RESEARCH LETTER

Copper deficiency in potato dextrose agar causes reduced pigmentation in cultures of various fungi

Garth W. Griffith[1], Gary L. Easton[1], Andrew Detheridge[1], Kevin Roderick[1], Arwyn Edwards[1], Hilary J. Worgan[1], Jean Nicholson[1] & William T. Perkins[2]

[1]Institute of Biological Sciences, Aberystwyth University, Penglais, Aberystwyth, Ceredigion, Wales, UK; and [2]Institute of Geography and Earth Sciences, Aberystwyth University, Penglais, Aberystwyth, Ceredigion, Wales, UK

Correspondence: G.W. Griffith, Institute of Biological Sciences, Aberystwyth University, Penglais, Aberystwyth, Ceredigion SY23 3DD Wales, UK. Tel.: 0044 1970 622325; fax: 0044 1970 622350; e-mail: gwg@aber.ac.uk

Present address: G.L. Easton, H.J. Worgan, Institute of Rural Sciences, Aberystwyth University, Aberystwyth, UK.

Received 18 May 2007; accepted 20 August 2007.
First published online October 2007.

DOI:10.1111/j.1574-6968.2007.00923.x

Editor: Nina Gunde-Cimerman

Keywords
fungal media; phenoloxidase; laccase; micronutrient; melanin; organic farming.

Abstract

Potato dextrose agar (PDA) is one of the most commonly used media for the isolation and cultivation of fungi, with morphological features and pigmentation in culture often being important for identification of cultures. Cultivation of various fungi on different brands and batches of powdered (commercial) potato dextrose media revealed deficient pigmentation in five of 10 media tested. Reduced pigmentation on these media was correlated with low levels of copper and colony colour was restored by the addition of copper. Deficient pigmentation was most pronounced when copper levels in the medium were below 50 ng mL^{-1} (50 p.p.b.; 0.8 μM). Differences in pigmentation and laccase activity of spore and mycelial preparations were quantified for representative species belonging to the genera *Aspergillus*, *Fusarium*, *Trichoderma*, *Cladosporium* and *Penicillium* grown on PDA containing different amounts of copper. A strong positive correlation between laccase activity and copper levels was observed. Differences were also found between batches of raw potatoes, with organically cultivated tubers having higher copper levels than those grown by conventional methods, possibly because of the use of copper-based fungicides in the former case. Routine addition of 1000 ng mL^{-1} copper (or standard trace element solutions) to PDA and other undefined media is advised to avoid atypical culture pigmentation and possibly other consequences of reduced activity of copper-requiring enzymes.

Introduction

Identification of fungi cultured from natural substrata (soils, plant materials etc.) is based on the identification of asexual and sexual reproductive structures. The formation of pigments, present on and in spores, sporophores and mycelia, or secreted into the medium, is also an important aid in identification. The choice of medium used for isolation and identification of microfungi affects not only the groups of fungi that are isolated but also the ease with which they can be identified.

Most mycologists, however, routinely use a narrow range of media, of which potato dextrose agar (PDA) is the best known and most widely used (> 18 000 hits for 'Potato Dextrose Agar' in Google Scholar), with reference to use of this medium dating back to the early part of the 20th century (Edgerton, 1908; Duggar *et al.*, 1917). PDA contains 1.5% agar, 2% glucose, with nitrogen, phosphorus, vitamins and micronutrients being derived from a crudely filtered extract of macerated potatoes (57.5 g potatoes L^{-1} of medium; Gams *et al.*, 1998). The high carbon: nutrient ratio of PDA allows good growth and more importantly sporulation and pigmentation for a wide range of fungal taxa.

Copper is a micronutrient essential for fungal growth, playing a role as a cofactor for a range of oxidases and oxygenases, including laccases and other 'blue-copper' oxidases that are involved in pigmentation and catabolism of aromatic compounds such as lignin (Griffith, 1994; Thurston, 1994). Although more attention has been paid to mechanisms of copper resistance in fungi in relation to the use of copper as an agricultural fungicide or in response to heavy-metal contamination (Phelan *et al.*, 1990; Fomina *et al.*, 2000), the effects of copper deficiency on fungal growth and development have also been long appreciated. Mulder (1938) found conidial pigmentation in *Aspergillus*

niger to be influenced by copper levels in the medium, with defective pigmentation (buff, yellow or brown conidia) at lower copper concentrations (5–50 ng mL^{-1}) while normal (black) pigmentation required $\geq$75 ng mL^{-1} copper. Similarly, Caesar-Tonthat *et al.* (1995) found that melanin formation by *Gaeumannomyces graminis* was enhanced by the addition of low levels (10 μM = 670 ng mL^{-1}) of copper.

