Journal of Industrial Microbiology & Biotechnology (2000) 24, 227–230

www.nature.com/jim

ORIGINAL PAPERS

Heat resistance of *Paecilomyces variotii* in sauce and juice

E Piecková[1,2] and RA Samson[1]

[1]*Centraalbureau voor Schimmelcultures, Baarn, The Netherlands*

It has been demonstrated that some anamorphic fungi (*Paecilomyces variotii*, *Fusarium* sp) could cause spoilage of food products after pasteurisation. Four food-borne and one clinical isolate of *P. variotii* were cultivated on one solid medium and three liquid media. Their survival after heating at 80–100°C for 0.25–15 min in sterile distilled water and curry sauce or fruit juice was investigated. Heat resistance was determined by the thermal death method in a thermostatically-controlled oil bath. The most resistant spores of *P. variotii* from curry sauce cultivated on malt extract agar survived 100°C for 0.5 min in sauce; cultivated in curry sauce survived 100°C for 15 min in water and cultivated in malt broth survived 100°C for 5 min in water and sauce. The most resistant spores of *P. variotii* from juice cultivated on malt extract agar were able to survive 100°C for 15 min in water; cultivated in juice survived 100°C for 0.5 min in juice and suspensions from cultivation in malt broth survived 100°C for 1.5 min in juice. Spores of the clinical strain of *P. variotii* from malt extract agar survived 95°C for 0.33 min in water, and orange juice cultures survived 96°C for 10 min in orange juice. It was thus found that *P. variotii* strains cultivated in food were better adapted to heat stress, suggesting that fungal biomass suspensions were able to survive the higher temperatures for longer time intervals than spore suspensions. *Journal of Industrial Microbiology & Biotechnology* (2000) **24,** 227–230.

Keywords: fungi; heat resistance; *Paecilomyces variotii*; sauce; juice; thick-walled propagules

Introduction

Several heat-resistant fungi have been reported, which are able to survive temperatures >90°C for several minutes, depending on the species and/or products. The heat resistance is due mainly to ascospores and/or thick-walled chlamydospores or hyphae. *Byssochlamys nivea*, *Neosartorya fischeri*, *Talaromyces flavus* and *T. macrosporus* are important heat-resistant fungi for the food industry, causing spoilage problems, especially in fruit products, and some of them are able to produce mycotoxins. These species produce heat-resistant ascospores [2,8].

Some anamorphic fungi—like *Botrytrichum piluliferum*, *Gilmaniella humicola*, *Nodulisporium* sp survive heating even at 90°C. These species form sclerotia or thick-walled alleuriospores which are probably responsible for their heat resistance [3–5]. *Paecilomyces variotii* and some *Fusarium* sp can survive heat treatment of 95°C for 10–20 s, probably due to thick-walled structures such as hyphae and/or chlamydospores [8]. The significance of these species' heat resistance for the canning industry has not been investigated sufficiently, although *P. variotii* strains have been isolated from spoiled foods pasteurised at 93°C for 5 min. *P. variotii* is an ubiquitous contaminant of foods and raw materials, especially those containing oils (cereals, nuts, meat products, margarine, edible oils, cheeses, dried fruits and seeds). The species is sorbate-resistant. It has not been reported to produce any important secondary metabolites or mycotoxins [7].

[2]Correspondence to E Piecková at her present address: Institute of Preventive and Clinical Medicine, Limbová 14, SK-833 01 Bratislava, Slovakia
Received 2 June 1999; accepted 5 December 1999

The aim of this investigation was to study the ability of *P. variotii* (two strains isolated from pasteurised sauces, two strains from pasteurised juices and one clinical isolate cultivated on solid and various liquid media, to survive heating at 80–100°C for 0.25–15 min in different media. The study was divided into three experiments: in the first, the fungal strains were cultivated on a solid medium [malt extract agar (MEA)] and spores were harvested for the heat resistance test. In the other two experiments, the fungi were grown in liquid media—food products or malt broth.

