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BACTERIAL ACTIVITY IN DIFFERENT TYPES OF CASING DURING MUSHROOM CULTIVATION (*AGARICUS BISPORUS* (LANGE) IMBACH)

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The influence of casing type and raking on the behaviour of bacterial populations during the growth cycle of mushroom (*Agaricus bisporus* (Lange) Imbach) was studied. After the induction of fructification there was a significant increase in the total bacterial count except for the casing containing composted vine shoots. These high counts were maintained after the second flush in most cases. The count of fluorescent pseudomonads also increased after the casing was added until the induction of fructification and remained high or decreased after the second flush. The results point to a significant ($P \leq 0.05$) interaction between casing type and raking as regards total bacteria and fluorescent pseudomonads in all three experiments both after the induction of fructification and after the second flush.

Keywords: bacteria, casing, fluorescent pseudomonads, mushroom

In mushroom cultivation (*Agaricus bisporus* (Lange) Imbach), the initiation and development of the carpophores depend on the genetic capacity of the mycelium to fructify, physical and environmental factors, chemical and nutritional factors and microbiological factors (COUVY, 1972; BAZERQUE & LABORDE, 1975; FORET, 1989).

As far as microbiological factors are concerned, numerous studies have confirmed that the presence of bacteria in the casing, particularly of *Pseudomonas putida* (Trevisan) Migula, stimulates mushroom fructification (EGER, 1963; HAYES et al., 1969; ARROLD, 1972; HUME & HAYES, 1972; SAMSON et al., 1987; MASAPHY et al., 1987). These bacteria, when present in sufficient numbers, are capable of inhibiting mycelium growth, thereby promoting carpophore formation. There is also a reciprocal effect between the mycelium and the microflora present in the casing: the greater the number of bacteria, the stronger the inhibition of growth and the earlier the primordia formation (EGER, 1962, 1963). The interaction between *Pseudomonas* sp. and *A. bisporus* (Lange)

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Imbach during primordia formation has been confirmed by electron microscopic studies (MASAPHY et al., 1987).

HAYES and co-workers (1969) showed that the casing, besides acting as mechanical support for the sporophores and maintaining a sufficiently moist environment, also provides a substrate which stimulates bacteria. The same authors found that among the microorganism populations isolated from unsterilised casing material, there were certain bacteria that, when added to pure cultures, were capable of stimulating fructification. These Gram negative bacteria, which use glucose for oxidation and fluorescent bright green in King's B medium, were identified as *Pseudomonas putida* (Trevisan) Migula, or some very closely related species. CRESSWELL and HAYES (1979) demonstrated that the number of bacteria in the casing material increased significantly after application to a spawned compost, with *Pseudomonas* being the predominant group.

HAYES (1974) also showed that high microbial activity in the casing brought forward the time when mushrooms could be harvested and increased primordia formation and the number and weight of the harvested crop. It is clear, that such activity is very important.

Although the influence of bacterial populations in mushroom fructification has been demonstrated, as has the importance of the casing which, among other things, provides a suitable substrate for the bacteria, little attention has been paid to the joint influence of different types of casing and raking (mixing the casing with the mycelium growing therein) on bacterial activity during the mushroom (*A. bisporus* (Lange) Imbach) growth cycle. This interaction was the object of the present study, along with an analysis of the possible effect of these bacterial populations on growth parameters. La Manchuela is situated in the provinces of Cuenca and Albacete, Spain, where 40000–50000 tons of mushroom (45–50% of the Spanish production) are produced annually (PARDO et al., 2001). Besides the casings traditionally used in this region, a mineral soil with *sphagnum* or black peat, we analysed alternative mixtures containing composted pine bark, coconut or wood fibre, composted vine shoots and a casing commonly used in La Rioja, another mushroom growing region of Spain.

1. Materials and methods

1.1. Material

The experiment was carried out in the Mushroom Research Centre (CIES) of Quintanar del Rey, Cuenca, Spain, during the 1997–98 (Experiment 1) and 1998–99 (Experiments 2 and 3) growing seasons.

The mineral soil with no additives came from "La Cerrasa" (Villanueva de la Jara, Cuenca, Spain) and was supplied by Oviedo Soler Hnos., S.L. (Villanueva de la Jara, Cuenca, Spain). The casing used for mushroom cultivation in La Rioja consisting of a mixture of fine limestone gravel and *sphagnum* peat (PEÑA & SANZ, 1993) was provided by Gohercu, S.L. (Quel, La Rioja, Spain). Besides these casings, we also studied binary mixtures of mineral soil (80%, v/v) as base material mixed with each one

of the following materials (20%, v/v): *sphagnum* peat of German origin (Protorf), coconut fibre pith (Cocopeat®) and wood fibre (Hortifibra) supplied by Projar, S.A. (Valencia, Spain), black peat from the coastal formations southeast of Torreblanca (Castellón, Spain) supplied by Industrias Fertilizantes Orgánicas, S.A. (Valencia, Spain), composted pine bark provided by Masecor S.L. (Motilla del Palancar, Cuenca, Spain), and composted vine shoots previously produced in CIES (PARDO et al., 1999).

Three of the most widely used varieties of mycelium in La Manchuela (PARDO, 1999) were chosen: "Pla 8.9" (mid-range hybrid strain) for Experiment 1, "Blancochamp BL-40" (smooth white hybrid strain) in Experiment 2 and "Gurelan-45" (large off-white hybrid strain) in Experiment 3. A spawn rate of 12 g kg^{-1} fresh mushroom compost was used.

The composts used in the three experiments were based on winter cereal straw and were of the type denominated semisynthetic. They showed the following analytical characteristics: moisture content 66–69%, organic matter 73.5–77.5%, total nitrogen 2.20–2.36%, C/N ratio 18.5–19.6, pH 7.3–7.5 and ash content 22.6–26.5%. All the values fell within the range considered optimum for mushroom cultivation (VEDDER, 1978; PARDO, 1993; HEARNE, 1994). The absence of nematodes and mites indicated that the pasteurisation process had been sufficient (FLETCHER et al., 1989).

