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ORIGINAL PAPER

New solid-state fermentation chamber for bulk production of aerial conidia of fungal biocontrol agents on rice

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Abstract A novel solid-state fermentation apparatus, namely an upright multi-tray conidiation chamber, was developed to facilitate the production of aerial conidia of fungal biocontrol agents, such as *Beauveria bassiana*. The chamber with 25 bottom-meshed metal trays had a capacity of $\geqslant$50 kg rice with each tray holding $\geqslant$2 kg. In repeated trials, a mean yield of 2.4 (1.8–2.7) $\times 10^{12}$ conidia kg^{-1} rice was harvested from the 7-day cultures of *B. bassiana* in a fully loaded chamber. The new apparatus has a high potential for bulk production of fungal conidia.

Keywords Aerial conidia · *Beauveria bassiana* · fungal biocontrol agents · microbial control · solid-state fermentation

Introduction

Hyphomycetous biocontrol agents are of increasing importance in modern crop protection since integration of their formulations into pest management systems may reduce the dependence of agriculture on chemical pesticides. Many fungal agents, such as *Beauveria bassiana* and *Metarhizium anisopliae*, can be readily produced *in vitro* in the form of either aerial conidia or mycelia and blastospores. Solid-state fermentation (SSF) enables aerial conidia to be produced, which are similar to those produced naturally on the surface of insect cadavers and are superior to mycelia and blastospores produced under submerged fermentation conditions (Feng et al. 1994; Wraight et al. 2001; Roberts and St. Leger 2004). Submerged mycelia and blastospores have cell walls that are too thin to tolerate environmental stresses such as desiccation after fermentation and solar irradiation after application in the field (Jenkins and Prior 1993). Aerial conidia are also preferred for fungal biocontrol agents of some plant diseases (Masangkay et al. 2000; Wyss et al. 2001; Jones et al. 2004).

Maximization of surface area is a major concern for enhancement of conidial production by SSF technology. However, common SSF bioreactors often do not suit to conidial production for pest control at affordable cost because they are usually designed to produce 'low volume-high value' products (Krishna 2005). Production of high-quality conidia requires not only huge surface area but also optimal temperature, high humidity and natural light, as does on insect cadavers (Luz and Fargues 1998; Arthurs and Thomas 2001; Dalla Santa et al. 2004). Small grains, such as rice or barley, are excellent

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substrates with large surface area per weight and desired moisture for conidiation (Alves and Pereira 1989; Aregger 1992). However, vessels used in case studies hold no more than a few kilograms (Feng et al. 1994, Roberts and St. Leger 2004). Enlarged production is laborious, often accompanied with contamination. Thus, new SSF devices and methods are needed to ease conidial production at low costs. This study sought to develop a new SSF apparatus for facilitating bulk production of fungal conidia on rice.

Materials and methods

Upright multi-tray conidiation chamber

The new solid-state fermentation apparatus we designed to maximize surface area in a limited space is an upright multi-tray conidiation chamber (Feng et al. 2005), as illustrated in Fig. 1. The

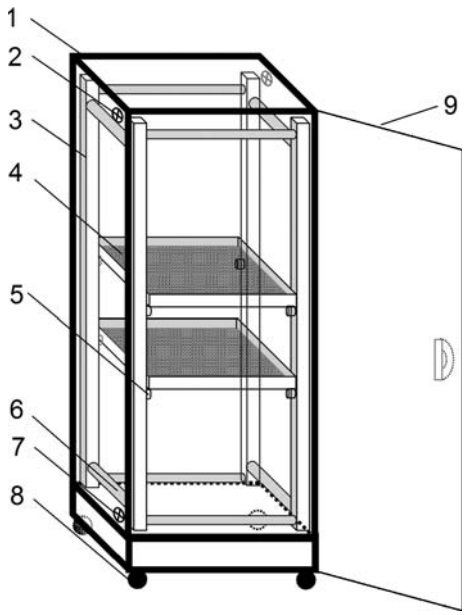

Fig. 1 Diagram for the structure of an upright multi-tray conidiation chamber. (1) Outside metal frame to support consolidated glass walls. (2) Moisture inlet that is piped to a moisture generator outside. (3) Inside rust-proof metal frame to support trays. (4) Rust-proof metal tray with meshed bottom. (5) Tray supporter that makes it easy to move the tray in or out. (6) Metal connection to strengthen the inside frame. (7) Moisture outlet. (8) Universal wheel to ease the movement of the whole chamber. (9) Consolidated glass door

frame has dimensions of $60 \times 60 \times 200$ cm, yielding an SSF volume of 0.72 m^3 on a 0.36 m^2 area. The chamber is equipped with 25 trays parallel to each other equally spaced. Each tray, 4 cm depth and 58 cm length, accommodates 2–3 kg solid substrate. The bottom of each tray is an open mesh (with 0.5-mm pores) to maintain temperature and relative humidity (RH) as uniformly as possible in the chamber during SSF. Thus, the chamber has an overall SSF area of 8.4 $(0.58 \times 0.58 \times 25)$ m^2 and may hold $\geqslant 50$ kg solid substrate. The transparency of its door and walls permits entry of natural light, favoring fungal conidiation and solid culture observation during SSF. A sample chamber with all trays was manufactured at the cost of *ca* US $450 in China.

