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ALTERNATIVE *AGARICUS*. THE CULTURE OF *AGARICUS* W4 AND *AGARICUS ARVENSIS*

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Summary

The culture of two wild *Agaricus* species, *A. subfloccosus* (a brown woodland mushroom designated W4) and *A. arvensis* (the horse mushroom) was further examined on an experimental and semi-commercial scale.

The cropping performance of the previous best *Agaricus* W4 isolate, W4-IV, was compared with those of several single spore isolates taken from W4-IV. Single spore isolate 15 produced a higher yield than W4-IV in semi-commercial size trays. During a four-flush harvesting period (35 days), the best single spore isolates from W4-IV produced 70% of the yield of a commercial brown strain of *A. bisporus*.

A wild isolate of *A. arvensis*, 93-7 produced a slightly higher yield than a control strain R20 in both crate and tray experiments, although the number of fruitbodies was slightly lower. The commercial trialling of *Agaricus subfloccosus* W4-IV ssi 15 and *Agaricus arvensis* 93-7 in bags and trays is recommended.

Introduction

An *Agaricus* species (W4) in the *A. subfloccosus* complex, with an attractive appearance and distinct flavour, considered to be better than that of *A. bisporus*, has been identified. As a result of HDC Projects M4 and M4a, an isolate has been found (W4-IV) which is capable of producing 60-70% of the yield of a commercial brown strain of *A. bisporus* (Table 1). Single spore isolates have been obtained from W4-IV, including isolates which on a small scale produced a higher yield than W4-IV. The cropping performance of these isolates has yet to be evaluated and compared with W4-IV in semi-commercial size trays.

As part of Projects M4 and M4a, the cropping performance of a commercial strain of the horse mushroom, *Agaricus arvensis* (Somycel R20) was examined. Yields of up to 177 kg/tonne compost at spawning were achieved on a semi-commercial scale. *A. arvensis* isolates have been obtained from the wild in the UK, and one of these, 93-7, produced a 10% higher yield than R20 on a small scale. Since project M4a, several other *A. arvensis* isolates have been collected from the wild, although their yield potential on composted substrates has not been determined.

The objectives of the present project were:

1. To compare the yields of single spore isolates of *Agaricus* W4-IV with those of the original *Agaricus* W4IV isolate and commercial *A. bisporus* strains, in semi-commercial size trays.

2. To examine the cropping performance of several wild isolates of *A. arvensis* in small containers (5 kg substrate).

3. To compare the cropping performance of the best wild *Agaricus arvensis* isolates with that of the commercial strain, Somycel R20 in semi-commercial size trays.

Part I - *Agaricus* W4

Experiment I - Strain evaluation in cropping trays

Materials and Methods

Agaricus strains/isolates

Agaricus W4	(i) W4-IV	
Single spore isolates	(ii) 13	(iv) 15
	(iii) 14	(v) 16
Single spore isolate combinations	(vi) 13/14	
	(vii) 13/15	
	(viii) 14/16	

A. bisporus(vii)Somycel 856, brown

Cultural procedure

Spawn of the different W4 isolates was produced using rye grain. A commercial Phase II compost was spawned at 2% w/w and filled into wooden trays, each filled with 45 kg of substrate. The analysis of the substrate is shown in Appendix I.

Spawned compost was hydraulically pressed into the trays which were then covered with paper and stacked in a spawn-running room where the compost temperature was maintained at 25°C. After full mycelial colonization of the substrate, 20 days after spawning, the compost was cased with a ready-mixed casing (a moist mixture of peat and 20% v/v sugar beet lime) to a depth of 35 mm. The trays were transferred to a cropping shed where the relative humidity of the air was maintained at 95% and the compost temperature kept at 25°C. The casing was covered with paper until mycelium became visible at the surface, 14 days after the casing was applied. Fresh air was then introduced in the cropping shed and the environmental conditions were altered to encourage pinhead initiation and fruitbody