Although potato dextrose broth (PDB) and PDA are very widely used in microbiological research, their preparation from raw materials is rather tedious. Many labs now use powdered commercial preparations, containing lyophilized potato extract mixed with glucose and (for PDA only) agar. The aim of this study was to test the hypothesis that incomplete pigmentation of several fungal species on several commercial brands and batches of PDA/PDB was due to differences in the medium copper content, which altered the activity of phenoloxidase enzymes involved in pigment formation.

Table 1. Final copper concentrations in batches of PDA and PDB from different sources

Product	Supplier	Batch number	Final concentration in medium (ng mL^{-1})*
PDB	A	1	6†
PDB	A	2	50†
PDB	A	3	7†
PDA	B	1	44
PDB	C	1	32†
PDA	D	1	139
PDA	D	2	89
PDA	D	3	41
PDA	E	1	32
PDA	F	1	110

*For PDA and PDB media 39 and 25 g L^{-1} of powder were added, according to the manufacturers' instructions.

†For comparison of copper levels between PDA and PDB media, 20 ng mL^{-1} should be added to the latter to account for the copper present in the added agar.

Materials and methods

Fungal strains

The wild type (Glasgow wild type – G051; genotype *biA1 veA1*) and a yellow–green mutant strain (G0247; *ygA6; biA1*; *veA1*) of *Aspergillus nidulans* were kindly supplied by Dr John Clutterbuck, University of Glasgow. Cultures of *Trichoderma harzianum*, *Fusarium culmorum*, *Stachybotrys atra*, *Cladosporium herbarum* and *Penicillium roqueforti* (ATCC 34908) were from the Aberystwyth University fungal culture collection. Cultures of *Magnaporthe grisea* and *Botrytis cinerea* were kindly supplied by Dr Luis Mur (Aberystwyth University).

Preparation of PDA

Commercial PDA/PDB powders were obtained from various commercial sources (Table 1). The recipe for making PDA from fresh potatoes follows that described by Gams *et al.* (1998) in which 100 g peeled, sliced potatoes are homogenized with 300 mL tap water in a kitchen blender, allowed to steep overnight at 4 °C, before crude filtration through muslin (yielding ca. 300 mL potato extract). A total of 230 mL potato extract, 20 g glucose, 15 g agar (No.2 Agar, Lab Ltd) are mixed before making up to a final volume of 1 L. Commercial and in-house preparations were sterilized by autoclaving (121 °C/15 min) before dispensing into Petri dishes. In all cases distilled water was used to avoid confounding effects associated with varying copper levels in tap water.

Copper analysis

Copper analysis was conducted using inductively coupled plasma MS (ICP-MS). Powdered medium (50 mg; three replicates) or pieces of fresh-peeled potatoes (400 mg; three different tubers analysed per sample) were disrupted (with three 3-mm-diameter acid-washed glass beads) for 60 s in a 2 mL microcentrifuge tube using a dental amalgamator (OralB), before the addition of 1 mL concentrated HNO_3. Blackening of the extract was reduced by addition of 50 μL of the acid before disruption of the tuber sample, with the remaining 950 μL added afterwards. Following digestion at 40 °C for 4 days, samples were evaporated to dryness and resuspended in a final volume of 2 mL 10% HNO_3 containing 100 μg L^{-1} ruthenium chloride as an internal standard. Analysis was conducted under standard conditions using a VG plasma Quad PQII ICP-MS (Thermo Electron Corporation, Winsford, Cheshire, UK).

Measurement of conidial pigmentation and conidial production in *A. nidulans*

Conidial suspensions in 0.01% Nonidet P-40 (a surfactant to reduce clumping of conidia) were prepared separately from 5 days-old cultures (37 °C incubation) of the wild type and *ygA* strains grown on PDA (from Supplier D, batch 1; Table 1). After measuring conidial concentration with a haemocytometer and adjusting the concentration to 3×10^6 conidia mL^{-1} with 0.001% Nonidet P-40, 200 μL of spore suspension (6×10^5 conidia per plate) was spread onto the surface of each 85-mm-diameter Petri dish. Plates were incubated inverted in the dark at 37 °C for 40 h before assessment.

In order to provide a quantitative measure of differences in the pigmentation of the upper surface of cultures growing on different media, 40 h cultures were scanned (face down with lids removed) with an Epson GT12000 scanner. Scanning was conducted at 200 dpi in greyscale (256 levels) and scanned images were saved as TIFF files before importing

into ADOBE PHOTOSHOP (version 5.0 LE). A 4 cm × 4 cm square was cut from the central portion of the image of each plate and image size was reduced to four pixels. For each pixel, darkness ('*K*') was measured and the value averaged to give a single value for each plate. Three replicate plates were assessed for each treatment.