Materials and methods

Experiment I: Paecilomyces variotii strains cultivated on agar medium

Five strains of *P. variotii* [No. 1 and 2 isolated from curry sauce containing 1% protein, 1.8% saccharides, 9.4% fat and vitamins B_1, B_6, B_{12}, C and folic acid; No. 3 isolated from apple juice; CBS 902.97 isolated from tropical fruit juice (pineapple, banana, mango, passion fruit, orange, grape) and one clinical isolate (eye tumor) CBS 124.97] were cultivated on MEA (Oxoid, Unipath, Basingstoke, UK) slants at 24°C for 2 weeks.

Preparation of spore suspensions: Spores were scraped gently from the mycelium with a sterile needle and suspended in sterile water with Tween 80 (0.05–0.01%) in a sterile bottle with glass beads. After intensive hand-shaking (about 3 min) the suspension was filtered through sterile glass wool. A viable count of the suspension was determined by counting colony forming units (CFU) ml^{-1} on MEA plates inoculated with 0.2 ml from the suspension tested, diluted at least 4-times tenfold (always in duplicate).

The suspension was incubated at 24°C for 4–5 days. Freshly prepared suspensions were used in all experiments.

Experiment II: Paecilomyces variotii strains cultivated in food products

P. variotii strains were cultivated in 200 ml of food product—strains No. 1 and 2 in curry sauce (pH 4.05), strain No. 3 in apple juice (pH 3.77), strains CBS 902.97 and 124.97 in orange juice (pH 3.54)—in a 500-ml Erlenmeyer flask on a rotary shaker (rotation speed 100 rpm) at room temperature (22–24°C) for 2 weeks.

Preparation of suspensions of fungal propagules (fragments of hyphae, spores, chlamydospores): Liquid cultures of *P. variotii* were fragmented in a blender (Homogenizer type MPW-302 by Mechanyka Precyzyjna, Warszawa, Poland). The suspension was cooled in an ice bath during fragmentation to avoid heating. The suspension obtained was diluted 1:2 with sterile distilled water and the viable count was determined as described above.

Experiment III: Paecilomyces variotii strains cultivated in malt both

P. variotii strains No. 1–3 were cultivated in 200 ml of malt broth (pH 5.6; Oxoid) in a 500-ml Erlenmeyer flask on a rotary shaker. The fungal particle suspensions were prepared as in experiment II.

Heating media: Sterile distilled water, curry sauce (pH 4.05) diluted with sterile distilled water 1:1, apple juice (pH 3.77) or orange juice (pH 3.54) were used as heating media for heat resistance studies.

Determination of heat resistance: Heat resistance was determined by the thermal death method [9] using glass test tubes containing 4.5 ml of preheated heating medium, kept in a thermostatically-controlled oil bath (NESLAB GP-400, Neslab Instruments, Portsmouth, USA) at specific temperatures of 80, 84, 88, 92, 93, 94, 95, 96, 97 and 100°C for at least 10 min. After warming the heating medium in the test tube, 0.5 ml of the *P. variotii* inoculum was added to each tube by an automatic pipette (Finnpipette Digital, 0.2–1 ml by Labsystems, Helsinki, Finland). Inoculated medium was stirred three times by air flow from the pipette. At predetermined intervals (0.25, 0.33, 0.5, 1, 1.5, 3, 5, 7.5, 10 and 15 min), test tubes were removed and immediately cooled in ice water (for a minimum of 10 min). The heated suspension (0.2 ml) and one diluted tenfold with cold heating medium were inoculated onto malt extract agar plates (in duplicate), spreading with a glass rod in the shape of a hockey-stick. The number of surviving fungal spores and hyphal fragments was determined by counting CFU on malt extract agar plates after incubation at 24°C for 4–5 days. The longest heating times at the highest temperatures with *P. variotii* are recorded in the tables.

Results

The number of *P. variotii* propagules (an average of four agar plates—two plates from two replicate experiments) recovered after the highest temperature for the longest heating period is reported in Tables 1–3.

Experiment I

The *P. variotii* spores' survival after heat treatment in water and sauces or juices is shown in Table 1. Spores of the two strains of *P. variotii* from sauce were able to survive 100°C max for 0.5 min in sauce; spores of the two strains from juice survived 100°C for 15 min in water, and spores of the clinical strain survived 95°C for 0.33 min in water.