Cultivation was carried out in a walk-in growth chamber measuring 3.7×3.1×2.6 m (20.2 m^3), with a controlled heating/cooling and recirculation system/exterior ventilation so that temperature, relative humidity and CO_2 concentration could be controlled automatically.

1.2. Experimental design

The experimental design used was a 8×2 Equilibrated Factorial Plan, with 4 repetitions (randomised blocks with two factorial factors). The first factor, with eight levels, was the casing type. The second factor, with two levels, was the application or absence of the raking technique. Repetitions corresponding to four blocks were placed in two levels on both sides of the crop chamber.

In each experiment, 64 PVC trays measuring 29.5×29.5×18.5 cm (16 l in volume, 870 cm^2 in area) were used. Experimental trays were filled with 6 kg of compost compacted at 450 kg m^{-3} (69 kg compost m^{-2}). The volume of the casing was 2.61 l per tray, giving a depth of 3 cm.

1.3. Cultivation cycles

The growth cycles were carried out according to the growth chamber conditions (air temperature, relative humidity and carbon dioxide concentration) suggested for each of the selected strains (CIES, 2000).

The casing was applied 14 days after spawning and the treatments normally used in the area were carried out: disinfectant (18 ml m^{-2} formaline), insecticide (3.6 g m^{-2}

diflubenzuron 25%) and fungicide (0.62 g m^{-2} procloraz 46%). When used, raking was carried out 7 days after casing, and at the beginning of fructification four days later. Each cultivation cycle lasted 80 days.

The casing was moistened to between 60% and 70% of its water-holding capacity, by regular and uniform watering with between 0.5 l m^{-2} and 1.5 l m^{-2}, depending on necessity, and according to the usual cultivation technique (WUEST, 1982).

1.4. Sampling

Casing samples were taken by means of sterilised copper tubes (12 cm long × 2.5 cm diameter). One end of the tubes was sharpened to help penetration and minimize disturbance of the casing. The resulting orifices were filled with the same type of casing.

Samples were taken at the same time the casing was applied, one day after induction (12–13 days after casing) and after the second flush (34–39 days after casing). Four samples were taken for each combination of factors, one per repetition. The samples were mixed and two subsamples of 9–10 g were taken.

1.5. Sample preparation and microbiological analysis

The samples were homogenized by magnetic stirrer for 30 min.

The diluent used both for the mother suspension (10^{-1}) and dilutions (up to 10^{-7}) was sterile buffered peptone water (Cultimed). The culture media used were Plate Count Agar (Merck) for the total count and King's B medium (Cultimed) enriched with 25 mg kg^{-1} of cycloheximide (Sigma) to count fluorescent pseudomonads.

The bacterial counts were determined by spreading. The plates were inverted to prevent water condensation and placed in a refrigerated incubator (Hotcold, J.P. Selecta, S.A., Abrera, Barcelona, Spain) and incubated for 72 h at 20 °C.

To detect fluorescence in the pseudomonads count, the colonies were exposed to ultraviolet light at 254 nm (VL-4C lamp, Vilber-Lourmat, Marne La Vallée, France).

The results for both counts were expressed as number of microorganisms (colony forming units) per gram of dry matter (CFU g^{-1}) (SAMSON et al., 1987; MASAPHY et al., 1989; MILLER et al., 1995).

1.6. Statistical analysis

The software package Statgraphics Plus v. 2.1 (Statistical Graphics Corp., Princeton, NJ, USA) was used for the statistical analysis. In order to resolve the variability of the experimental results into independent components, the "Multifactor ANOVA" tool of the "Analysis of Variance" option of Statgraphics was used, based on a significance level of 5%. The Tukey-HSD test (P=0.05) was used to establish significant differences, while in order establish correlations between changes in the bacterial populations and production parameters a linear correlation matrix was constructed alongside the p-values, which tests the statistical significance of the correlations measured, using the "Multiple-Variable Analysis" tool of Statgraphics.

2. Results and discussion

Table 1 shows the total bacterial counts and those of fluorescent pseudomonads at the time each type of casing was applied and the mean count in all the experiments. The lowest count was found in Experiment 3, more specifically in the casings containing *sphagnum* peat, composted pine bark and coconut fibre (4.2×10^4, 9.1×10^4 and 9.3×10^4 CFU g^{-1}, respectively). The mean number of total bacteria in these samples was two logarithmic units below the others (10^5 as opposed to 10^7). The highest initial counts were made in Experiments 1 and 2 when composted vine shoots were used in the casing mixture (9.0×10^7 and 7.8×10^7 CFU g^{-1}, respectively), followed by the La Rioja mixture (6.1×10^7 and 1.4×10^7 CFU g^{-1}, respectively). These initial counts were higher than those found previously by other investigators, who mention figures of around 10^4 CFU g^{-1} (HAYES, 1974; HAYES & NAIR, 1976), but similar to those found by DOORES and co-workers (1987) if we only bear in mind the figures for Experiment 3, or CRESSWELL and HAYES (1979) and MASAPHY and co-workers (1989), if the other two experiments are considered.

As regards the fluorescent pseudomonads they represented 0.0–3.1% of the total bacteria counted (Fig. 1), a similar proportion to that found by DOORES and co-workers (1987), but below the 50% mentioned by SAMSON and co-workers (1987), which emphasizes the extreme variability of the results. Experiment 2 showed the greatest number of casings containing pseudomonads (5 out of 8), although Experiment 1 showed the highest mean count (1.1×10^4 CFU g^{-1}) (Table 1).

