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Growth and mycotoxin production by fungi in atmospheres containing 80% carbon dioxide and 20% oxygen

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ABSTRACT

The effect of atmosphere containing 80% CO_2 and 20% O_2 on growth of *Mucor plumbeus*, *Fusarium oxysporum*, *Byssochlamys fulva*, *Byssochlamys nivea*, *Penicillium commune*, *Penicillium roqueforti*, *Aspergillus flavus*, *Eurotium chevalieri* and *Xeromyces bisporus* was investigated. Production of aflatoxin by *A. flavus*, patulin by *B. nivea*, roquefortine C by *P. roqueforti*, and cyclopiazonic acid by *P. commune* was also studied. Fungal growth was evaluated by three methods: colony diameter, hyphal length or mycelium dry weight and ergosterol content. Among the nine fungal species examined, two *E. chevalieri* and *X. bisporus*, did not grow under these conditions. In this study, fungi differed in their response to modified atmospheres in biomass, ergosterol content, mycotoxin production and morphology. Reductions of 57.8–96.9%, 73.7–99.6% and 91.5–99.9% were obtained in colony diameter, hyphal length and ergosterol content, respectively, under this atmosphere compared to air. Ergosterol content was more affected in most species than other measurements. Patulin, cyclopiazonic acid and roquefortine C were produced in this atmosphere, although levels were very low and aflatoxin was not produced at all. Growth was quite extensive as measured by colony diameters, but hyphal lengths were low and ergosterol production was also affected in all species of this study.

1. Introduction

Carbon dioxide is known to inhibit growth of many microorganisms, including fungi (Daniels et al. 1985; Hoogerwerf et al. 2002; Zardetto, 2005; Giorni et al. 2008; Taniwaki et al. 2009), but inhibitory concentrations vary markedly among species. Levels of CO_2 from 4 to 20% can be stimulatory to growth of many fungi in atmospheres containing low levels of O_2 (Wells and Uota, 1970; Gibb and Walsh, 1980), but other reports indicate that atmospheres containing higher than 40% CO_2 substantially inhibit growth of most spoilage fungi (Zardetto, 2005; Taniwaki et al. 2009). Some *Rhizopus*, *Mucor* and *Fusarium* species appear to be very tolerant of high levels of CO_2, reportedly growing in atmospheres containing as much as 96–100% CO_2 (Stotzky and Goos, 1965).

Mechanisms of inhibition of fungal growth under atmospheres with either high CO_2 or depletion of O_2 are not well understood. Studies have demonstrated that environments with elevated CO_2 concentrations are generally much more effective in restricting fungal growth than those of low O_2 concentration (Stotzky and Goos, 1965; Yang and Lucas, 1970). However, some moulds have been reported to grow in the presence of high levels of CO_2 provided O_2 is present (Ellis et al. 1993; Taniwaki et al. 2009).

Mycotoxin formation by fungi has reportedly been controlled by enriching atmospheres with CO_2 or decreasing O_2, even when fungal growth is not greatly affected (Paster and Lisker, 1985; Paster, 1990; Paster et al. 1995). Giorni et al. (2008) showed that 25% CO_2 did not reduce aflatoxin production by *Aspergillus flavus*, either on culture media or maize grain and that at least 50% CO_2 was required to inhibit aflatoxin production to any extent.

Taniwaki et al. (2009) showed that *Mucor plumbeus*, *Fusarium oxysporum*, *Byssochlamys fulva* and *B. nivea* can grow in atmospheres containing up to 60% CO_2 in the presence of <0.5% residual O_2. In this study, we report the effect of an atmosphere of 80% CO_2 and 20% O_2 on growth and mycotoxin production by *Mucor plumbeus*, *Fusarium oxysporum*, *Byssochlamys fulva*, *B. nivea* (capable of producing patulin), *Penicillium roqueforti* (roquefortine C), *P. commune* (cyclopiazonic acid), *Aspergillus flavus* (aflatoxin B_1 and B_2), *Eurotium chevalieri* and *Xeromyces bisporus*. The response of fungi to this atmosphere can provide practical information on methods for prevention of fungal growth and mycotoxin production in food using a high CO_2 concentration without the expense or complexity of removing residual O_2.

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2. Materials and methods

2.1. Fungi

Nine species of fungi were chosen for this study, and represent examples of heat resistant, xerophilic and toxigenic food spoilage fungi. Most were isolated from processed foods of low O_2 content, such as Ultra High Temperature (UHT) and vacuum packaged products. The species studied were: *Mucor plumbeus* FRR 2414, isolated from spoiled, fermenting apple juice, Sydney, NSW, Australia; *Fusarium oxysporum* FRR 3414, from spoiled, fermenting orange juice, Sydney; *Byssochlamys fulva* FRR 3792, from fermenting strawberry puree, Sydney; *B. nivea* FRR 4421 from strawberries, Sao Paulo, SP, Brazil, capable of producing patulin; *Penicillium roqueforti* FRR 2162, from spoiled cheddar cheese, Lincoln, Nebraska, USA, capable of producing roquefortine C and reported to be tolerant of low O_2 and high CO_2; *P. commune* FRR 3932, from spoilage of Cheddar cheese packed in low O_2 atmospheres, Sydney, capable of producing cyclopiazonic acid; *Aspergillus flavus* FRR 2757, from peanuts, Kingaroy, Queensland, Australia, a producer of aflatoxin B_1 and B_2; *Eurotium chevalieri* FRR 547, from animal feed, Sydney; and *Xeromyces bisporus* FRR 2351, from dates, Sydney. FRR is the acronym for the culture collection at CSIRO Food and Nutritional Sciences, North Ryde, NSW.