Laccase assays

Cultures were grown on PDA (Supplier E, batch 1; Table 1) with or without copper amendment for 3 days at 37 °C (*A. nidulans*) or 7 days at 25 °C (other species) following spread inoculation of plates with a lawn of 10^6–10^7 conidia. A suspension of conidia in water was prepared by gently scraping the surface of colonies with a toothpick. Conidia were pelleted by centrifugation (13 000 ***g*** for 1 min) and resuspended in 1 mL of 100 mM citrate–200 mM phosphate buffer (pH 5.0) at a concentration of ca. $1–2 \times 10^8$ conidia mL^{-1}, following the method of Pokorny *et al.* (2005). The laccase substrate 2,2′-azino-bis-[3-ethyl-benzthiazoline-6-sulfonic acid] (ABTS; Sigma) was added to a concentration of 0.6 mM and the tubes left for 36 h at 25 °C. Following incubation, the conidia were removed by centrifugation (13 000 ***g*** for 1 min) and the absorbance of the supernatant measured at 405 nm.

A second assay using *N*,*N*-dimethyl-*p*-phenylenediamine sulphate (DMP; Acros 181430250), devised by Clutterbuck (1972), was also used to measure laccase activity. Mycelium and conidia were scraped from a 1 cm^2 area of sporulating cultures. These were disrupted in 700 μL of 100 mM acetate buffer (pH 6.0) for 1 min (four cycles of 15 s; OralB Amalgamator) and centrifuged for 10 min at 13 000 ***g***. Twenty microliters of the supernatant was mixed with 150 μL of DMP (0.8 mg mL^{-1}) in the acetate buffer (in a 96-well microplate) and incubated in a microplate reader (Biotek ELX800IU) at 25 °C, measuring $A_{490\,nm}$ at 2 min intervals for up to 4 h. The effect of adding copper at 200 ng mL^{-1} to the assay mixture was also tested for the wild type and *ygA* mutant strains of *A. nidulans*, using the same assay conditions.

Statistical analyses

Parametric (ANOVA) and nonparametric (Kruskal–Wallis) tests were conducted using MINITAB v 12.23. For ANOVA, Tukey's test (at $P < 0.05$) was used to identify significantly different treatment means.

Results and discussion

Copper levels in different commercial PDA preparations

This study was initiated following the observation of defective conidial pigmentation (lime-green) of colonies of *Aspergillus nidulans* on a batch of PDB (Supplier A, batch 3; supplemented with agar to make PDA). Addition of 100 ng mL^{-1} copper (as $CuSO_4$) to the medium allowed normal pigmentation whilst addition of 1 mM EDTA to this and other brands and batches of PDA inhibited the formation and pigmentation of conidia, though mycelial growth did occur. Analysis of copper levels in the PDB revealed a copper level equivalent to 7 ng mL^{-1} in the final liquid medium (Table 1). The contribution to copper levels in the final medium from addition of 15 g L^{-1} agar powder (from Supplier D) was 20 ng mL^{-1} (analysis of three other batches of standard microbiological agar from two suppliers found copper levels in agar to range from 17 to 24 ng mL^{-1}). Other batches of PDA/PDB from several suppliers were found to vary in their copper content, ranging from 6 to 50 ng mL^{-1} for PDB and 32 to 139 ng mL^{-1} in PDA. In five of the 10 commercial media tested, even accounting for the copper contribution from the agar, the final copper concentration was < 50 ng mL^{-1} and several fungi cultivated on these media showed defective pigmentation. There was no correlation between particular manufacturers or products and it is likely that the variability in PDA products related to the source of potatoes rather than to any artefact of the manufacturing process.

Effect of copper level on conidial pigmentation in *A. nidulans*

In *A. nidulans*, the final step in conidial pigment formation involves a conidial-specific laccase (product of the *yA* gene), which converts a yellow precursor to the dark-green melanin pigment and requires copper as a cofactor (Clutterbuck, 1990). Conidial laccase purified from *A. nidulans* cultures grown on media containing < 64 ng mL^{-1} (1 μM) copper was found to have reduced specific activity (Kurtz & Champe, 1981). Differences in pigmentation were visible when the wild type of *A. nidulans* was plated on different brands and batches of PDA. The effect of copper deficiency was more pronounced in the *ygA* mutant, forming partially pigmented conidia on media that supported normal conidiation of the wild type, as reported by Clutterbuck (1990). Addition of low levels of copper to these PDA media allowed normal pigmentation, with the *ygA* mutant requiring greater supplementation (200 ng mL^{-1}) for normal pigmentation, as shown in Fig. 1. Pigmentation differences were quantified using greyscale scanned images of plates incubated for 40 h (Fig. 2), showing that >100 ng mL^{-1} copper was required to achieve full pigmentation of the *ygA* mutant.