Table 2 shows the total bacteria and fluorescent pseudomonads counts after the induction of fructification for each type of casing with and without raking and the final mean count in all the experiments. Note the significant increase in the total count after the induction of fructification, which agrees with the findings of other authors (CRESSWELL & HAYES, 1979; OLIVER & GUILLAUMES, 1979; HAYES, 1981; INGRATTA & PATRICK, 1987; REDDY & PATRICK, 1992; MILLER et al., 1995). Only the casing containing composted vine shoots in Experiment 1 and 2 produced a different result, since the counts decreased after induction (Tables 1 and 2). The total counts were between 7.6×10^6 CFU g^{-1} in Experiment 2 with composted vine shoots and 9.2×10^7 CFU g^{-1} in Experiment 1 with the La Rioja casing (Table 2). Mean counts were identical in all the experiments, between 1.6×10^7 CFU g^{-1}, in Experiment 3 and 2.8×10^7 CFU g^{-1} in Experiment 1 (Table 2). These figures are higher than those found by HAYES (1974), HAYES and NAIR (1976) (about 10^5 CFU g^{-1}) and below those cited by DOORES and co-workers (1987), CRESSWELL and HAYES (1979), INGRATTA and PATRICK (1987), MASAPHY and co-workers (1989) and MILLER and co-workers (1995) (about 10^8).

Table 1. Total bacteria and fluorescent pseudomonads counts at casing for each casing type in the three experiments

	Count (CFU g^{-1})					
	Experiment 1		Experiment 2		Experiment 3	
	TC	PC	TC	PC	TC	PC
Casing 1 (S)	1.0×10^6 c	0 c	2.2×10^5 d	0 d	6.8×10^5 a	0 b
Casing 2 (S+SP)	1.1×10^6 c	0 c	2.4×10^6 cd	0 d	4.2×10^4 d	0 b
Casing 3 (S+BP)	8.8×10^5 c	7.7×10^3 b	1.6×10^5 d	3.9×10^2 d	4.0×10^5 bc	0 b
Casing 4 (S+CB)	1.4×10^6 c	0 c	6.5×10^5 d	7.7×10^2 d	9.1×10^4 d	0 b
Casing 5 (S+CF)	3.5×10^6 c	1.5×10^4 a	4.9×10^5 d	0 d	9.3×10^4 d	0 b
Casing 6 (S+WF)	5.4×10^6 c	0 c	4.1×10^6 c	1.6×10^4 a	2.8×10^5 c	8.8×10^3 a
Casing 7 (S+CV)	9.0×10^7 a	0 c	7.8×10^7 a	1.2×10^4 b	7.6×10^5 a	0 b
Casing 8 (RI)	6.1×10^7 b	0 c	1.4×10^7 b	8.6×10^3 c	4.6×10^5 b	0 b
Mean	2.1×10^7	1.1×10^4	1.3×10^7	4.7×10^3	3.5×10^5	1.1×10^3

Values followed by a different letter within a column are significantly different at 5% level according to Tukey's test

TC: Total bacteria count
PC: Fluorescent pseudomonads count
S: Soil
S+CF: Soil +coconut fibre pith (4:1, v/v)
S+SP: Soil + sphagnum peat (4:1, v/v)
S+WF: Soil + wood fibre (4:1, v/v)
S+BP: Soil + black peat (4:1, v/v)
S+CV: Soil + composted vine shoots (4:1, v/v)
S+CB: Soil + composted pine bark (4:1, v/v)
RI: La Rioja type casing