2.2. Media and culture conditions

Fungi were grown on Czapek Yeast Extract agar (CYA) and Potato Dextrose agar (PDA), except for *Eurotium chevalieri*, which was grown on Czapek Yeast Extract 20% Sucrose agar (CY20S), and *Xeromyces bisporus*, grown on Malt Yeast 50% Glucose agar (MY50G). The media were freshly prepared according to the formulation and directions in Pitt and Hocking (2009).

The fungi were inoculated in the centre of each plate using a needle. Sterile tooth picks were placed between the base and the lid of each plate to facilitate gas exchange into the medium.

2.3. Growth under modified atmospheres

Gas concentrations were maintained within narrow limits, as detailed by Taniwaki et al. (2009). Inoculated media were incubated in anaerobe jars under a gaseous atmosphere mixture of 80% CO_2 with 20% O_2 and <1% N_2, that was obtained by flushing several times with this gas mixture. The gas composition was checked every day and the jars were flushed with the gas mixture if the concentration of either CO_2 or O_2 varied by 2% or N_2 increased to 1%. Changes were minimal over the 30 day period of the experiment. Fungal colonies from culture plates in each jar were sampled in duplicate. However the experiment was carried out only once because of the paramount requirement for control of the atmospheric conditions, and the large number of anaerobe jars required. Despite the lack of replication of each experiment, the fact that fungal growth for each experiment was determined by several different methods served as a good control on the reliability of the conclusions that were obtained.

2.4. Growth measurement

Colony diameters, ergosterol concentration and biomass (hyphal length or mycelial dry weight) were measured at various periods as determined by the observed rate of fungal growth. Cultures were considered to have failed to grow if visible changes to the inoculum had not occurred after 30 days, except for *E. chevalieri* and *X. bisporus* which were incubated for 60 days. After 30 or 60 days, the inocula were examined microscopically for signs of germination or growth. Absence of germination was the criterion used for recording a negative result. The procedures to measure colony diameter, ergosterol content, hyphal length and mycelium dry weight were described in Taniwaki et al. (2009) and have been discussed in Taniwaki et al. (2006).

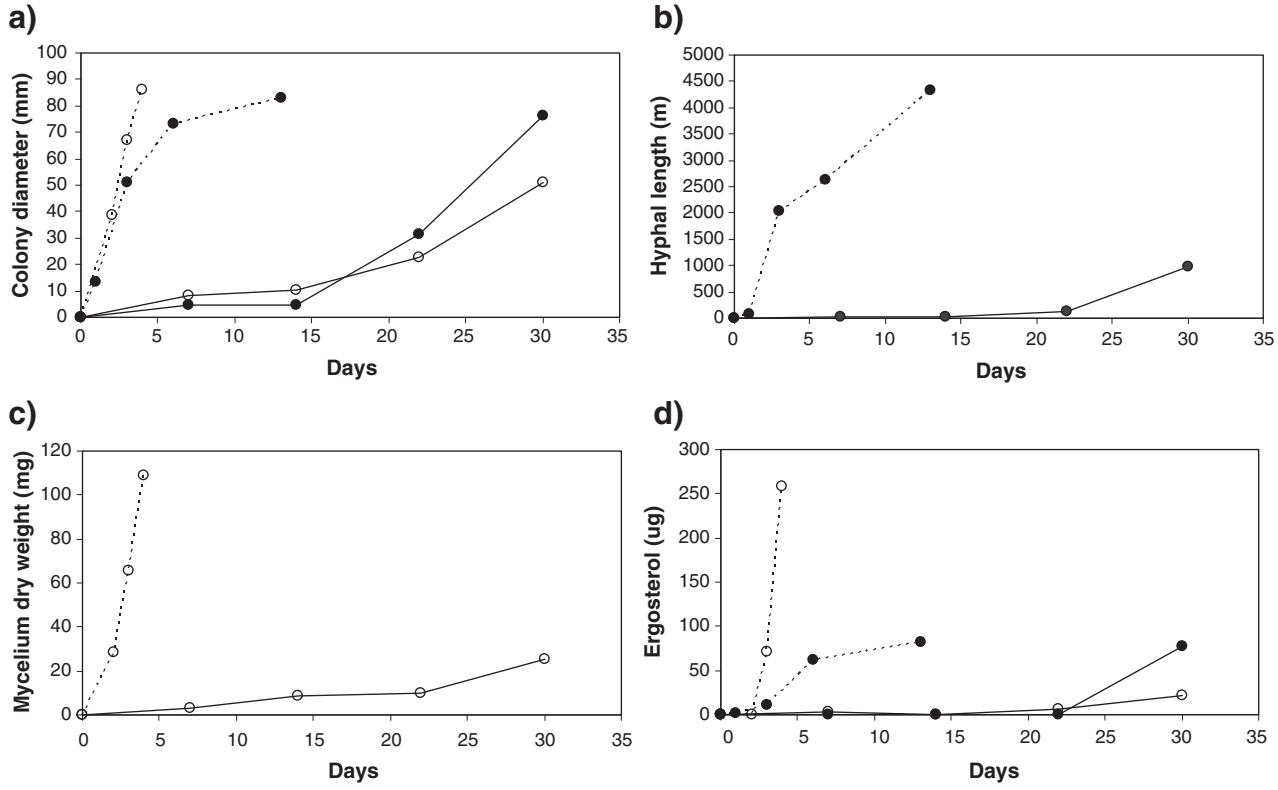

Fig. 1. Growth of *Mucor plumbeus*: ——, in air, and ___, in atmosphere of 80% CO_2 plus 20% O_2 and <1% N_2, after various periods of incubation at 25 °C. (a) Colony diameter (mm); (b) hyphal length (m); (c) mycelium dry weight (mg) and (d) ergosterol content (μg). Growth on ● CYA; growth on ○ PDA.