The number of conidia formed by both the wild type and *ygA* mutant strains was also affected by different copper levels ($P < 0.001$; data not shown), though to a lesser extent than conidial pigmentation. A PDA containing 26 ng mL^{-1} copper (Supplier A; batch 1) conidiation of the wild type was reduced to such an extent that the grey pigmentation of the underlying conidiophores (Clutterbuck, 1990) was visible.

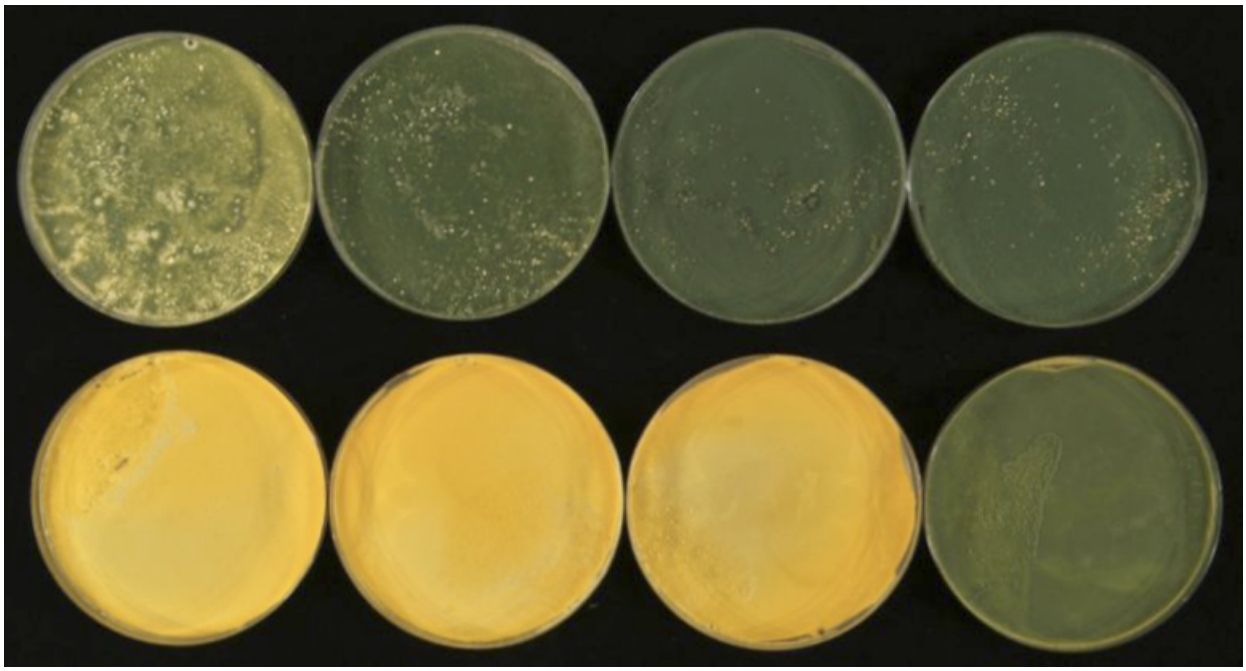

Fig. 1. Effect of addition of copper sulphate to a commercial PDA formulation (Supplier D, batch 3 with 41 ng mL^{-1} copper; Table 1) on pigmentation of wildtype (above) and *ygA* mutant (below) of *Aspergillus nidulans*. From left to right, the amount of copper sulphate added was 0, 5, 15 and 200 ng mL^{-1}.

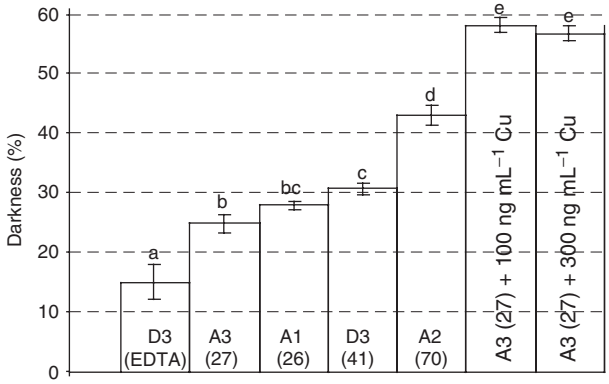

Fig. 2. Pigmentation of the *ygA* mutant strain in media prepared with different levels of added copper as assessed by image analysis in greyscale (darkness level of 100% corresponds to greyscale level 256). Codes for different PDA media correspond to Table 1 and numbers in brackets indicate the copper concentration (ng mL^{-1}) of the basal medium. SD error bars are for replicate samples ($n = 3$). Columns with different superscripts are significantly different (ANOVA $P < 0.001$; $F = 268.87$).