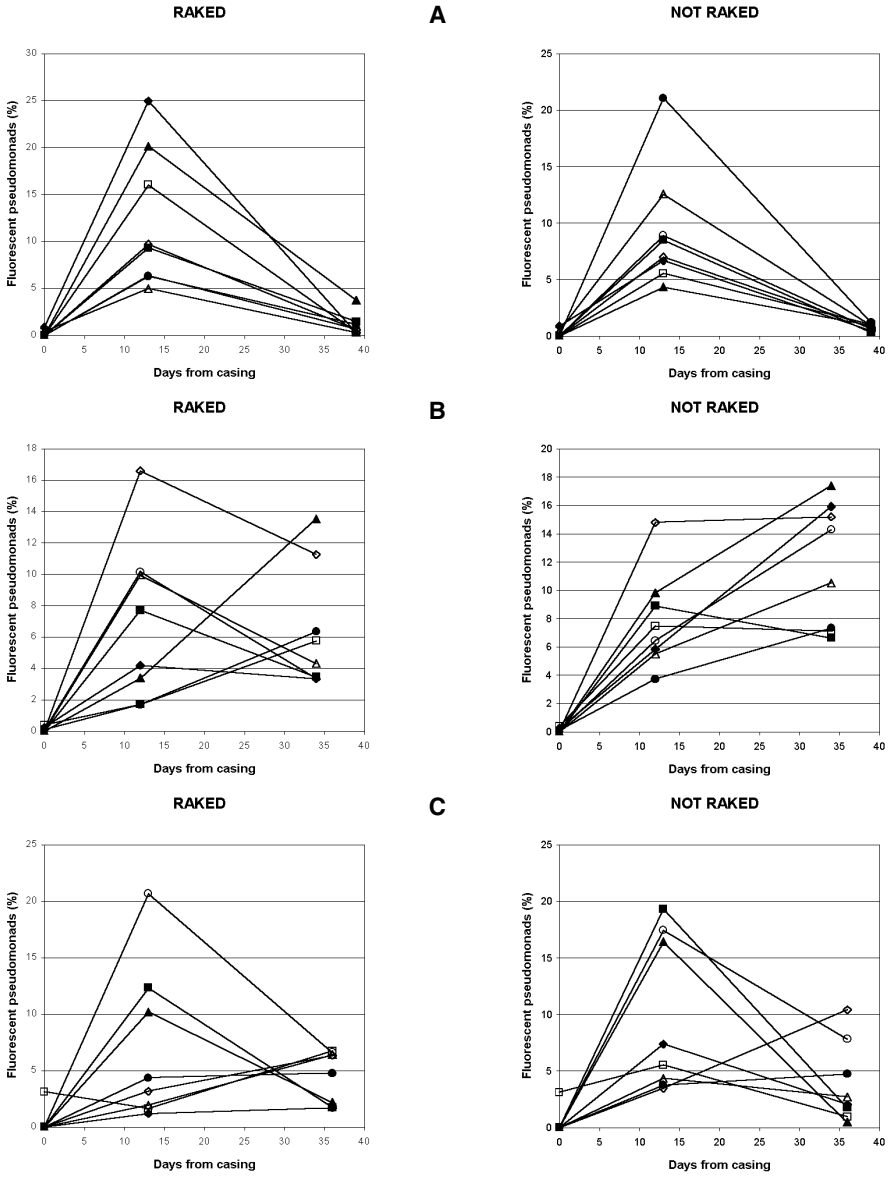

Fig. 1. Evolution of fluorescent pseudomonads percentage in relation to total bacterial count with different types of casing in each one of the experiments. A: Experiment 1; B: experiment 2; C: experiment 3. S: Soil (▲); S+SP: Soil + sphagnum peat (4:1, v/v) (■); S+BP: Soil + black peat (4:1, v/v) (◆); S+CB: Soil + composted pine bark (4:1, v/v) (●); S+CF: Soil +coconut fibre pith (4:1, v/v) (Δ); S+WF: Soil + wood fibre (4:1, v/v) (□); S+CV: Soil + composted vine shoots (4:1, v/v) (◊); RI: La Rioja type casing (○)

Table 2. Total bacteria and fluorescent pseudomonads counts after the induction of fructification for each casing type and raking in each one of the experiments

	Count (CFU g^{-1})					
	Experiment 1		Experiment 2		Experiment 3	
	TC	PC	TC	PC	TC	PC
Casing 1 (S)	1.3×10^7 ef	1.8×10^6 cd	3.1×10^7 a	1.6×10^6 cd	1.5×10^7 bc	1.9×10^6 c
Casing 2 (S+SP)	1.5×10^7 e	1.3×10^6 d	3.7×10^7 a	2.9×10^6 ab	1.6×10^7 bc	2.5×10^6 b
Casing 3 (S+BP)	8.5×10^6 f	1.5×10^6 cd	3.5×10^7 a	1.8×10^6bcd	1.7×10^7 b	6.4×10^5 d
Casing 4 (S+CB)	2.7×10^7 bc	3.0×10^6 b	3.0×10^7 a	9.9×10^5 cd	1.6×10^7 bc	6.4×10^5 d
Casing 5 (S+CF)	3.2×10^7 b	2.6×10^6 bc	3.0×10^7 a	1.9×10^6 bc	2.4×10^7 a	6.2×10^5 de
Casing 6 (S+WF)	1.7×10^7 de	1.7×10^6 cd	1.1×10^7 b	5.5×10^5 d	1.3×10^7 cd	4.3×10^5 de
Casing 7 (S+CV)	2.3×10^7 cd	1.8×10^6 cd	7.6×10^6 b	1.2×10^6 cd	1.1×10^7 d	3.7×10^5 e
Casing 8 (RI)	9.2×10^7 a	7.0×10^6 a	4.5×10^7 a	3.7×10^6 a	1.7×10^7 b	3.1×10^6 a
Raked	2.8×10^7	2.5×10^6	2.7×10^7	1.8×10^6	1.7×10^7 a	1.1×10^6 b
Not raked	2.8×10^7	2.7×10^6	3.0×10^7	1.9×10^6	1.5×10^7 b	1.5×10^6 a
Mean	2.8×10^7	2.6×10^6	2.8×10^7	1.8×10^6	1.6×10^7	1.3×10^6

Values followed by a different letter within a column are significantly different at 5% level according to Tukey's test

TC: Total bacteria count
PC: Fluorescent pseudomonads count
S: Soil
S+CF: Soil +coconut fibre pith (4:1, v/v)
S+SP: Soil + sphagnum peat (4:1, v/v)
S+WF: Soil + wood fibre (4:1, v/v)
S+BP: Soil + black peat (4:1, v/v)
S+CV: Soil + composted vine shoots (4:1, v/v)
S+CB: Soil + composted pine bark (4:1, v/v)
RI: La Rioja type casing

The increase in the pseudomonads count over the initial count was even more pronounced and significant than the total bacteria count (Tables 1 and 2), ranging from 3.7×10^5 CFU g^{-1} in Experiment 3 with the vine shoot casing to 7.0×10^6 CFU g^{-1} in Experiment 1 with the casing from La Rioja (Table 2). The highest fluorescent pseudomonads count after induction in all three experiments was observed with La Rioja casing, which also produced the highest total count in Experiments 1 and 2 and the second highest (after coconut fibre) in Experiment 3. At this stage of the growth, the presence of fluorescent bacteria is evident with all the casing types (Table 2).

The raking operation did not significantly ($P\leq0.05$) affect the counts made in Experiments 1 and 2, although it did affect the results of Experiment 3, in which the total count was greater and the pseudomonads count lower (Table 2). The results point to a significant ($P\leq0.05$) casing type-raking interaction for both counts in all three experiments (Figs 2 and 3).

As a proportion of total bacteria, the percentage of fluorescent pseudomonads in Experiment 1 varied from 5.0 to 25.0% in the raked casings and between 4.3 and 21.1% when not raked. In Experiment 2 these proportions varied between 1.7 and 16.6% (raked) and 3.7–14.8% (unranked) (Fig. 1). The corresponding figures for Experiment 3 were 1.2–20.9% (raked) and 3.5–19.3% (unraked) (Fig. 1). These figures are higher than those found by DOORES and co-workers (1987) but below those mentioned by SAMSON and co-workers (1987) and MASAPHY and co-workers (1989). According to MILLER and co-workers (1995), the increase in fluorescent pseudomonads with respect to total bacteria which occurs after the casing is placed over the germinated compost, (Fig. 1) may be due to the fact that in a nutrient-poor environment (such as that provided by mushroom casing) the production of metabolites by *Agaricus bisporus* (Lange) Imbach may give rise to nutrient gradients to which chemotactic bacteria can respond. The capacity of fluorescent pseudomonads to move rapidly towards these nutrients probably gives them an advantage over less mobile or non-chemotactic bacteria. GREWAL and RAINEY (1991) have previously demonstrated the migration of fluorescent pseudomonads towards exudates of *A. bisporus* mycelium.