2.5. Mycotoxin analysis

Mycotoxin analyses were carried out on cultures grown under the 80% CO_2 plus 20% O_2 atmosphere and for parallel cultures grown in the presence of air.

Cultures of *A. flavus* were analysed for production of aflatoxins B_1 and B_2, *P. roqueforti* for roquefortine C, *B. nivea* for patulin, and *P. commune* for cyclopiazonic acid (CPA). These fungi were grown on plates of CYA and PDA at 25 °C and tested for mycotoxin production after various periods of incubation in the modified atmosphere or air. Except for CPA, the methods for extraction, analyses and recovery rates of these mycotoxins, are described elsewhere (Taniwaki et al. 2001; Taniwaki et al. 2009).

2.5.1. Cyclopiazonic acid assay

For analysis of cyclopiazonic acid (CPA) from *Penicillium commune*, the entire contents of a Petri dish (medium and colony) were transferred to a Stomacher bag with 50 ml of chloroform: methanol (85 + 15 v/v/) plus 0.5 ml of 20% sulphuric acid. Extracts were filtered and concentrated in a rotary evaporator to near dryness, resuspended in chloroform, then filtered through polypropylene membrane filters (0.45 μm pore size, 13 mm diam., Activon Scientific Products, Thornleigh, Australia). CPA was assayed by HPLC (Model 10A, Shimadzu Corporation, Japan) fitted with a OD-224 column (22 cm length and 4.6 mm internal diam.), filled with Spheri-5 RP-18 beads (Brownlee Labs, USA). CPA was quantified by HPLC according to the method of Urano et al. (1992). The column was eluted with two solvents of different mobilities: A, methanol: water (85:15 v/v); and B, methanol: water (85:15 v/v) with 4 mM $ZnSO_4 \cdot 7H_2O$. The elution followed a linear gradient from100% A to 100% B in 10 min, followed by 100% B for 10 min, at 1.0 ml/min. CPA was detected by absorption at 279 nm about 9–11 min after injection. CPA (1 μg/ml; Sigma, St. Louis, MO, USA) was used as standard.

3. Results

3.1. Effect of 80% CO_2 and 20% O_2 on fungal growth

Eurotium chevalieri and *Xeromyces bisporus* did not grow in an atmosphere of 80% CO_2 plus 20% O_2 during incubation for 60 days. Both species had been inoculated onto optimal media: *E. chevalieri* onto Czapek Yeast Extract agar with 20% added sucrose and *X. bisporus* on Malt Yeast 50% Glucose agar. Growth on the plates did not occur after they were removed from this atmosphere and incubated for 2 weeks in air. This suggested that the atmosphere of 80% CO_2 plus 20% O_2 affected the viability of the spores.

All of the other fungi grew in this atmosphere, on both CYA and PDA. Colony diameters, hyphal lengths and ergosterol concentrations for the other seven fungi after growth on either CYA or PDA are shown in Figs. 1–5. Data for growth in air are included for comparison. As reported in our previous experiments (Taniwaki et al. 2009), dry weight was recorded for growth of *Mucor plumbeus* on PDA because it consisted predominantly of chlamydoconidia with little mycelium.

The effect of this gas mixture on growth of each of these species is described in the following sections.

3.2. Mucor plumbeus

Thin hyaline colonies were developed by *M. plumbeus* on CYA, whereas on PDA the colonies were more compact and gelatinous in appearance. No production of sporangiospores was observed on either medium. Colony diameters increased quite rapidly in the presence of air, and after about 15 days in the presence of 80% CO_2 plus 20% O_2 (Fig. 1a). This extensive late growth under the modified atmosphere was also evident when growth was measured by other methods (Fig. 1b,c,d). For growth in air, the ergosterol content on PDA was higher than on CYA, but in atmospheres with 80% CO_2, the ergosterol content on CYA was higher than on PDA (Fig. 1d, 30 day data). *M. plumbeus* developed chlamydoconidia on both media under these conditions.