Differences in pigmentation of other microfungi on PDA

A range of microfungi, including several species where distinctive pigmentation patterns are important for identification of taxa, were grown on various PDA media. Differences in pigmentation were readily observed compared with the same media supplemented with additional copper (Fig. 3). This was most noticeable on PDA formulations containing < 50 ng mL^{-1} copper. In addition to the cultures of *T. harzianum*, *F. culmorum*, *S. atra* and *C. herbarum* shown in Fig. 3, atypical pigmentation on copper-deficient media was also observed for *M. grisea*, *B. cinerea* and several *Penicillium* spp.

Influence of copper level in PDA media on laccase activity

The final stages of pigment formation in many fungi, for example *Fusarium* spp. (Kim *et al.*, 2005) and *Trichoderma* spp. (Holker *et al.*, 2002), are known to involve laccases. To explore the possibility that the deficient pigmentation described above was the result of reduced phenoloxidase activity, owing to reduced availability of the copper cofactor, the laccase activity was measured using both ABTS (for conidial suspensions) and DMP (conidial and mycelial extracts). Laccase activity measured using both assays was significantly higher (ANOVA; $P < 0.001$) for cultures grown on PDA containing an additional 30 or 200 ng mL^{-1} copper, compared with unamended PDA (Supplier E; batch 1; containing a basal level of 32 ng mL^{-1} copper) (Table 2).

There was considerable variation in laccase activity between fungal species and some indication that laccase activity was tightly associated with conidia, and thus not detected in supernatants, as noted previously for

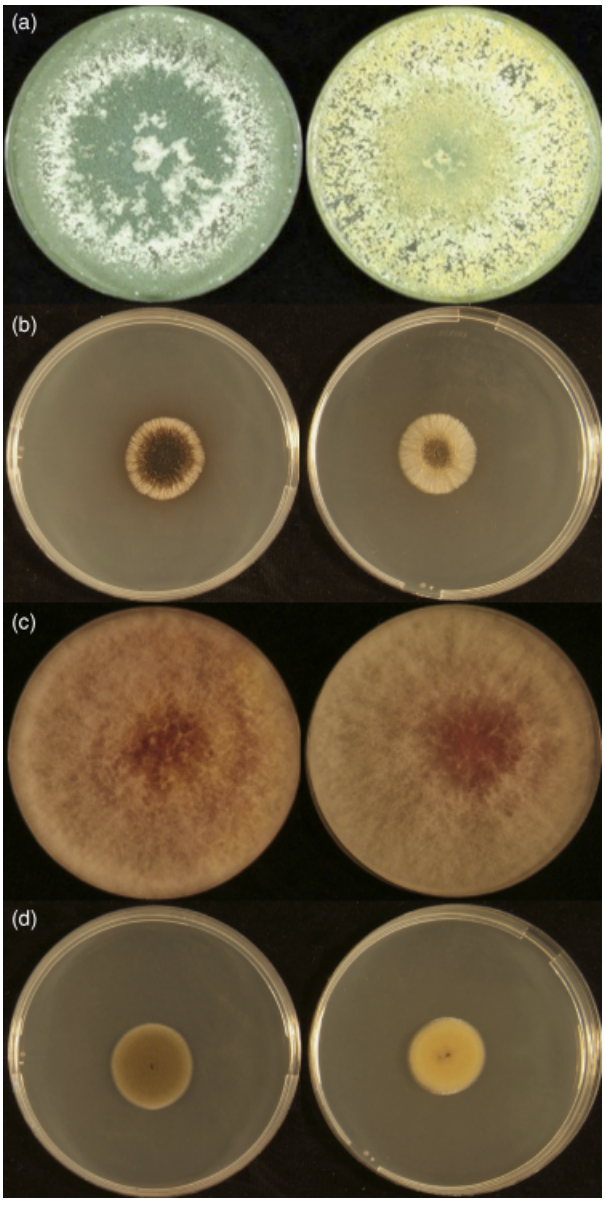

Fig. 3. Sporulation of a range of microfungi on PDA. Plates on the right contain PDA from Supplier E (batch 1; 32 ng mL^{-1} copper in medium), those on the left contain the same medium amended with 1000 ng mL^{-1} copper sulphate. (a) *Trichoderma harzianum*; (b) *Stachybotrys atra*; (c) *Fusarium culmorum*; (d) *Cladosporium herbarum*. Plates were incubated in ambient daylight at 22 °C for 6 days.

