and hemi-cellulase activity at 5 and 15°C and cellulase activity at 5°C). *P. hordei* Stolk, isolates separate from the ser. *Corymbifera* taxa by positive loading along PC 2 as represented by protease activity at 5, 15 and 15°C. *Penicillium allii* Vincent and Pitt isolates also separate from the ser. *Corymbifera* taxa due to high negative loadings along PC 1, represented by high β-glucanase and hemi-cellulase activity at 25°C.

Growth rate data for taxa representing the ser. *Corymbifera* are presented in Table 2. None of the ser. *Corymbifera* species grew markedly at 30°C; however all of the species grew consistently at 0°C with *P. radicicola* having the greatest colony diameter (19 mm after 30 days). Vigorous growth occurred for all ser. *Corymbifera* species at 5°C. Colony diameters for all species grown for 14 days at 5°C reached or exceeded the colony diameter obtained at optimal growth temperatures after 7 days (34 to 44 mm). Optimal growth temperature for all ser. *Corymbifera* species lay between 20 and 25°C.

When extracellular enzyme activity was compared for ser. *Corymbifera* taxa to the remainder of examined *Penicillia* (Table 3), ser. *Corymbifera* species demonstrated a 1.5 to 2-fold increase in arabino-xylanase, β-glucanase, hemi-cellulase and xylanase production at 5°C compared to other *Penicillium* series species. Extracellular protease production differed considerably between ser. *Corymbifera* species and the other Penicillia tested. Protease production ranged from no enzyme production at 5°C to weak enzyme production at 25°C for ser. *Corymbifera* strains whereas protease activity for the other Penicillia at 15 and 25°C was more than double the measured value for ser. *Corymbifera* strains.

Table 2. Average colony diameter (mm) for *P.* series *Corymbifera* strains incubated on CYA at 10, 15, 20, 25 and 30°C after 7 days, 5°C after 14 days and 0°C after 30 days.

Species	0°C, 30 d (±SD)	5°C, 14 d (±SD)	10°C, 7 d (±SD)	15°C, 7 d (±SD)	20°C, 7 d (±SD)	25°C, 7 d (±SD)	30°C, 7 d (±SD)
P. albocoremium (n=5)	14.9 (±4.0)	37.9 (±4.5)	20.3 (±3.1)	36.0 (±2.4)	38.6 (±3.1)	36.0 (±5.0)	3.5 (±3.5)
P. allii (n=3)	14.5 (±3.7)	37.5 (±8.0)	13.0 (±3.2)	36.8 (±3.2)	37.5 (±4.8)	44.2 (±4.9)	1.7 (±1.5)
P. hirsutum (n=5)	15.4 (±7.0)	36.7 (±10.3)	19.5 (±3.1)	35.5 (±1.4)	39.2 (±5.4)	37.1 (±4.2)	2.0 (±1.8)
P. hordei (n=3)	8.2 (±2.6)	37.7 (±3.5)	12.8 (±1.8)	34.3 (±1.4)	35.2 (±1.2)	40.0 (±3.9)	2.8 (±2.6)
P. radicicola (n=5)	19.0 (±2.3)	40.2 (±8.1)	14.3 (±2.2)	33.5 (±2.7)	34.8 (±5.3)	35.7 (±3.1)	0.7 (±0.5)
P. tulipae (n=3)	13.3 (±1.5)	43.7 (±5.3)	15.7 (±2.3)	32.3 (±2.5)	40.0 (±4.2)	42.8 (±3.0)	0.5 (±0.5)
P. venetum (n=4)	11.0 (±3.4)	30.8 (±10.1)	11.2 (±1.5)	31.6 (±0.8)	33.7 (±1.8)	27.0 (±3.8)	0.7 (±0.7)

Table 3. Comparison of average extracellular enzyme production between ser. *Corymbifera* taxa (n=16) and the other *Penicillium* species (n=20) screened at 5, 15 and 25°C (0: no enzyme production, 1: weak enzyme production, 2: moderate enzyme production, 3: strong enzyme production).