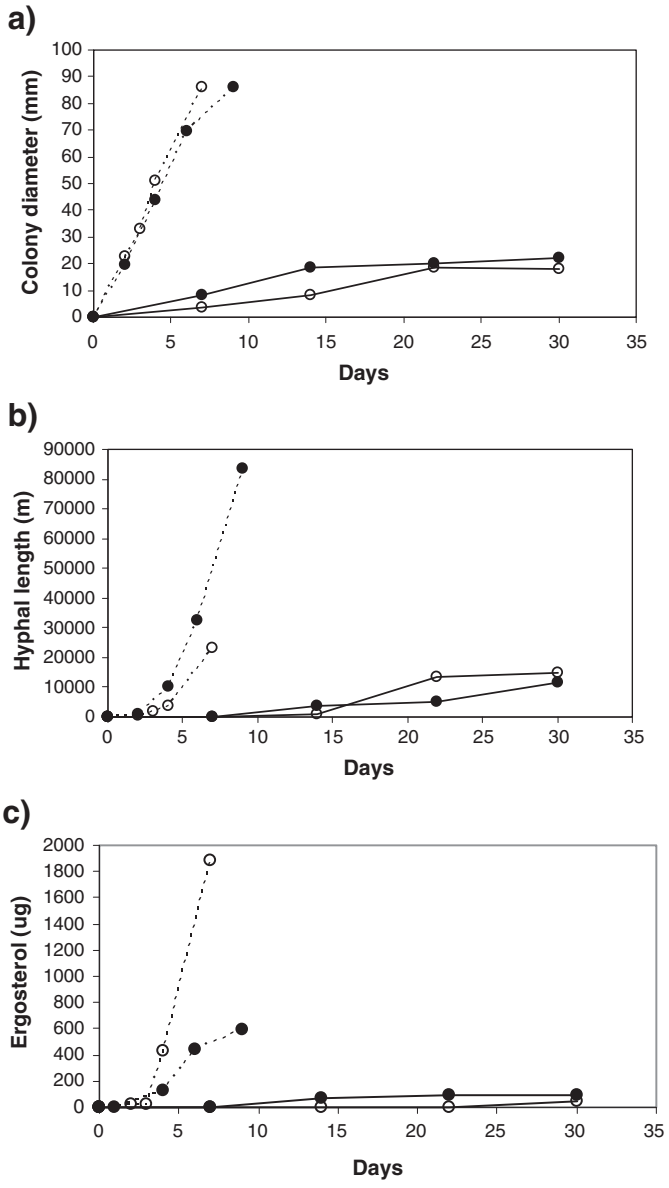

Fig. 2. Growth of *Fusarium oxysporum*: --, in air, and ___, in atmosphere of 80% CO_2 plus 20% O_2 and <1% N_2, after various periods of incubation at 25 °C. (a) Colony diameter (mm); (b) hyphal length (m) and (c) ergosterol content (μg). Growth on ● CYA; growth on ○ PDA.

3.3. Fusarium oxysporum

Fusarium oxysporum grew compactly on both media, producing pinkish colonies and abundant production of microconidia and chlamydoconidia. Growth was retarded under 80% CO_2 plus 20% O_2 compared with that in air as clearly evidenced from colony diameter, hyphal length and ergosterol content (Fig. 2a,b,c). Surprisingly, while the ergosterol content from growth on PDA was 3 fold higher than for growth on CYA in air (Fig. 2c), the hyphal length on CYA was 3 fold higher than that on PDA (Fig. 2b).

3.4. Byssochlamys fulva

On CYA, colonies of *Byssochlamys fulva* had a soft, white, and plane morphology, but on PDA they were cottony. Aleurioconidia were

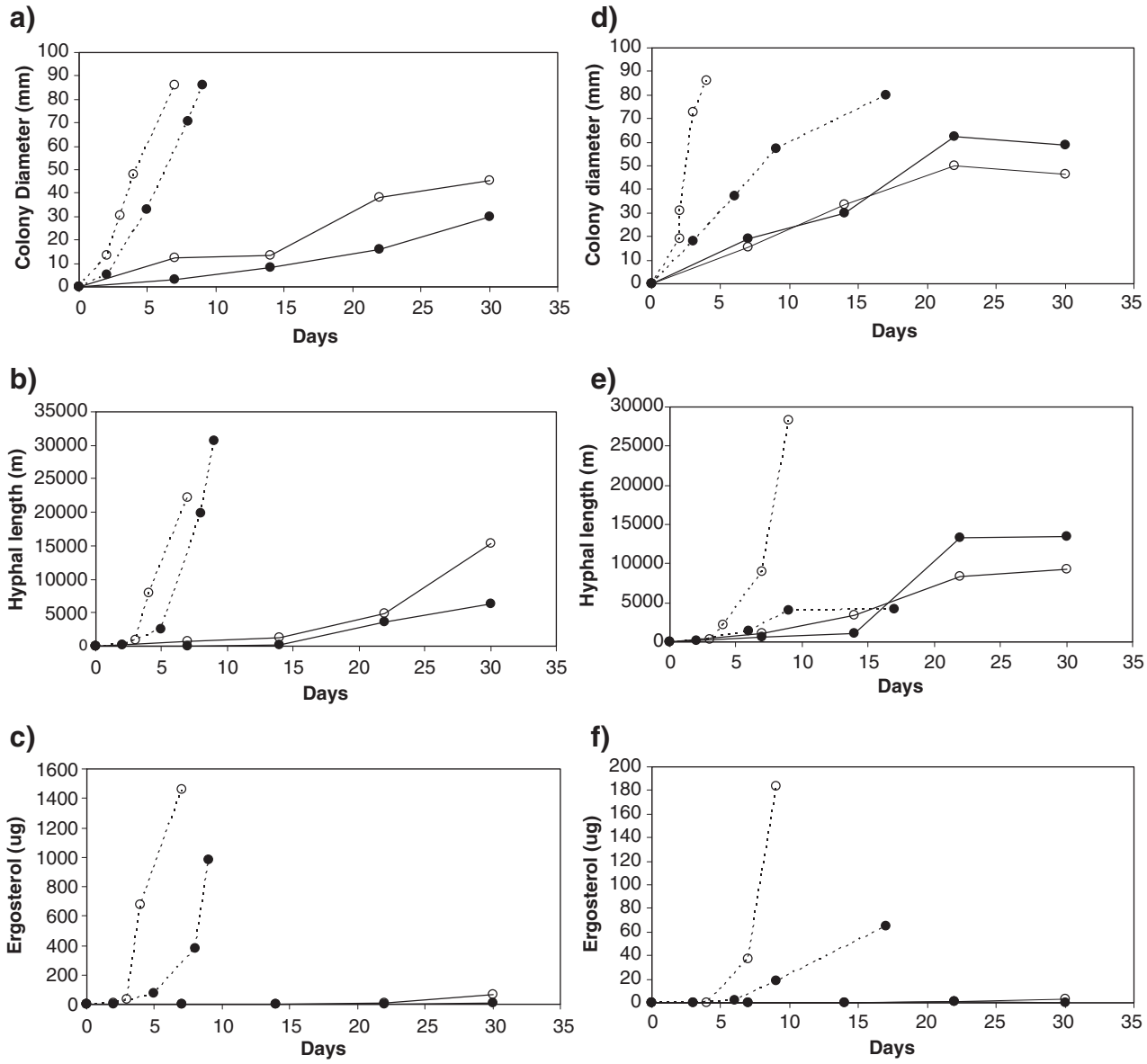

Fig. 3. Growth of *Byssochlamys fulva* and *B. nivea*: --, in air, and ___, in atmosphere of 80% CO_2 plus 20% O_2 and <1% N_2, after various periods of incubation at 25 °C. (a) Colony diameter (mm) of *B. fulva*; (b) hyphal length (m) of *B. fulva;* (c) ergosterol content (μg) of *B. fulva;* (d) colony diameter (mm) of *B. nivea*; (e) hyphal length (m) of *B. nivea* and (f) ergosterol content (μg) of *B. nivea*. Growth on ● CYA; growth on ○ PDA.

produced on both media. Substantial growth occurred under atmospheres of 80% CO_2 plus 20% O_2, with colony diameters exceeding 40 mm on PDA after 30 days (Fig. 3a). Hyphal development was extensive (Fig. 3b), especially on PDA, but ergosterol production was very limited (Fig. 3c).