Trichoderma spp. (Pokorny *et al.*, 2005). In some cases there was evidence of substrate specificity of the laccase activity, for instance in the case of *C. herbarum* and *P. roqueforti*, which oxidized DMP but not ABTS (the standard laccase substrate). *Stachybotrys atra* showed no oxidizing activity with either substrate. For all the species investigated, oxidization of DOPA was also poor (data not shown). In the case of the *ygA* mutant of *A. nidulans*, copper amendment did not alter laccase activity, despite the fact that conidial pigmentation was visibly different between the three treatments. However, addition of copper (to a final concentration of 200 ng mL^{-1} in the DMP assay mixture) to conidial extracts of *ygA* mutant strains of *A. nidulans*, caused a twofold increase in laccase activity ($P < 0.001$), suggesting that the low activity detected for this strain was related to the absence of the required cofactor rather than to the absence of the enzyme.

The poor conidial pigmentation of *ygA* mutants is believed to be due to deficiency in copper uptake (Clutterbuck, 1972). Comparison of the classical linkage map and genome sequence of *A. nidulans* strongly suggests that the *ygA* locus corresponds to the auto-called gene AN3624.2 (Genbank: AACD01000061). The putative peptide sequence from this gene (1182 aa; Swissprot accession Q5B756_EMENI) shows high similarity (> 60% identity) with several other fungal proteins, all of which contain copper ion-binding domains and ATP-binding sites. Best studied of these genes is the *clap1* gene in *Colletotrichum lindemuthianum*, listed as a copper-transporting ATPase, with mutants at this locus having defective spore pigmentation remediated by the addition of 3200 ng mL^{-1} (50 μM) copper (Parisot *et al.*, 2002).

Copper levels in extracts from different potato samples

Most mycologists now use commercial preparations of PDA in which potato extract prepared, dried and powdered (usually by a third party) is mixed with glucose and agar. Older recipes for PDA reveal a range of methods for the preparation of the medium, for example, Stevens (1974) recommended the use of peeled 'old white' potatoes autoclaved with water before straining, while Booth (1971) recommended that new potatoes should be avoided. Ainsworth *et al.* (2001) state that potatoes should be scrubbed (not peeled) and that the variety Red Desirée is found to be the best. There is also anecdotal evidence suggesting that PDA should be made using 'organically' cultivated potatoes (J.C. Frankland, pers. commun., 2005).

Copper levels in 'organically' cultivated potatoes were higher than in conventionally cultivated tubers (ANOVA $P < 0.001$; $F = 24.3$), with the means of 1194 and 715 ng mL^{-1}, respectively (Table 3). Analysis of filtered extracts made from 27 tuber samples prepared according to a standard recipe for PDA (Gams *et al.*, 1998) revealed a sixfold range of copper concentrations and confirmed the significantly higher copper content of extracts from tubers grown under organic management regimes (supplementary Table S1 and supplementary Fig. S1). By comparison with copper levels in these potato extracts, it was estimated that copper levels in 'homemade' PDA would range from 33 to

Table 2. Laccase activity measured in conidial suspension (ABTS) or conidium/mycelium extracts (DMP) of fungal cultures grown on PDA (batch E1) with different levels of added copper

	Absorbance in ABTS assay (405 nm)*				Absorbance in DMP assay (490 nm)*			
Species	0†	30†	200†	*P* value	0†	30†	200†	*P* value
Aspergillus nidulans WT	0.169[a]	0.204[b]	0.178[a]	0.005	0.439[a]	0.716[b]	0.748[b]	< 0.001
WT+Cu in assay‡	ND	ND	ND		0.691[a]	0.888[b]	0.951[c]	< 0.001
Aspergillus nidulans ygA1	0.171	0.168	0.170	NS	0.409	0.412	0.409	NS
ygA1+Cu in assay‡	ND	ND	ND		0.639[a]	0.720[b]	0.802[c]	< 0.001
Fusarium culmorum	0.586[a]	1.645[b]	2.718[c]	< 0.001	0.297[a]	0.509[b]	0.720[c]	< 0.001
Trichoderma harzianum	0.157[a]	0.418[c]	0.340[b]	< 0.001	0.290[a]	0.298[a]	0.333[b]	< 0.001
Penicillium roqueforti	0.000	0.000	0.000	NS	0.143 [a]	0.200 [b]	0.210 [b]	< 0.001
Cladosporium herbarum	0.000	0.000	0.000	NS	0.207[a]	0.204[a]	0.216[b]	0.003