	Arabino-Xylanase	Beta-Glucanase	Proteinase	Hemi-Cellulase	Xylanase
Series *Corymbifera* (5°C)	1.9	8.0	0	1.5	1.3
Penicillia excl. Series *Corymbifera* (5°C)	1.6	5.0	0.1	1.0	0.6
Series *Corymbifera* (15°C)	1.9	1.6	0.4	1.7	1.7
Penicillia excl. Series *Corymbifera* (15°C)	1.8	1.0	0.8	1.0	1.3
Series *Corymbifera* (25°C)	2.1	2.1	0.7	2.0	1.6
Penicillia excl. Series *Corymbifera* (25°C)	2.7	2.0	1.8	1.5	2.5

Table 4. Average extracellular enzyme production for series *Corymibfera* species at 5 / 15 / 25°C (0: no enzyme production, 1: weak enzyme production, 2: moderate enzyme production, 3: strong enzyme production).

Species	Arabino-Xylanase	β-Glucanase	Proteinase	Hemi-Cellulase	Xylanase
P. albocoremium (n=3)	2 / 2 / 2	1 / 2 / 2.5	0 / 0.5 / 1	2 / 2 / 2.5	1.5 / 1.5 / 1.5
P. allii (n=2)	0 / 0 / 1.5	0 / 1 / 2.5	0 / 0 / 0	0 / 2 / 2.5	1 / 1 / 1.5
P. hirsutum (n=3)	2/ 2 / 2	0.5 / 1.5 / 2	0 / 0 / 0	1 / 1.5 / 2	1 / 1.5 / 1.5
P. hordei (n=2)	2.5 / 2.5 / 2.5	0.5 / 1 / 1.5	1.5 / 2 / 2	1 / 1 / 1	2 / 2.5 / 2
P. radicicola (n=2)	3 / 3 / 2.5	1 / 2 / 2.5	0 / 0 / 0.5	2 / 2 / 2	2 / 3 / 2
P. tulipae (n=2)	2 / 1.5 / 1	1 / 1.5 / 2	0 / 0.5 / 1	1 / 1.5/ 2	0 / 0 / 0
P. venetum (n=2)	1 / 1.5 / 2	2 / 2 / 2	0 / 0 / 0	2 / 2 / 2	1 / 1.5 / 2

Extracellular enzyme activity for ser. *Corymbifera* species over 5-25°C are detailed in Table 4. In general, extracellular enzymes for all of the ser. *Corymbifera* taxa were active at 5°C with the exception of *P. allii*. Arabino-xylanase and xylanase production remained consistent from 5 to 25°C for most ser. *Corymbifera* species; however, production increased for *P. venetum* and *P. allii* between 5 and 25°C (15-25°C for *P. allii*) while *P. tulipae* strains decreased production of arabino-xylanase and did not produce xylanase when grown between 5 and 25°C. Both β-glucanase, and to a lesser extent, hemi-cellulase production increased on average between 5 and 25°C for ser. *Corymbifera* species, with the exception of *P. radicicola* for hemi-cellulase and *P. venetum* for hemi-cellulase and β-glucanase production, where production remained constant over the 5-25°C range. Within the ser. *Corymbifera*, protease production was limited to *P. hordei* with consistent, moderate production between 5 and 25°C and to very weak production by *P. albocoremium*, *P. radicicola* and *P. tulipae* at 25°C.

DISCUSSION

Results obtained in this survey support the classification of the ser. *Corymbifera* taxa as being related and unique when grouped against other Penicillia based upon extracellular enzymatic activity and temperature tolerance. Enzymatic activity and temperature profiling have been proven in the past as an efficient means of evaluating and/or testing taxonomic characterizations based upon colony appearance and micromorphology (Pitt, 1973). Frisvad *et al.* (2000) classified the ser. *Corymbifera* based predominantly upon shared characteristics in secondary metabolite production and a common habitat: the rhizosphere of vegetables and bulbs (especially *Allium* species). In addition to those characteristics, the ser. *Corymbifera* taxa differ from the other Penicillia tested in this study in the production and activity of hemi-cellulase and cellulase enzymes at lower temperatures and the lack of protease production at most temperatures. *P. hordei* strains differed from the ser. *Corymbifera* in their moderate production of proteases at all temperatures. When describing the series, Frisvad *et al.* (2000) noted that all ser. *Corymbifera* species share the same habitat except *P. hordei*, which is predominantly found on cereals, a substrate which is higher in protein content than most bulbs.