3.5. Byssochlamys nivea

Large, cottony colonies, that had a white with orange pigmentation when observed in reverse plates, were produced by *Byssochlamys nivea* on both media. This species grew strongly in the 80% CO_2 plus 20% O_2 atmospheres (Fig. 4d,e,f) on PDA, but took twice as long to reach a diameter of 60 mm as in air. On CYA, the colony spread across the plate, and reached 80 mm in 15 days (Fig. 3d), but produced very low hyphal length after 30 days (Fig. 3e). Considering the extensive nature of its growth, ergosterol production by *B. nivea* was greatly reduced under the modified atmosphere in comparison with air (Fig. 3f).

3.6. Penicillium commune

Colonies of *Penicillium commune* in the 80% CO_2 plus 20% O_2 atmospheres on both media were compact and wrinkled, with greenish colouration from conidial formation. Growth in the 80% CO_2 plus 20% O_2 atmospheres was restricted over 70% compared to that in air. Colony diameters (Fig. 4a) and hyphal length (Fig. 4b) in this modified atmosphere were similar on both media. However, ergosterol content (Fig. 4c) was twice as high on CYA as on PDA after 30 days. *P. commune* produced higher levels of ergosterol in the modified atmosphere compared with *P. roqueforti.*

3.7. Penicillium roqueforti

Green, velutinous (i.e. with a surface texture like velvet) colonies with abundant sporulation were produced by *Penicillium roqueforti* in an atmosphere of 80% CO_2 plus 20% O_2. Colony diameters were reduced over 80% and 60% on CYA and PDA, respectively, compared with those in air. Visible colonies had developed after 7 days on both