Table 3 shows the total and fluorescent pseudomonads counts after the second flush for each casing type (with and without raking) and the final mean count in all the experiments. The total bacteria counts continued to increase in all three experiments except in Experiment 2 with the soil alone, *sphagnum* peat and La Rioja casings, when they fell (Tables 2 and 3). Except for these three cases, there was no tendency for the total count to decrease or stabilize as the growth cycle progressed, as was observed by other authors (DOORES et al., 1987; MASAPHY et al., 1989; MILLER et al., 1995). Total counts were between 1.6×10^7 CFU g^{-1} in Experiment 3 with soil alone and 1.8×10^8 CFU g^{-1} in Experiment 1 with coconut fibre (Table 3). Mean counts were identical in Experiments 2 and 3 (3.3×10^7 CFU g^{-1}), but higher in Experiment 1 (1.4×10^8 CFU g^{-1}) (Table 2).

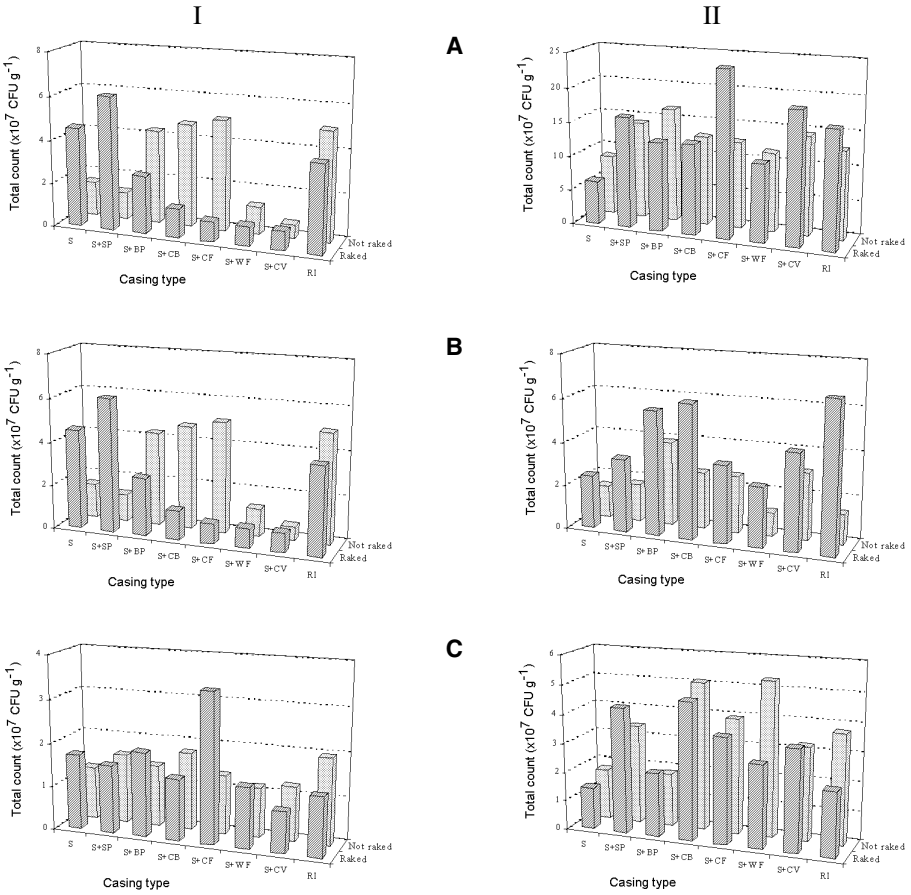

Fig. 2. Interaction raking-casing type over the total bacteria count, after the induction of fructification (I) and after the second flush (II), in each one of the experiments (confidence level: 95%).
A: Experiment 1; B: experiment 2; C: experiment 3. S: Soil; S+SP: Soil + sphagnum peat (4:1, v/v); S+BP: Soil + black peat (4:1, v/v); S+CB: Soil + composted pine bark (4:1, v/v); S+CF: Soil + coconut fibre pith (4:1, v/v); S+WF: Soil + wood fibre (4:1, v/v); S+CV: Soil + composted vine shoots (4:1, v/v); RI: La Rioja type casing

Fluorescent pseudomonads counts varied from 1.9×10^5 CFU g^{-1} in Experiment 3 with the soil alone as casing material (as observed for the total count) to 4.8×10^6 CFU g^{-1} in Experiment 2 for the composted vine shoot casing (Table 3). There was a tendency to decrease as the growth cycle progressed in Experiment 1 and to remain stable in Experiments 2 and 3 (Tables 2 and 3).