Preparation of solid substrate

To further increase conidiation area, low-quality rice (e.g., indica rice grown in early season in southern China or long stored) at the bulk price of *ca* 0.2 US $ kg^{-1} in market is chosen as solid substrate due to larger surface area in smaller particles of an equal volume. Prior to use in cultures, rice must be soaked in hot water for 30–40 min, rinsed with tap water, steamed for 15 min in a large autoclave at 121°C or in a special cooking cabinet, and then cooled to ambient temperature. The rice ready for inoculation is usually in moderate firmness with a water content of ~38%.

Pilot production trials

To test the performance of the conidiation chamber, four pilot trials were separately carried out for production of fungal conidia in Hangzhou, Zhejiang during late January (winter), late March (spring), late May (early summer) and early September (late summer) of 2005. Liquid culture of a *Beauvaria bassiana* strain with mycelial biomass of ~18 mg ml^{-1} was mixed with the steamed rice at the ratio of ~100 ml kg^{-1}. Uniform inoculation was achieved by agitating the mixture in a stirrer. Well mixed rice with no excessive water dripping was then spread *ca* 2.5 cm thick in trays (2 kg per tray in dry weight).

Each trial included 50 kg rice for conidiation in the sample chamber standing in a 20 m^2 room, in

which temperature was controlled around 25°C by a 1.18-kW air conditioner. Before use, all trays and tools in the room were treated overnight under ultraviolet light. During the first 3-day SSF, clean mist from a moisture generator containing 3.5 liter distilled water was slowly piped into the chamber to maintain nearly saturated RH. Subsequently, moisture supply was ceased but high relative humidity (RH) was retained in the following 2–3 days for conidiation. When rice grains were generally covered with a heavy layer of yellowish powder, conidiation was terminated by rapid RH reduction, which was achieved by opening moisture outlets or slightly opening the door. The resultant rice cultures were dried overnight in a ventilation chamber at 33°C, followed by harvest of fine conidial powder using a cyclone spore separator 'MK-1' (CABI Bioscience, Silwood Park, Ascot, Berks, UK). Three samples of the conidial powder from each tray were then taken for the estimate of percent water content (based on weight loss of 1 g powder dried at 120°C for 2 h) conidia g^{-1} powder (based on microscopic counts in hemocytometer) and percent viability (based on counts of germinated conidia in 24 h liquid culture shaken at 25°C). Rice consumption rate in each trial was estimated based on the weight of the rice residues after harvest. These estimates and calculations were used as indices for the production of aerial conidia.

To monitor temperature and RH inside the chamber during SSF, both variables were hourly recorded by three digital recorders (Zheda Electric Apparatus, Inc., Hangzhou, Zhejiang, China) hung in the upper, middle and lower layers of the chamber, respectively.

Results and discussion

Controllability of temperature and RH

Hourly records from the three digital recorders during SSF provide an overview to the controllability of both temperature and relative humidity (RH) within the chamber standing in the workshop-like room (Fig. 2). During fungal growth and conidiation (days 1–6), daily means (±SD) of the two variables were 24.4°C (±3.9) and 95.6% RH (±4.3) in trial 1, 25.1°C (±2.5) and 98.1% RH (±2.8) in trial 2, 25.9°C (±1.5) and 96.5% RH (±3.6) in trial 3, and 25.4°C (±0.8) and 98.0% RH (±3.3) in trial 4, respectively. Generally, both variables were well controlled in different seasons despite slight variation within the chamber. The RH was controlled more readily by the mist supply than was the temperature by air conditioner. Over 95% RH was achieved soon after initiation in the seasonal trials but a longer period was taken to reach the expected temperature in winter (trial 1). Moreover, the RH control tended to be more stable at less fluctuating temperatures.

Conidial production in pilot trials

Overall, the chamber worked very well in all trials. The first 3 days witnessed rapid growth of the fungal agent on rice, followed by desirable conidiation in the following 3 days. Under the controlled conditions, the whole production process was completed within 7 days (Fig. 2). Self-heating and contamination were not observed in the chamber. Fine powder of aerial conidia was readily separated from the dried cultures by the cyclone spore separator and automatically collected into a spore tray. Mycelial debris (coarse powder) not going through the filter above the tray was also collected.