Absorbance readings for each isolate × assay combination with different superscripts were significantly different (Tukey's test; $P < 0.05$).
*Absorbance readings were taken after incubation for 36 h (ABTS) or 2 h (DMP), except for *F. culmorum* (12 h with ABTS).
†Cultures were grown on PDA medium (containing 32 ng mL^{-1} copper) with 0, 30 or 200 ng mL^{-1} copper added to the medium (total copper content of media used are 32, 62, 232 ng mL^{-1}).
‡Indicates where 200 ng mL^{-1} copper was added to the assay mixture ('+Cu in assay') for the two *A. nidulans* strains.
WT, wild type; ND, not determined; NS, not significant.

Table 3. Final copper concentrations in fresh potato tubers[a] grown under conventional and organic management

Variety (batch number)	Origin	Management	Copper concentration[a] (ng g^{-1})
Saxon (Morrisons 9916/06)	UK (Scotland)	Conventional	788.2 ± 186.4*
Maris Piper (Co-op M112 106–900)	UK	Conventional	872.3 ± 98.8
Romano (Co-op M112 286–1800)	UK (Lincolnshire)	Conventional	503.4 ± 70.4
Nadine (Co-op M112 275–1800)	UK (Yorkshire)	Conventional	684.8 ± 212.9
Valor (Morrisons 3155/11)	Israel	Organic	1346.0 ± 80.9
Nicola (Co-op M081C)	Israel	Organic	1421.6 ± 214.0
Nicola (Tesco GV1 78444)	Egypt	Organic	1044.4 ± 106.9
Nicola (Tesco GV1 79239)	Israel	Organic	915.2 ± 138.6

Copper levels in 'organically' cultivated potatoes were higher than in conventionally cultivated tubers (ANOVA $P < 0.001$; $F = 24.3$).
*Mean of three replicates ± SD.

113 ng mL^{-1}, similar to those found in commercial preparations of PDA (26–139 ng mL^{-1}; Table 1).

The use of Bordeaux mixture and other copper-based fungicides for the control of late blight in potatoes was widespread from the 1880s until the advent of synthetic organic fungicides in the 1930s, the period when use of PDA medium became widespread (Edgerton, 1908; Duggar *et al.*, 1917). However, Bordeaux mixture is still used to control late blight in organically cultivated crops (Kuepper, 1998; Duncan, 2003) and it is possible that this accounts for the elevated copper levels in these tubers.

Conclusions

The fact that copper levels can vary considerably between different potato batches, and that such variations can cause significant alterations in conidial and mycelial pigmentation in fungal colonies grown on different batches of commercially prepared and 'home-made' PDA is a matter of concern for mycologists, especially if batch variability can give rise to morphological differences in certain fungal cultures. Other secondary metabolic processes, notably mycotoxin production, are also affected by trace element concentrations in media (Dombrink-Kurtzman & Blackburn, 2005), with O'Brien *et al.* (2006) reporting anecdotal evidence of differences between commercial PDA preparations.

It is recommended that a small amount of copper sulphate (1000 ng mL^{-1}; ca. 15 μM $CuSO_4 \cdot 5H_2O$) be added when making PDA. Alternatively, the addition of Hunter's (*Aspergillus*) trace element solution containing 4000 ng mL^{-1} copper (Hill & Kafer, 2001) or a similar formulation would additionally guard against this and other mineral deficiencies. It is further possible that batch variation of copper or other trace elements in the many other long-established and undefined natural media still in widespread use could be avoided by taking such simple precautions.

Acknowledgements

The authors are grateful to Mr Robert Jones for assistance with copper analysis, to Dr Jim Jefferies for assistance with

scanning, to Ms Gwen Jenkins for photographic expertise and to Drs John Clutterbuck and Martin O'Brien for useful discussions.

References

Ainsworth GC, Bisby GR, Staplers JA, David JC & Kirk PM (2001) *Dictionary of the Fungi*, 9th edn. CABI Publishing, Wallingford, UK.

Booth C (1971) *The Genus Fusarium*. Commonwealth Mycological Institute, Kew, London.

Caesar-Tonthat TC, Kloeke FV, Geesey GG & Henson JM (1995) Melanin production by a filamentous soil fungus in response to copper and localization of copper sulfide by sulfide-silver staining. *Appl Env Microbiol* **61**: 1968–1975.