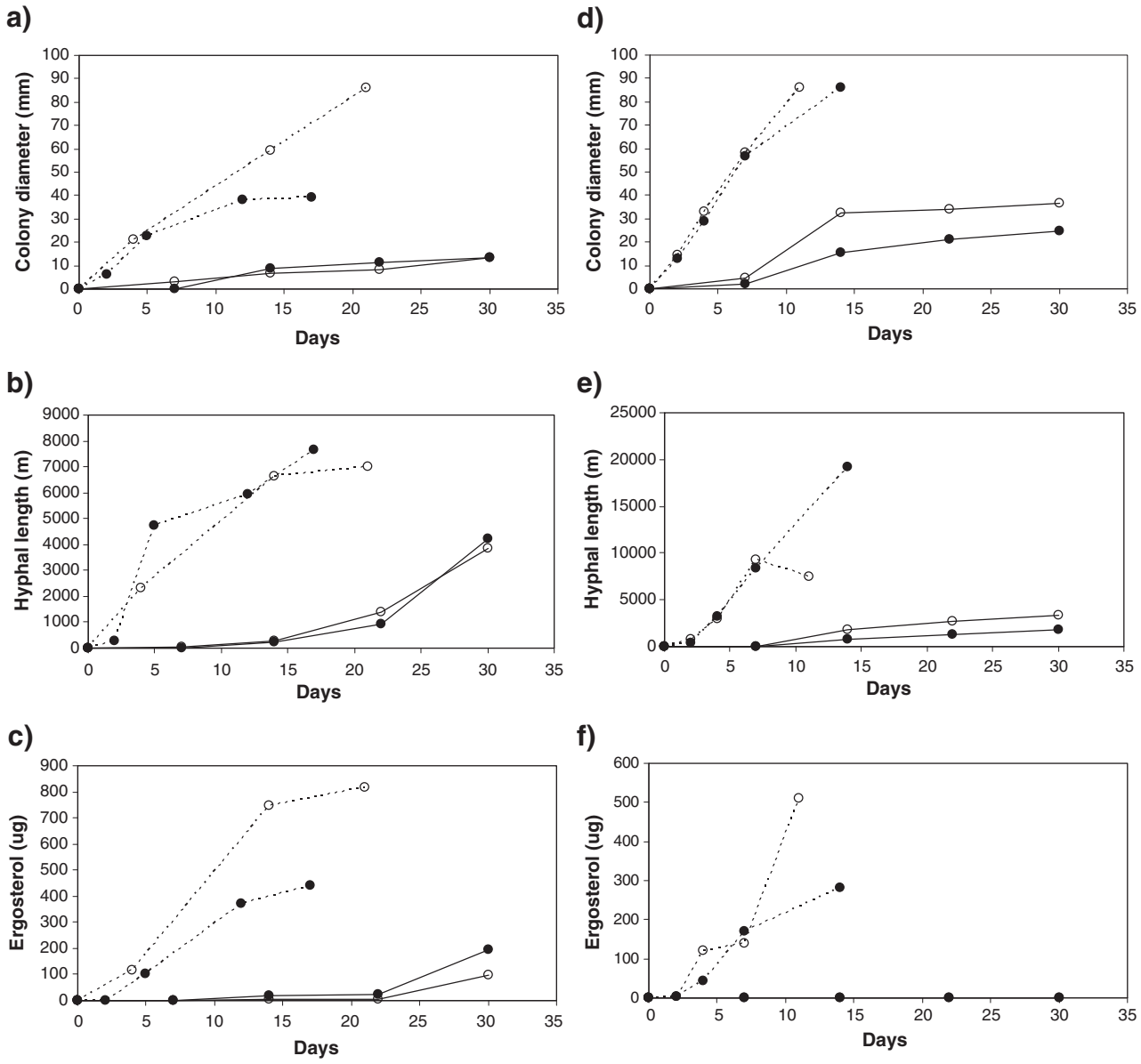

Fig. 4. Growth of *Penicillium commune* and *P. roqueforti*: --, in air, and ___, in atmosphere of 80% CO_2 plus 20% O_2 and <1% N_2, after various periods of incubation at 25 °C. (a) colony diameter (mm) of *P. commune*; (b) hyphal length (m) of *P. commune*; (c) ergosterol content (μg) of *P. commune*; (d) colony diameter (mm) of *P. roqueforti*; (e) hyphal length (m) of *P. roqueforti* and (f) ergosterol content (μg) of *P. roqueforti*. Growth on ● CYA; growth on ○ PDA.

CYA and PDA (Fig. 4d,e,f). Colony diameters on PDA were 1.5 times larger than those on CYA (Fig. 4d), with twice the hyphal length (Fig. 4e). Ergosterol production was very low on both media (Fig. 4f).

3.8. *Aspergillus flavus*

Growth of *Aspergillus flavus* was very slow on both media in the 80% CO_2 plus 20% O_2 atmospheres, with colonies reaching only 10 mm after 30 days of incubation compared with 90 mm after 10 days in air (Fig. 5a). Colonies were white and without sporulation. Similarly, hyphal length and ergosterol values were drastically decreased for growth in the presence of the CO_2 (Fig. 5b,c).

3.9. *Effect of 80% CO_2 plus 20% O_2 on mycotoxin production*

Patulin, cyclopiazonic acid and roquefortine C were produced by *B. nivea*, *P. commune* and *P. roqueforti*, respectively, when grown in 80% CO_2 plus 20% O_2 on both CYA and PDA (Fig. 6). However, levels were very low and, from any practical point of view, insignificant. For growth in air, these species, respectively, gave maximum production of 300 μg, 175 μg and 100 μg per plate after 9, 12 and 14 days. No aflatoxin production by *A. flavus* was detected on either medium in this atmosphere, but in air, 20 μg per plate was produced after 11 days growth.

4. Discussion

The failure of *Xeromyces bisporus* to grow, or of its ascospores even to survive, during 60 days incubation in 80% CO_2 with 20% O_2 is surprising. It was reported by Dallyn and Everton (1969) to tolerate CO_2 levels as high as 95% with 1% O_2 however; these authors did not quantify growth nor compare it with growth in air.

Facultative anaerobic growth in Mucorales has been observed in several studies (Bartnicki-Garcia and Nickerson, 1962; Hawker, 1966; Hesseltine et al. 1985; Pitt and Hocking, 2009 and Taniwaki et al. 2009). *M. plumbeus* grew strongly under 80% CO_2 plus 20% O_2 on both

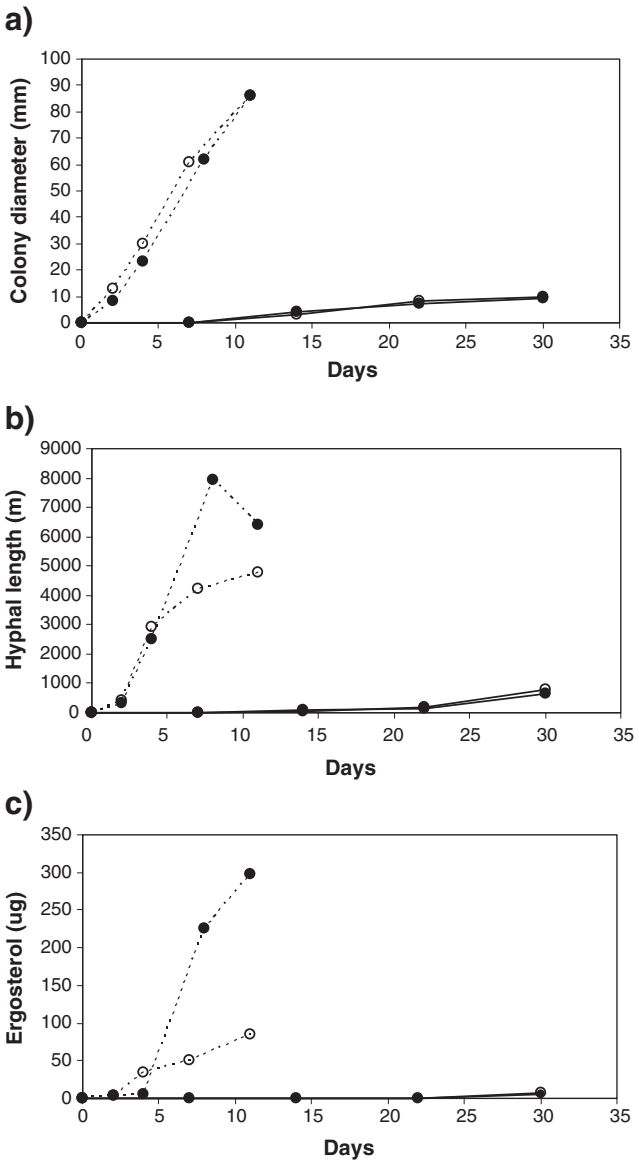

Fig. 5. Growth of *Aspergillus flavus*: --, in air, and ___, in atmosphere of 80% CO_2 plus 20% O_2 and <1% N_2, after various periods of incubation at 25 °C. (a) Colony diameter (mm); (b) hyphal length (m) and (c) ergosterol content (μg). Growth on ● CYA; growth on ○ PDA.