Indices for conidial production in the chamber are listed in Table 1. The conidial powder harvested from 50-kg rice cultures weighed 649.5, 727.5 683.0 and 854.5 g in trials 1–4, respectively and had a variable range of water content. The high quality of the harvested conidial powder was featured with the purity of 1.7 (1.4–1.9) $\times 10^{11}$ conidia g^{-1}, the viability of 94.0% (92.1–95.7%), and the water content of 12.8% (9.9–18.1%). On average, conidial yield reached 14.6 (13.0–17.1) g powder kg^{-1} rice, including 2.4 (1.8–2.7) $\times 10^{12}$ conidia g^{-1} powder. This conidial powder can be easily dried to ~5% water content in a vacuum drier for long-term storage under cool conditions and then used to prepare oil formulation for ultra-low volume spray or emulsifiable formulation for low or normal volume spray onto crops (Pu et al. 2005).

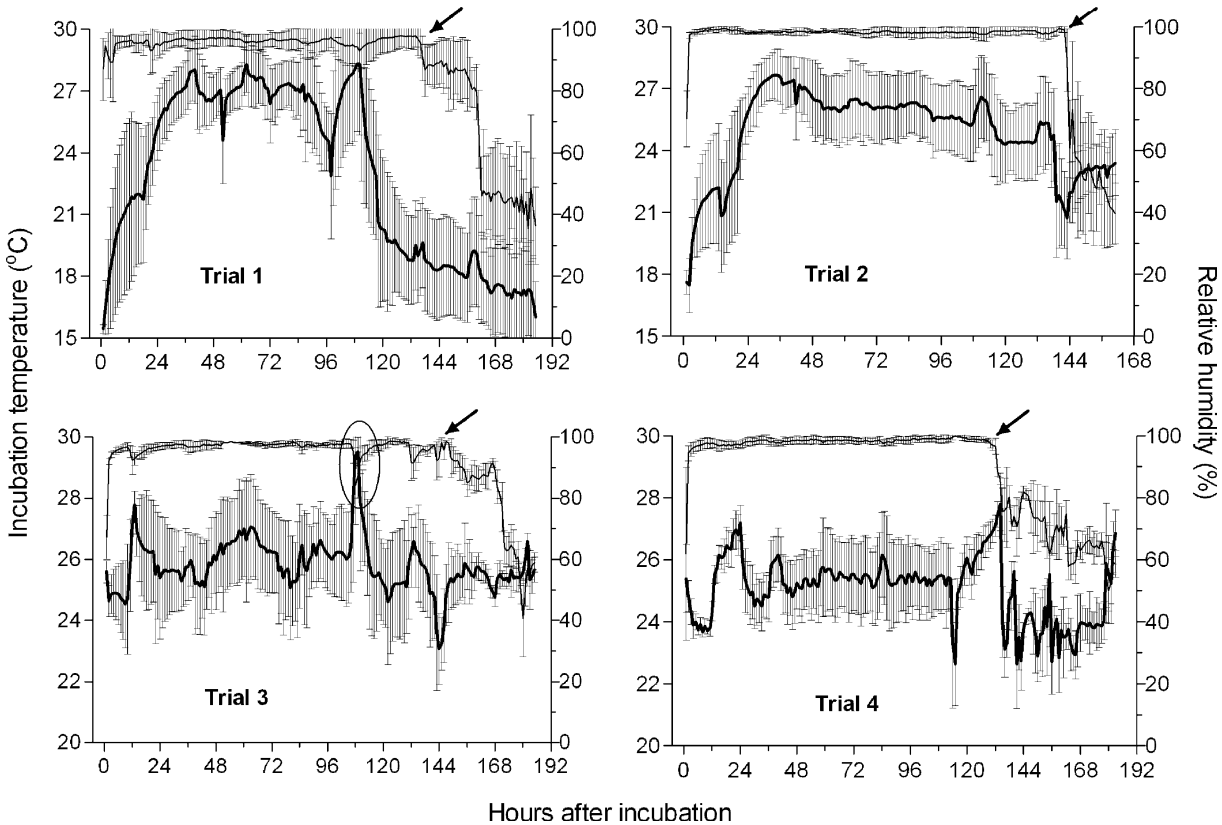

Fig. 2 Trends in the hourly mean records of temperature (bold solid lines) and relative humidity (solid lines) within the upright multi-tray conidiation chamber in trials 1–4. Each bar denotes a variability of the records within the chamber. The arrow in each graph indicates the time at which the conidiation was terminated by opening the moisture outlets or slightly opening the door. Note that the circled high temperature records in trial 3 affected the stability of the relative humidity control due to accidentally switching off the air conditioner for a short time