Experiment II

Final pH values of cultivation media were estimated: curry sauce pH 3.78 (strain No. 1), pH 3.61 (strain No. 2), apple juice pH 3.24 (strain No. 3), orange juice pH 3.44 (strains CBS 902.97 and 124.97) The *P. variotii* propagules' survival after heat treatment in water and sauce or juice is presented in Table 2. The propagules of the two strains from sauce survived 100°C for 15 min in water; the propagules of the two strains from juice survived 100°C max for 0.5 min in juice and propagules of the clinical strain survived 96°C max for 10 min in orange juice.

Experiment III

Final pH values of cultivation medium—malt broth were estimated: for strain No. 1 cultivation pH 4.56, strain No. 2 pH 4.48 and strain No. 3 pH 5.52.

The survival of *P. variotii* propagules after heat treatment of suspensions prepared from fluid cultures in malt broth in water and juice is shown in Table 3. Propagules of the two strains from sauce survived 100°C max for 5 min in water and also in sauce, and propagules of the strain from apple juice survived 100°C max for 1.5 min in apple juice.

The structure of the fungus after growing in malt broth and food products and comparison of these results with the morphology after growth on solid agar media were made using light microscopy. Many thick-walled fungal structures (chlamydospores, hyphal fragments)—rare in the agar cultures—were observed in liquid cultures.

Discussion

Mechanisms of heat resistance in certain micromycete species are not well understood. In cells, the mechanism could be based on the composition of fatty acids, higher or lower levels of some substances (eg minerals) in the cell, lower intracellular value of a_w, special structure of the cell membrane and/or the effects of heat-shock proteins. Heat resistance, halotolerance or osmotolerance of fungal propagules is influenced under laboratory conditions by a number of factors, such as age of test-culture, incubation temperature and aeration, composition of the medium as well as heating medium composition (pH, a_w, presence of saccharides, organic acids, proteins and products of their digestion [6,8].

From this study it can be concluded that the five *P. variotii* strains were able to produce structures which could survive heat treatment even at 100°C for different time intervals in water and foods. Heated fungal biomass suspensions obtained from liquid cultures in malt broth survived high temperatures for shorter times than biomass suspensions from fluid cultures in foods, sauces and juices. From this

Table 1 Effect of heat treatment on survival of *Paecilomyces variotii* spores from malt extract agar

Strain	Density of suspensions (CFU ml^{-1})		Density of suspensions (CFU ml^{-1})	
	Initial	Final	Initial	Final
	Water		**Curry sauce**	
No. 1 (from sauce)	1.75×10^5	100 93°C/7.5 min[a]	1.75×10^5	25 100°C/0.5 min
No. 2 (from sauce)	2.4×10^5	787.5 93°C/0.5 min	2.4×10^5	12.5 93°C/7.5 min
	Water		**Apple juice**	
No. 3 (from apple juice)	2.65×10^5	12.5 100°C/15 min	2.65×10^5	12.5 100°C/10 min
	Water		**Orange juice**	
CBS 902.97 (from tropic juice)	2.9×10^5	12.5 96°C/10 min	2.9×10^5	8975 92°C/10 min
CBS 124.97 (clinical isolate)	4.9×10^5	12.5 95°C/0.33 min	4.9×10^5	1825 92°C/10 min

[a]The highest temperature survived/the longest period survived.

Table 2 Effect of heat treatment on survival of *Paecilomyces variotii* propagules from fluid cultures in sauce or juice

Strain	Density of suspensions (CFU ml^{-1})		Density of suspensions (CFU ml^{-1})	
	Inital	Final	Initial	Final
	Water		**Curry sauce**	
No. 1 (from sauce) pH 3.78[b]	6.4×10^4	12.5 100°C/7.5 min[a]	6.4×10^4	87.5 100°C/7.5 min
No. 2 (from sauce) pH 3.61	6.2×10^4	12.5 100°C/15 min	6.2×10^4	12.5 100°C/10 min
	Water		**Apple juice**	
No. 3 (from apple juice) pH 3.24	1.7×10^6	687.5 84°C/15 min	1.7×10^6	412.5 100°C/0.5 min
	Water		**Orange juice**	
CBS 902.97 (from tropic juice) pH 3.44	1.4×10^6	125 100°C/0.25 min	1.4×10^6	1300 96°C/10 min
CBS 124.97 (clinical isolate) pH 3.44	4.9×10^6	0 93°C/<0.25 min	4.9×10^6	25 96°C/10 min

[a]The highest temperature survived/the longest period survived.
[b]Final pH of cultivation media.