Clutterbuck AJ (1972) Absence of laccase from yellow-spored mutants of *Aspergillus nidulans*. *J Gen Microbiol* **70**: 423–435.

Clutterbuck AJ (1990) The genetics of conidiophore pigmentation in *Aspergillus nidulans*. *J Gen Microbiol* **136**: 1731–1738.

Dombrink-Kurtzman MA & Blackburn JA (2005) Evaluation of several culture media for production of patulin by *Penicillium* species. *Int J Food Microbiol* **98**: 241–248.

Duggar BM, Severy JW & Schmitz H (1917) Studies in the physiology of fungi. IV. The growth of certain fungi in plant decoctions preliminary account. *Ann Missouri Bot Gardens* **4**: 165–173.

Duncan JM (2003) Breeding to tackle blight without copper or GM. *Nature (London)* **425**: 15.

Edgerton CW (1908) The physiology and development of some anthracnoses. *Bot Gaz* **45**: 367–408.

Fomina M, Ritz K & Gadd GM (2000) Negative fungal chemotropism to toxic metals. *FEMS Microbiol Lett* **193**: 207–211.

Gams W, Hoekstra ES & Aptroot A (1998) *CBS Course on Mycology*. Centraalbureau voor Schimmelcultures, AG Baarn, the Netherlands.

Griffith GW (1994) Phenoloxidases. *Aspergillus nidulans: 50 Years On*, *Vol. 29*. (Martinelli SD & Kinghorn JR, eds), pp. 763–788. Elsevier Scientific Publishers, London, UK.

Hill TW & Kafer E (2001) Improved protocols for *Aspergillus* minimal medium: trace element and minimal medium salt stock solutions. *Aspergillus Newslett* 48: 20–21 (http://www.fgsc.net/fgn48/tofc48.htm)

Holker U, Dohse J & Hofer M (2002) Extracellular laccases in ascomycetes *Trichoderma atroviride* and *Trichoderma harzianum*. *Folia Microbiol* **47**: 423–427.

Kim J, Han K, Jin J, Kim H, Kim J, Yun S & Lee Y (2005) Putative polyketide synthase and laccase genes for biosynthesis of aurofusarin in *Gibberella zeae*. *Appl Env Microbiol* **71**: 1701–1708.

Kuepper G (1998) Alternative controls for late blight in potatoes. Pest Management Technical note. ATTRA Alternative Technology Transfer for Rural Areas. (http://www.attra.org/attra-pub/lateblight.html).

Kurtz MB & Champe SP (1981) Dominant spore colour mutants of *Aspergillus nidulans* defective in germination and sexual development. *J Bacteriol* **148**: 629–638.

Mulder MEG (1938) Sur l'influence du cuivre sur le croissance des microorganismes. *Annal Fementations* **IV**: 513–533.

O'Brien M, Nielsen KF, O'Kiely P, Forristal PD, Fuller HT & Frisvad JC (2006) Mycotoxins and other secondary metabolites produced *in vitro* by *Penicillium paneum* Frisvad and *Penicillium roqueforti* Thom isolated from baled grass silage in Ireland. *J Agric Food Chem* **54**: 9268–9276.

Parisot D, Dufresne M, Veneault C, Lauge R & Langin T (2002) *clap1*, a gene encoding a copper-transporting ATPase involved in the process of infection by the phytopathogenic fungus *Colletotrichum lindemuthianum*. *Mol Gen Genom* **268**: 139–151.

Phelan A, Thurman DA & Tomsett AB (1990) The isolation and characterization of copper-resistant mutants in *Aspergillus nidulans*. *Curr Microbiol* **21**: 255–260.

Pokorny R, Vargovic P, Holker U, Janssen M, Bend J, Hudecova D & Varecka L (2005) Developmental changes in *Trichoderma viride* enzymes abundance in conidia and the light-induced conidiation signalling pathway. *J Basic Microbiol* **45**: 219–229.

Stevens RB (1974) *Mycology Guidebook*. Mycological Society of America, University of Washington Press, Seattle.

Thurston CF (1994) The structure and function of fungal laccases. *Microbiol UK* **140**: 19–26.

Supplementary material

The following supplementary material is available for this article online:

Table S1. Origins of potato tubers and the copper content of extracts derived from these.

Fig. S1. Copper levels in potato extract prepared from 27 potato samples from a range of sources.

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/abs/10.1111/j.1574-6968.2007.00923.x (This link will take you to the article abstract).

Please note: Blackwell Publishing is not responsible for the content or functionality of any supplementary materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.