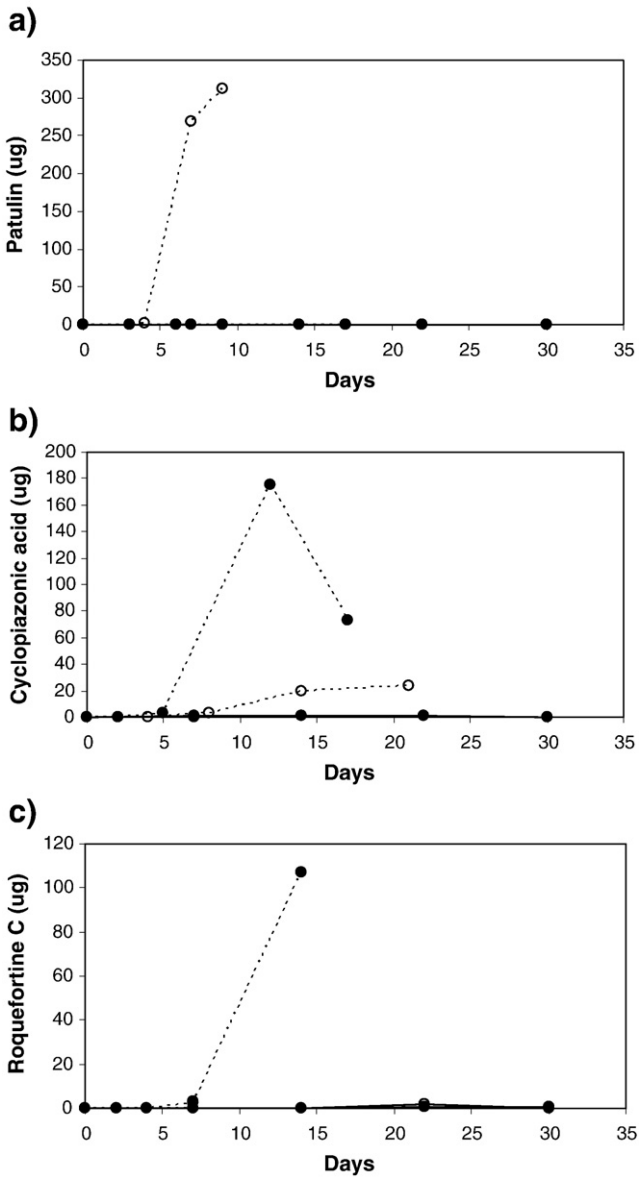

Fig. 6. Mycotoxin production: --, in air and ___, in atmosphere of 80% CO_2 plus 20% O_2 and <1% N_2, after various periods of incubation at 25 °C. (a) Patulin (μg/Petri dish) by *Byssochlamys nivea*, (b) cyclopiazonic acid (μg/Petri dish) by *Penicillium commune* and (c) roquefortine C (μg/Petri dish) by *Penicillium roqueforti*. Growth on ● CYA; growth on ○ PDA.

media. Under these conditions, *M. plumbeus* developed chlamydoconidia, an effect seen previously (Pitt and Hocking, 2009). This effect of anaerobic conditions was more pronounced on CYA than on PDA, in contrast with previous results on growth patterns in high CO_2 and <0.5% O_2 (Taniwaki et al. 2009) where chlamydoconidia production was more evident on PDA. Hyphal length was used as one measure of growth on CYA in 80% CO_2 plus 20% O_2 to be consistent with the previous work, but hyphal length was very low, and inappropriate for measuring growth of this species here.

As expected, *Fusarium oxysporum* grew strongly under 80% CO_2 plus 20% O_2 with abundant sporulation. Previous observations have shown that this species tolerates very high CO_2 levels and low O_2 concentrations (Hollis, 1948; Newcombe, 1960; Stotzky and Goos, 1965) and even of anaerobic conditions (Gunner and Alexander, 1964; Marchant et al. 1994; Abe et al. 2007). Taniwaki et al. (2009) observed anaerobic growth of *F. oxysporum*, *M. plumbeus*, *B. fulva* and *B. nivea*, all of which grew under the same conditions as the obligately anaerobic bacterium, *Clostridium sporogenes*.

Byssochlamys fulva developed strongly under 80% CO_2 plus 20% O_2, with abundant sporulation. King et al. (1969) observed that this fungus had the capacity for growth under nitrogen with a low concentration of O_2 (0.27%), but not under strictly anaerobic conditions. However, Taniwaki et al. (2009) showed that *B. fulva* could grow under facultative anaerobic conditions, as discussed previously.

Hyphal production by *Byssochlamys nivea* on CYA under 80% CO_2 plus 20% O_2 was slow but extensive, and ergosterol content was very low. Perhaps *B. nivea* may produce other sterols under atmospheres with high CO_2 levels. *B. nivea* has been reported previously to grow in low O_2 and high CO_2 (King et al. 1969). Yates et al. (1967) reported that in 100% CO_2, *B. nivea* produced only 4% of the mycelial dry weight produced in air. In 80% CO_2 plus 20% O_2, the weight was 85%, and in 80% CO_2 mixed with air, the weight was 16% of that in air. In our study, this species produced three times the hyphal length in 80% CO_2 plus 20% O_2 than in air on CYA, but over a longer period. Patulin production was decreased but not completely inhibited.