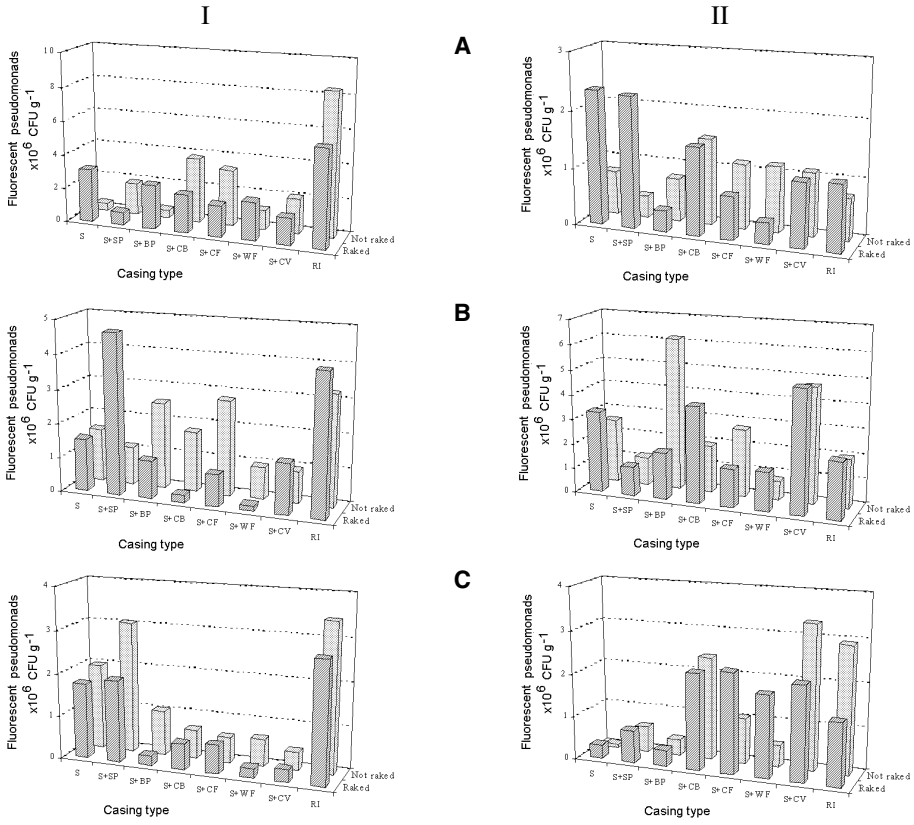

Fig. 3. Interaction raking-casing type over the count of fluorescent pseudomonads, after the induction of fructification (I) and after the second flush (II), in each one of the experiments (confidence level: 95%). A: Experiment 1; B: experiment 2; C: experiment 3.
S: Soil; S+SP: Soil + sphagnum peat (4:1, v/v); S+BP: Soil + black peat (4:1, v/v); S+CB: Soil + composted pine bark (4:1, v/v); S+CF: Soil + coconut fibre pith (4:1, v/v); S+WF: Soil + wood fibre (4:1, v/v); S+CV: Soil + composted vine shoots (4:1, v/v); RI: La Rioja type casing

Raking significantly ($P \leq 0.05$) affected the total counts although in an opposite sense in Experiment 3 to that observed in the other two experiments, in which they were higher in the raked material (Table 3). The results point to a significant casing type-raking interaction for both total and fluorescent pseudomonad counts in all three Experiments (Figs 2 and 3).

Table 3. Total bacteria and fluorescent pseudomonads counts after the second flush for each casing type and raking in each one of the experiments

	Count (CFU g^{-1})					
	Experiment 1		Experiment 2		Experiment 3	
	TC	PC	TC	PC	TC	PC
Casing 1 (S)	7.4×10^7 e	1.6×10^6 a	2.0×10^7 e	2.9×10^6 b	1.6×10^7 d	1.9×10^5 e
Casing 2 (S+SP)	1.5×10^8 bc	1.3×10^6 ab	2.5×10^7 de	1.1×10^6 c	3.9×10^7 b	6.7×10^5 de
Casing 3 (S+BP)	1.5×10^8 bc	5.6×10^5 b	4.8×10^7 a	4.0×10^6 a	2.0×10^7 d	3.7×10^5 e
Casing 4 (S+CB)	1.3×10^8 cd	1.5×10^6 ab	4.4×10^7 ab	2.9×10^6 b	4.9×10^7 a	2.3×10^6 ab
Casing 5 (S+CF)	1.8×10^8 a	9.4×10^5 ab	3.1×10^7 cd	2.1×10^6 b	3.8×10^7 b	1.7×10^6 bc
Casing 6 (S+WF)	1.1×10^8 d	7.6×10^5 ab	1.9×10^7 e	1.2×10^6 c	4.1×10^7 b	1.2×10^6 cd
Casing 7 (S+CV)	1.7×10^8 ab	1.1×10^6 ab	3.8×10^7 bc	4.8×10^6 a	3.3×10^7 bc	2.8×10^6 a
Casing 8 (RI)	1.5×10^8 bc	9.5×10^5 ab	4.1×10^7 ab	2.1×10^6 b	3.0×10^7 c	2.2×10^6 ab
Raked	1.5×10^8 a	1.2×10^6	4.4×10^7 a	2.6×10^6	3.1×10^7 b	1.4×10^6
Not raked	1.3×10^8 b	9.4×10^5	2.2×10^7 b	2.7×10^6	3.5×10^7 a	1.4×10^6
Mean	1.4×10^8	1.1×10^6	3.3×10^7	2.7×10^6	3.3×10^7	1.4×10^6

Values followed by a different letter within a column are significantly different at 5% level according to Tukey's test

TC: Total bacteria count
PC: Fluorescent pseudomonads count
S: Soil
S+CF: Soil +coconut fibre pith (4:1, v/v)
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S+BP: Soil + black peat (4:1, v/v)
S+CV: Soil + composted vine shoots (4:1, v/v)
S+CB: Soil + composted pine bark (4:1, v/v)
RI: La Rioja type casing

Table 4. Correlations matrix of the bacterial populations during the growth cycle with the principal quantitative production parameters