Table 3 Effect of heat treatment on survival of *Paecilomyces variotii* propagules from malt broth

Strain	Density of suspensions (CFU ml^{-1})		Density of suspensions (CFU ml^{-1})	
	Initial	Final	Initial	Final
	Water		**Curry sauce**	
No. 1 (from sauce) pH 4.56[b]	3.8×10^6	575 100°C/1 min[a]	3.8×10^6	12.5 100°C/0.5 min
No. 2 (from sauce) pH 4.48	5.45×10^6	12.5 100°C/5 min	5.45×10^6	25 100°C/5 min
	Water		**Apple juice**	
No. 3 (from apple juice) pH 5.52	7.15×10^6	12.5 97°C/5 min	7.15×10^6	37.5 100°C/1.5 min

[a]The highest temperature survived/the longest period survived.
[b]Final pH of cultivation media.

observation it can be concluded that when cultures were cultivated in foods, they were more stressed during growth (pH, presence of spices, possible weak antifungals), in comparison with cultivation in complete rich nutrient medium, and therefore they were better adapted to heat stress.

Generally, the expectation that fungal biomass suspensions would survive higher temperatures for longer time intervals than suspensions of spores has been confirmed—the thick-walled fungal propagules (chlamydospores, clusters of hyphae) appear to be responsible for heat resistance of *P. variotii*.

It was further demonstrated that the composition of the heating media (heat conductance, protective effect of proteins and/or saccharides) influenced the resistance of the fungal propagules to heating. Some discrepancies observed in numbers of colonies surviving particular heat treatment could be caused by natural uneven distribution of the thick-walled propagules in the tested suspensions [1].

It seemed that the origin of the strains influenced their heat resistance only when water was used as the heating medium. The clinical isolate was more sensitive as might be expected.

These results represent a good starting-point for more detailed scientific investigation of the heat resistance of the anamorphic fungi important for the food industry and for hygiene.

Acknowledgements

The study was made with financial support by the Federation of European Microbiological Societies and Master Foods BV, Oud Beijerland, The Netherlands. Thanks are due to Dr K Wilkins for language revision.

References

1 Fujikawa H and T Itoh. 1996. Tailing of thermal inactivation curve of *Aspergillus niger* spores. Appl Environ Microbiol 62: 3745–3749.
2 Jesenská Z and E Piecková. 1995. Heat-resistant fungi. Czech Mycol 48: 73–76.
3 Jesenská Z, E Piecková and D Bernát. 1992. Heat-resistant fungi in the soil. Int J Food Microbiol 18: 209–214.
4 Jesenská Z, E Piecková and D Bernát. 1993. Heat resistance of fungi from soil. Int J Food Microbiol 19: 187–192.
5 Piecková E, D Bernát and Z Jesenská. 1994. Heat resistant fungi isolated from soil. Int J Food Microbiol 20: 297–299.
6 Pitt JI. 1989. Food mycology: an emerging discipline. J Appl Bact Symp Suppl 67: 1S–9S.
7 Pitt JI and Hocking AD. 1997. Fungi and Food Spoilage, 2nd edn. Blackie Academic & Professional, London, 593 pp.
8 Samson RA, ES Hoekstra, JC Frisvad and O Filtenborg. 1996. Introduction to Food-borne Fungi, 5th edn. Centraalbureau voor Schimmelcultures, Baarn-Delft, 322 pp.
9 Tchango Tchango J, R Tailliez, P Eb, T Njine and JP Hornez. 1997. Heat resistance of the spoilage yeasts *Candida peliculosa* and *Kloeckera apis* and pasterurisation values for some tropical jucies and nectars. Food Microbiol (London) 14: 93–99.