Table 1 Indices for the production of *B. bassiana* conidia in repeated pilot trials of the upright multi-tray conidiation chamber fully loaded with rice

Production indices	Mean ± SD[B]				Overall Mean ± SD
	Trial 1	Trial 2	Trial 3	Trial 4	
Conidial powder (g kg^{-1} rice)	13.0 ± 1.7c	14.6 ± 2.1b	13.7 ± 1.5bc	17.1 ± 2.4a	14.6 ± 1.8
Water content in conidia (%)	11.2 ± 1.3b	9.9 ± 3.2b	12.0 ± 2.1b	18.1 ± 4.7a	12.8 ± 3.6
No. conidia g^{-1} powder ($\times 10^{11}$)	1.4 ± 0.1c	1.9 ± 0.3a	1.9 ± 0.2a	1.6 ± 0.1b	1.7 ± 0.2
No. conidia kg^{-1} rice ($\times 10^{12}$)	1.8 ± 0.3b	2.7 ± 0.6a	2.5 ± 0.4a	2.7 ± 0.4a	2.4 ± 0.4
Conidial viability (%)	92.1 ± 2.3b	94.1 ± 2.4a	94.1 ± 2.1a	95.7 ± 2.0a	94.0 ± 1.5
Coarse mycelia (g kg^{-1} rice)	1.4 ± 0.4b	2.2 ± 0.7a	1.7 ± 0.8ab	1.8 ± 0.6ab	1.8 ± 0.3
Overall biomass (g kg^{-1} rice)[A]	14.4 ± 1.8c	16.7 ± 2.0ab	15.4 ± 1.6bc	18.9 ± 2.8a	16.3 ± 2.0
Rice consumption rate (%)	20.3 ± 3.6a	21.8 ± 3.0a	21.3 ± 5.5a	16.2 ± 6.6b	19.9 ± 2.6

[A]The sum of the fine conidial powder and the coarse mycelial powder

[B]Each table entry was based on measurements or observations of three rice culture samples from each of the 25 trays (2 kg rice per tray) in each trial. Means with different lowercase letters in each line differed significantly (Tukey's HSDs, $P < 0.05$; df = 2, 24 for all F tests).

Moreover, a small amount of mycelial debris in the form of coarse powder was also obtained at the yield of 1.8 (1.4–2.2) g kg^{-1} rice (Table 1). This coarse powder was potentially useful for control of underground pests although it was unsuitable for making a formulation for conventional spray. Overall, the four trials generated 16.3 (14.4–18.9) g biomass kg^{-1} rice at the rice consumption rate of 19.9% (16.2–21.8%). The residue rice retained well the shape of grains after aerial conidia were harvested using the cyclone spore separator and could be partially recycled in conidial production with the chamber.

Conclusions

The new apparatus especially designed for conidial production of fungal biocontrol agents features a large SSF area in a very limited space, simple structure, low manufacture cost, and easy operation and monitoring. All it requires are air conditioning for control of ambient temperature and clean mist input for maintenance of nearly saturated humidity. Optimized firmness and water content of solid substrate and tray–tray spaces are crucial to desired conidiation.

Based on the harvested yield of aerial conidia in the repeated trials and a normal application rate of ~1.0×10^{13} conidia ha^{-1} for insect control in the field (Feng et al. 1994; Wraight et al. 2001), a single chamber can produce a quantity of *B. bassiana* conidia for a spray of 12.2 ha at the cost of 50 kg rice within ~7 days. This cost is likely to be decreased if the residual rice is recycled in production. Thus, the chamber would be of high potential for bulk production of aerial conidia of *B. bassiana* and other fungal biocontrol agents. Since the pilot trials were conducted in a workshop-like room, the new apparatus can be relied upon for designing a real workshop if facilities for rice treatment in large quantity are integrated. For instance, no more than 200 chambers are needed to warrant a daily loading of 1 ton rice (i.e., 20 chambers per day) for fermentation. Since each chamber at the recommended size occupies only 0.36 m^2, the entire SSF workshop would be no more than 200 m^2. Such a workshop may produce 244×10^{13} conidia per day with an annual yield for a spray coverage of at least 80,000 ha. The UK-derived cyclone technology (spore separators in the MycoHarvester series) would suit well to harvest the conidia from the rice cultures. Therefore, the new SSF apparatus would have great potential for application to bulk production of aerial conidia of *B. bassiana* and other fungal biocontrol agents.

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