	Experiment 1				Experiment 2				Experiment 3			
	BIOEF	EARL	NUM	UNITW	BIOEF	EARL	NUM	UNITW	BIOEF	EARL	NUM	UNITW
TC_1	n.s	-0.3273^{++}	n.s.	-0.2945^{+}	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
PC_1	0.4313^{+++}	n.s.	0.3879^{++}	n.s.	n.s	n.s.	n.s.	n.s.	n.s.	n.s.	-0.2979^{+}	0.3055^{+}
PP_1	0.2528^{+}	n.s.	0.2929^{+}	n.s.	-0.3295^{++}	n.s.	n.s.	n.s.	n.s.	n.s.	-0.2979^{+}	0.3055^{+}
TC_2	-0.3191^{+}	-0.4496^{+++}	n.s.	-0.3308^{+}	n.s.	n.s.	n.s.	n.s.	0.3225^{++}	n.s.	n.s.	n.s.
PC_2	-0.3322^{++}	-0.4490^{+++}	n.s.	-0.2572^{+}	n.s.	n.s.	0.3305^{++}	-0.3150^{+}	n.s.	n.s.	n.s.	n.s.
PP_2	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
TC_3	0.3531^{++}	-0.3526^{++}	0.4922^{+++}	-0.4876^{+++}	0.4352^{+++}	-0.5967^{+++}	0.5047^{+++}	-0.5075^{+++}	n.s.	n.s.	n.s.	n.s.
PC_3	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.2713^{+}	-0.3602^{++}	0.3327^{++}	-0.2820^{+}
PP_3	n.s.	0.2543	-0.2653^{+}	0.4074^{+++}	n.s.	0.5834^{+++}	-0.2668^{+}	0.3564^{++}	0.2648^{+}	-0.3244^{++}	0.3623^{++}	-0.2949^{+}

n.s.: not significant ($P>0.05$); +: significant at 95% ($0.01<P\leq0.05$); ++: significant at 99% ($0.001<P\leq0.01$); +++: significant at 99.9% ($P\leq0.001$)
BIOEF: Biological efficiency (kg 100 kg^{-1} compost); EARL: Earliness (days from casing); NUM: Number of harvested mushrooms per surface unit (mushrooms m^{-2}); UNITW: Mushroom unitary weight (g)
TC_1: Total count at casing (CFU g^{-1}); PC_1: Fluorescent pseudomonads count at casing (CFU g^{-1}); PP_1: Fluorescent pseudomonads percentage at casing; TC_2: Total count after the induction of fructification (CFU g^{-1}); PC_2: Fluorescent pseudomonads count after the induction of fructification (CFU g^{-1}); PP_2: Fluorescent pseudomonads percentage after the induction of fructification; TC_3: Total count after the the second flush (CFU g^{-1}); PC_3: Fluorescent pseudomonads count after the second flush CFU g^{-1}); PP_3: Fluorescent pseudomonads percentage after the second flush

The percentage of fluorescent pseudomonads with respect to total bacteria fell in Experiment 1 with all casing types, whether or not raked, varying from 0.3 to 3.7% in raked casings and from 0.3 to 1.2% in unraked casings (Fig. 1). In Experiment 2 the proportions varied irregularly depending on the material and raking was out, varying between 3.3 and 13.5% in raked casing and 6.6–17.4% in unraked casings (Fig. 1). The corresponding figures for Experiment 3 were 1.7–6.7% (raked) and 0.4–10.4% (unraked) (Fig. 1).

The data referring to the bacterial populations in the different types of casing (raked and unraked) and with the different mushroom strains (Experiments 1–3) during the growth cycle of *A. bisporus* depicted in Tables 1–3 can be correlated with the principal quantitative production parameters (biological efficiency, earliness, number of mushrooms harvested per surface unit and unitary weight) in the casings studied (PARDO, 1999) to give Table 4.

The most notable aspects of this matrix are the following: i) the higher total bacterial count after induction of fructification was correlated with an earlier harvesting date in Experiment 1; ii) after the second flush, the greater bacterial activity in Experiments 1 and 2 were correlated significantly ($P \leq 0.01$) with an earlier harvest, greater biological efficiency and a higher number of mushrooms obtained (although of lower unitary weight).

3. Conclusions

These results with respect to the principal quantitative parameters provide no confirmation of the conclusions reached by other authors. For example, HAYES (1974) and HAYES and NAIR (1976) affirmed that greater microbial activity in the casing prior to harvesting brought harvesting forward, encouraged the formation of primordia and increased the number and weight of mushrooms. MASAPHY and co-workers (1989) also found that a higher level of favourable bacteria such as *Pseudomonas* spp. stimulated the formation of primordia, resulting in larger and earlier harvests (although mushroom size decreased). The differences in behaviour observed in this study are probably mainly due to the use of materials of a diverse nature and, as mentioned above, to the complex interaction between different factors on which carpophore initiation and growth depend. Among these are microbiological factors, the genetic capacity of a mycelium to fructify, physical and environmental factors and, lastly, chemical and nutritional factors.

References

ARROLD, N. P. (1972): Confirmation of the ability of *Pseudomonas putida* to cause fruiting of the cultivated mushroom. *M.G.A. Bull*., *269*, 200–201.

BAZERQUE, A. & LABORDE, J. (1975): Contribution a l'étude des terres de couverture utilisées en France pour la culture du champignon couche. *Bull. FNSACC*, *4*, 941–956.

CIES (2000): Relación de variedades comerciales de micelios de champiñón y setas *Pleurotus*. El champiñón en Castilla-La Mancha n° 15. Centro de Investigación, Experimentación y Servicios del Champiñón, Quintanar del Rey, Cuenca, Spain.

COUVY, J. (1972): Les facteurs de la fructification de l'*Agaricus bisporus* (champignon de couche). *Bull. FNSACC*, *14*, 585–604.

CRESSWELL, P. A. & HAYES, W. A. (1979): Further investigation on the bacterial ecology of the casing layer. *Mushroom Sci*., *10*, 347–359.

DOORES, S., KRAMER, M. & BEELMAN, R. (1987): Evaluation and bacterial populations associated with fresh mushrooms (*Agaricus bisporus*). -in: WUEST, P. J., ROYSE, D. J. & BEELMAN, R. B. (Eds.), *Developments in crop science 10: Cultivating edible fungi*. Elsevier Science Publishing Company Inc., New York, pp. 283–294.