development. The air temperature, humidity and CO_2 concentration were reduced to levels of 16-17°C, 90-92% and 0.07-0.08% v/v respectively. The casing was kept moist by regular light watering after the first fruitbodies had developed to 10 mm diam. About 15% more water was applied to the *A. bisporus* treatments than to the *Agaricus* W4 treatments since previous trials had shown that over-watering inhibited pinhead formation of *Agaricus* W4. Fruitbodies, diam 35-45 mm, were harvested at the 'stretched veil' stage over a 35 day period. The stipes were trimmed (about 13% of the sporophore weight was removed) and the weight of fruitbodies harvested from each tray was recorded. A randomized block design was used, with four replicate trays of each strain.

Results

Four flushes of mushrooms were produced during the harvesting period. The yields of *Agaricus* W4 isolates and *A. bisporus* Somycel S609 are shown in Table 2. There were significant differences in yield between single spore isolates of W4-IV. Isolates 14 and 16 produced significantly lower yields and isolate 15 produced a significantly higher yield than the original culture W4-IV. Cultures taken from the zone between single spore isolate cultures resulted in yields similar to one or other of the constituent isolates.

The best isolates (W4-IV ssi 15 and ssi 13/15) produced on average 70% of the yield of the brown *A. bisporus* strain, Somycel 856.

Part II - *Agaricus arvensis*

Materials and Methods

Experiment II/1 - Strain evaluation in small containers

Agaricus isolates and strains

The original sources of the wild isolates are shown in Table 1.

A. arvensis

(i) 93-7
(ii) 94-1
(iii) 94-22
(iv) 94-26
(v) Somycel R20

Single spore isolates

(vi) 93-7A
(v) 93-7B
(vi) 93-7C
(vii) 93-7D
(viii) R20A

(ix) *A. bisporus* Hauser A12

Four replicate crates of each strain were prepared.

Cultural procedure

Spawn of the different wild isolates was prepared using rye grain. The experiment was conducted in a controlled environment chamber. The substrate was filled into plastic crates each holding 5 kg substrate, and spawned at 2% w/w with grain spawn. The same substrate as shown in Appendix 1 was used. Cultural conditions were similar to those described for *Agaricus* W4, except a shallower layer of casing (20 mm) was used and lighting (150 lux) was used after fresh air was introduced into the chamber. Mushrooms were harvested closed with a cap diameter of 45 mm. The base of the stem was trimmed so that the average trimmed weight of fruitbodies was 15-20 g.

Experiment II/2 - Strain evaluation in cropping trays

Agaricus arvensis isolates and strains

(i) 93-7
(ii) 93-10
(iii) 94-22
(iv) 94-32
(v) 93-7 B) Single spore isolates
(vi) 93-7 D) from 93-7
(vii) Somycel R20
(viii) *A. bisporus* Hauser A12

Four replicate trays of each isolate/strain were prepared.

Cultural procedure

The cultural procedure was similar to that described for *Agaricus* W4, except that lighting (150 lux) was used after fresh air was introduced into the chamber. The analysis of the substrate used is shown in the Appendix. The procedure for harvesting the mushrooms was similar to that described for Experiment II/1. Average trimmed weight of the mushrooms was 30 g.

Results

Strain evaluation in small containers

The strains 93-7 and R20 produced a first flush about 25 days after casing and had a flushing interval of about 12 days. Four flushes were produced during a 40 day cropping period. The first flush in isolates 94-1, 94-22 and 94-26 appeared about 32 days after casing.

Yields from the different isolates and strains are shown in Table 3. The yield and number of fruitbodies from 93-7 were slightly higher than those from the control strain, R20. The yields from isolates 94-1, 94-22 and 94-26, and the single spore isolates were lower than from the control strain R20.

The yield from the best *A. arvensis* isolate, 93-7, was 71% of that obtained from *A. bisporus,* strain A12.