With the exception of *P. allii*, all of the ser. *Corymbifera* taxa were capable of producing the extracellular enzymes arabino-xylanase, xylanase, β-glucanase and hemi-cellulases at 5°C. Although the extracellular profile of *P. allii* differs from the remainder of the *Corymbifera* series at lower temperatures (5°C), the extracellular enzymatic profile is characteristic of the *Corymbifera* series at 25°C (i.e. strong hemi-cellulase and cellulase production while having no protease production). As *P. allii* was the least active producer of extracellular enzymes at temperatures of 15°C and lower, it is unlikely that this species will be problematic in a storage environment held at temperatures below 10°C; however, the pronounced extracellular enzymatic activity of this species at 25°C indicates that this species is a potentially problematic field pathogen. *P. allii* was first isolated and described from blue mold infected *Allium sativum* in 1989 (Vincent and Pitt, 1989). In recent damp chamber pathogenicity trials, *P. allii* was characterized as an aggressive pathogen of *A. sativum*; where as *P. hirsutum*, in comparision, produced only minor lesions of discoloration around the point of inoculation (Overy *et al.*, 2004). In an earlier study using controlled field experiments (Smalley and Hansen, 1962), blue mold rot of *A. sativum* demonstrated 80% destruction of bulbs inoculated with a strain of *P. corymbiferum* (a later synonym of *P. hirsutum*). In their study, optimal infection conditions were reported at 25°C and crop losses were minimal at 5°C which corresponds more correctly with the extracellular activity demonstrated here for *P. allii* rather than *P. hirsutum* (synonym *P. corymbiferum*). At the time of the Smalley and Hansen experiments, *P. allii*, which was once considered a variety of *P. hirsutum*

(*P. hirsutum* var. *allii*) (Frisvad and Filtenborg, 1989), had not yet been characterized as a species and would have been considered as a strain of *P. corymbiferum*.

All of the ser. *Corymbifera* taxa were capable of growth at storage temperatures (displaying growth at 0 and 5°C) upon CYA media. As is demonstrated here, cellulase and hemicellulase activity for *P. albocoremium*, *P. hirsutum*, *P. hordei*, *P. radicicola*, *P. tulipae* and *P. venetum* remained highly active at refrigeration temperatures. All six of these species may prove to be problematic in a refrigerated environment as refrigeration conditions provide an ideal environment for development and proliferation of these species. Bulbs that are commercially grown for sale as flowering bulbs have to be placed in refrigerated conditions for an extended period of time to prevent abnormal flower development (2-10°C for 8-15 weeks for dormancy release in *Tulipa gesneriana* (De Hertogh and Le Nard, 1993; Kamenetsky *et al.*, 2003). Blue mould rot in bulbs occurs via superficial wounding sites and from natural ruptures in the basal plate at the site of root emergence (Saaltink, 1971) where mucilage is excreted to form a protective barrier. In these regions, primary infection symptoms begin with a brown discoloration of the root initials, which is succeeded by fungal proliferation resulting in reduced root growth. Bulbs suffering from a ser. *Corymbifera* infection result in marketable crop losses due to an increased rate of floral abortion.

As all of the ser. *Corymbifera* taxa (with the exception of *P. allii*), can grow and proliferate at storage temperatures, all of these species pose a potential storage threat to bulb growers and therefore correct pathogen/host affiliations become relevant to aid in disease diagnostics. In addition to the potential for post harvest crop losses, the risk of mycotoxin contamination by these fungi should be considered and therefore correct identification of the agent causing a blue mold rot becomes even more relevant (Overy *et al.*, 2004). For example, high cellulase and hemicellulase activity along with low proteinase expression by ser. *Corymbifera* species corresponds to the cell wall composition of *Allium cepa* and therefore is ideal for the breakdown of these bulbs, even at storage temperatures of 10°C or lower. With the exception of *P. hordei*, all of the ser. *Corymbifera* taxa have been recently proven to be pathogenic upon *A. cepa* (Overy *et al.*, 2004). *A. cepa* crops that are used as flavorings in large scale food production are ground up whole in large batches where potential mycotoxin contamination of these products caused by the growth of ser. *Corymbifera* taxa might be overlooked. Future pathogenic studies involving ser. *Corymbifera* taxa infecting a variety of commercially grown flower and vegetable bulbs is recommended to further clarify outstanding relationships currently present in the literature.

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