EGER, G. (1962): The 'Halbschalentest', a simple method for testing casing materials. M.*G.A. Bulletin*, *148*, 159–168.

EGER, G. (1963): Untersuchungen zur Fruchtkörperbildung des Kulturchampignons. *Mushroom Sci*., *5*, 314–320.

FORET, V. (1989): Aspects physiologiques et métaboliques de la fructification d'*Agaricus bisporus*. Cahier Technique du Champignon nº 9. Centre Technique du Champignon, St. Paterne, France, 83 pp.

FLETCHER, J. T., WHITE, P. F. & GAZE, R. H. (1989): *Mushrooms: Pest and disease control*. Intercept Ltd., Andover, Hants, pp. 115–122.

GREWAL, S. I. S. & RAINEY, P. B. (1991): Phenotypic variation of *Pseudomonas putida* and *P. tolaasii* affects the chemotactic response to *Agaricus bisporus* mycelial exudate. *J. gen. Microbiol.*, *137*, 2761–2768.

HAYES, W. A. (1974): Micro-biological activity in the casing layer and its relation to productivity and disease control. -in: HAYES, W. A. (Ed.), *The casing layer.* Mushroom Growers' Association, W.S. Maney and Son Ltd., Leeds, pp. 37–47.

HAYES, W. A. (1981): Interrelated studies of physical, chemical and biological factors in casing soils and relationships with productivity in commercial culture of *A. bisporus*. *Mushroom Sci*., *11*, 103–129.

HAYES, W. A. & NAIR, N. G. (1976): Effects of volatile metabolic by-products of mushroom mycelium on the ecology of the casing layer. *Mushroom Sci*., *9*, 259–268.

HAYES, W. A., RANDLE, P. E. & LAST, F. T. (1969): The nature of the microbial stimulus affecting sporophore formation in *Agaricus bisporus* (Lange) Sing. *Ann. appl. Biol*., *64*, 177–187.

HEARNE, A. J. (1994): Mushroom compost analysis. A few practical suggestions. *Mushroom J.*, *539*, 18–19.

HUME, D. P. & HAYES, W. A. (1972): The production of fruit-body primordia in *Agaricus bisporus* (Lange) Sing. on agar media. *Mushroom Sci*., *8*, 527–532.

INGRATTA, F. J. & PATRICK, Z. A. (1987): Influence of microorganisms and fungistasis on sporophore initiation in *Agaricus brunnescens*. -in: WUEST, P. J., ROYSE, D. J. & BEELMAN, R. B. (Eds.), *Developments in crop science 10: Cultivating edible fungi*. Elsevier Science Publishing Company Inc., New York, pp. 27–40.

MASAPHY, S., LEVANON, D., TCHELET, R. & HENIS, Y. (1987): Scanning electron microscope studies of interactions between *Agaricus bisporus* (Lange) Sing hyphae and bacteria in casing soil. *Appl. environ. Microbiol*., *53*(5), 1132–1137.

MASAPHY, S., LEVANON, D., DANAI, O. & HENIS, Y. (1989): Nutritional supplementation to the casing soil: Ecological aspects and mushroom production. *Mushroom Sci., 12*, 417–426.

MILLER, N., GILLESPIE, J. B. & DOYLE, O. P. E. (1995): The involvement of microbiological components of peat based casing material in fructification of *Agaricus bisporus*. *Mushroom Sci*., *14*, 313–321.

OLIVIER, J. M. & GUILLAUMES, J. (1979): Évolution microbiologique des terres de gobetage. *Bull. FNSACC*, *1*, 9–16, *2*, 27–43.

PARDO, J. (1993): Productividad de un compost y economía de su elaboración: criterios de evaluación basados en una analítica sistemática. -in: *Jornadas Técnicas del Champiñón y otros Hongos Comestibles en Castilla-La Mancha*. Consejería de Agricultura y Medio Ambiente y Diputación Provincial de Cuenca, Cuenca, Spain, pp. 37–58.

PARDO, A. (1999): Respuestas agronómicas de diferentes materiales de cobertura para el cultivo del champiñón, *Agaricus bisporus* (Lange) Imbach. Ph. D. Thesis. Universidad de Castilla-La Mancha, Albacete, Spain, 334 pp.

PARDO, A., PARDO, J. E. & DE JUAN, J. A. (1999): Obtención y caracterización de sarmientos compostados como material de cobertura para el cultivo del champiñón. *Actas de Horticultura*, *26*, 100–105.

PARDO, A., DE JUAN, J. A., PARDO, J. E. & PARDO, J. (2001): Evolución histórica y problemática del sector del champiñón en Castilla-La Mancha. *Alimentaria*, *322*, 101–105.

PEÑA, M. L. & SANZ, A. (1993): Ensayo sobre tierras de cobertura en champiñón (alternativas a la tradicional). *La Rioja Agraria*, *13*, 34–44.

REDDY, M. S. & PATRICK, Z. A. (1992): Effect of bacteria associated with mushroom compost and casing on basidiomata formation in *Agaricus bisporus*. *Mushroom News*, *40*(5), 23–31.

SAMSON, R., HOUDEAU, G., KHANNA, P., GUILLAUMES, J. & OLIVIER, J. M. (1987): Variability of fluorescent Pseudomonads populations in composts and casing soils used for mushroom cultures. -in: WUEST, P. J., ROYSE, D. J. & BEELMAN, R. B. (Eds.), *Developments in crop science 10: Cultivating edible fungi*. Elsevier Science Publishing Company Inc., New York, pp. 19–25.

VEDDER, P. J. C. (1978): *Cultivo moderno del champiñón*. Mundi-Prensa, Madrid, pp. 161–210.

WUEST, P. J. (1982). How to manage the watering of a mushroom crop. -in: WUEST, P. J. & BENGTSON, G. D. (Eds.), *Penn state handbook for commercial mushroom growers*. The Pennsylvania State University, Pennsylvania, pp. 99–101.