Strain evaluation in cropping trays

The yield and number of fruitbodies obtained from the different isolates and strains are shown in Table 4. The isolate 93-7 produced a slightly higher yield than R20, although the number of fruitbodies was slightly lower. The other isolates and single spore isolates produced a lower yield than R20. The yield obtained in trays from *A. arvensis* isolate 93-7 was 53% of that produced by *A. bisporus* strain A12.

Conclusions

1. Previous results in small scale crates showing that the single spore isolate (15) produced a higher yield than the parent isolate of *A. subfloccosus*, W4-IV, were confirmed in semi-commercial trays.

2. The yields of the best single spore isolates taken from W4-IV were 70% of those obtained from a commercial brown strain of *A. bisporus*.

3. A wild isolate of *A. arvensis* 93-7 produced a slightly higher yield than a control strain R20 in both crate and tray experiments.

4. The strain R20 produced a larger number of smaller fruitbodies (at an equivalent stage of development) than the isolate 93-7.

Recommendations for further work

Commercial trials have shown that *Agaricus* W4 is suitable for bag cultivation. Further commercial trials should be conducted in bags and trays to assess the performance of W4-IV, single spore isolate 15.

Commercial trials should also be conducted to compare the performance of *Agaricus arvensis* strains 93-7 and R20.

Table 1. Source of *Agaricus subfloccosus* and *A. arvensis* isolates.

Isolate	Collected by	Location	Date/Year	Habitat
A. subfloccosus				
W4-IV	J.T. Fletcher	Olantigh, Kent	May 1992	Leaf litter beneath *Cupressus*
A. arvensis				
93-7	H. Grogan	Littlehampton, West Sussex	Sept 1993	Coniferous leaf litter
93-10	R.H. Gaze	Bedham, West Sussex	Oct 1993	Composting grass clippings
94-1	R. Noble	Kielce, Poland	Aug 1994	Grass beneath oak
94-22	J.F. Smith	Welford-On-Avon, Warwicks	Oct 1994	Grass beneath *Cupressus*
94-26	R. Noble	Houghton Wood, West Sussex	Oct 1994	Spruce litter
94-32	J.T. Fletcher	Olantigh, Kent	Nov 1994	*Cupressus*

Table 2. Yield of fruit bodies from W4-IV, single spore isolates of W4-IV and cultures taken from the zone between two single spore isolate cultures, Expt. I. Each value is the mean of 4 replicate trays.

Species	Isolate or Strain	Yield, kg/tonne
Agaricus W4	IV	166
Single spore isolates	13	158
	14	119
	15	177
	16	139
Single spore isolate 'junctions'	13/14	117
	13/15	188
	14/16	115
A. bisporus (brown)	Somycel 856	259

Table 3. Yield and number of fruitbodies from *Agaricus arvensis* isolates and strains. Each value is the mean of 4 replicate crates.

Isolate or strain	Yield, g/kg substrate	No. fruitbodies/crate
Parent cultures		
93-7	230	75
94-1	51	8
94-22	110	25
94-26	54	28
Somycel R20	208	68
Single spore isolates		
93-7A	111	24
93-7B	21	7
93-7C	182	52
93-7D	62	9
R20A	112	23
A. bisporus Hauser A12	322	164

Table 4. Yield and number of fruitbodies from *Agaricus arvensis* isolates and strains. Each value is the mean of 4 replicate trays.

Species	Isolate or strain	Yield, Kg/tonne	No. fruitbodies /tray
A. arvensis			
Parent cultures	93-7	192	243
	93-10	113	138
	94-22	50	49
	94-32	76	62
Single spore isolates	93-7B	85	120
	93-7D	169	211
Control strain	R20	181	271
A. bisporus	A12	361	-

Appendix I

Analysis of compost at spawning

	Percentage of dry weight			Moisture	pH
	N	NH_4^+	Ash	%	
Expts I & II/1	2.67	0.045	20.0	66.5	7.4
Expt II/2	2.68	0.059	19.1	62.7	7.3