As measured by colony diameter and hyphal length, growth of *P. commune* was decreased by an atmosphere of 80% CO_2 plus 20% O_2, but ergosterol content remained high compared with *P. roqueforti* and *Byssochlamys nivea*. *P. commune* did not grow in high CO_2 when O_2 was less than 0.5% (Taniwaki et al. 2009), so the factor limiting growth appears to be the availability of O_2 rather than the high CO_2 concentration. Haasum and Nielsen (1996) demonstrated that concentrations of O_2 between 2 and 18% had no significant effect on either lag time or growth rate of *P. commune*, but lower O_2 levels were not studied. Cyclopiazonic was produced in 80% CO_2 plus 20% O_2, but at very low levels. Taniwaki et al. (2001) reported CPA formation by *P. commune* in cheese packaged in gas mixtures of 20% $CO_2 + 5\%$ O_2 and 40% $CO_2 + 1\%$ O_2 as being 8% and 0.1% respectively, of that in air.

Penicillium roqueforti is well known as a cheese ripening fungus, so it should be well adapted to conditions where O_2 is limited and CO_2 levels may be elevated, such as inside cheese. We found that *P. roqueforti* grew well in the presence of 80% CO_2 plus 20% O_2 but, like *B. nivea*, the content of ergosterol was low. Only very low amounts of roquefortine C were produced.

High CO_2 and O_2 permitted very limited growth of *Aspergillus flavus*, with inhibition of sporulation and pigmentation. Aflatoxin production was not detectable. In reviewing several studies on aflatoxin production under modified atmospheres, Paster (1990) concluded that aflatoxin production is an aerobic process that can be inhibited at low O_2 concentration or blocked when CO_2 levels are increased.

When grown in an atmosphere of O_2 (0–20%) in balance with 60% CO_2/40%N_2, aflatoxin production by cultures of *A. flavus* increased up to a specific colony diameter (7 mm) and then decreased with further growth (Ellis et al. 1993). Depletion of available substrate for aflatoxin production or breakdown of aflatoxin as a substrate for further mycelial development and mould growth were suggested as reasons (Doyle et al., 1982). Aflatoxin production is greatest in young mycelium and, as colonies enlarge, mycelia that are less physiologically active contribute more to colony dry weight (Ellis et al. 1993). The observed decrease in the production of patulin, cyclopiazonic acid and roquefortine C in our study may also be due to these factors of depletion of substrate and metabolism of the toxin.

Mycotoxin production can also be reduced as a result of a decrease in the amount of mycelial growth or the effect of high CO_2 or low O_2 on toxin synthesis. The diversity in the biosynthesis mechanisms associated with the formation of different mycotoxins raises the possibility that these conditions may affect more than one specific biosynthetic pathway. The mechanisms of mycotoxin inhibition by high CO_2 concentration or O_2 depletion are not completely understood (Richard et al. 2004; Giorni et al. 2008; Schmidt-Heydt et al. 2009).

Very significant differences were observed in the effects of high CO_2 plus high O_2 on the four parameters used to measure growth. Colony diameters in some species were large, but the great decreases in hyphal length recorded indicated that biomass production was reduced in this atmosphere. Ergosterol production was markedly affected, and not uniformly affected among species. Ergosterol content does not appear to be a reliable indicator of fungal growth under modified atmospheres.

In this study, fungi differed in their response to modified atmospheres in biomass, metabolism and morphology. Reductions of 57.8–96.9%, 73.7–99.6% and 91.5–99.9% were obtained in colony diameter, hyphal length and ergosterol content, respectively, under this atmosphere compared to air. For most species, ergosterol content was more affected than other measurements. Among the species tested, the least to most affected by the 80% CO_2, 20% O_2 based on the measurements studied were: *Byssochlamys nivea*, *Penicillium roqueforti*, *P. commune*, *Fusarium oxysporum*, *B. fulva*, *Mucor plumbeus*, *Aspergillus flavus*, *Eurotium chevalieri* and *Xeromyces bisporus*.

In previous work, Taniwaki et al. (2009) reported the response of the same nine species under high carbon dioxide and low oxygen atmospheres. In light of the results reported in the present study, four groups could be distinguished with respect to their growth responses under modified atmospheres:

- Group I: species which did not grow in 20% CO_2 <0.5% O_2 nor 80% CO_2 with 20% O_2 (*E. chevalieri* and *X. bisporus*);
- Group II: species which grew in 20% CO_2 <0.5% O_2 and which grew in 80% CO_2 with 20% O_2 but not 40% CO_2 <0.5% O_2 (*P. roqueforti* and *A. flavus*);
- Group III: species which grew in 20%, 40% or 60% CO_2 <0.5% O_2, and which also grew in 80% CO_2 with 20% O_2 (*M. plumbeus*, *F. oxysporum*, *B. fulva* and *B. nivea*);
- Group IV: species which grew in 80% CO_2 with 20% O_2 but not in atmospheres where oxygen was limiting (*P. commune*).

Apparently species in Group I, *E. chevalieri* and *X. bisporus*, are sensitive to high CO_2 concentrations. Species in Group II, *P. roqueforti* and *A. flavus*, can be considered representative of fungi with a marginal ability to withstand high CO_2 and low O_2 concentrations: a high (80%) CO_2 concentration was tolerated in the presence of 20% O_2. *P. commune* was able to grow in elevated levels of CO_2 and 20% O_2, indicating that the principal factor limiting growth of *P. commune* is also the availability of O_2. Facultatively anaerobic behaviour was observed in *M. plumbeus*, *F. oxysporum*, *B. fulva* and *B. nivea* (Group III species) as previously reported (Taniwaki et al. 2009). Although growth response was delayed and reduced under high CO_2 atmospheres, the ability of these fungi to tolerate 60% CO_2 in the presence of low O_2 (<0.5%) or 80% CO_2 with 20% O_2, means they will be very difficult to control by modified atmosphere packaging. To control these species in minimally processed foods, the application of other parameters such as reduced water activity and/or temperature may be necessary. These studies have revealed new information about the effect of high CO_2 and low O_2 atmospheres on the growth and mycotoxin production of several fungi, paving the way for further fundamental studies to explain the physiological, biochemical and molecular bases of these observations.

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