GENETIC CHARACTERIZATION OF SINGLE SPORE ISOLATES OF *AGARICUS BISPORUS* (LANGE) IMBACH

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ABSTRACT

The yield and quality of the mushroom produced is determined by three factors, the genetic makeup of the mushroom strain, the environmental conditions in which the mushroom is grown, interaction of genotype and environment and the quality of nutrition. In India, introduction of exotic strains of button mushroom and their selection on the basis of their evaluation is a continuing process. The present investigation was undertaken to develop improved strains of *Agaricus bisporus* through single spore isolates (SSIs) and genetic characterization of developed SSIs using SDS-PAGE protein profiling. On the basis of yield performance, four strains of *Agaricus bisporus*, namely, S-11, MS-39, NCS-100 and NCS-101 were selected (designated as A,B,C and D, respectively) and used for the isolation of single spores. A total of 410 SSIs were developed out of which 255 SSIs performed below or at par to their respective parent while 155 of SSIs out yielded the parent. Further, on the basis of higher yields, 5 SSIs of A (S-11), 2 SSIs of B (MS-39), 5 SSIs of C (NCS-100) and 6 SSIs of D (NCS-101) were selected and analyzed by protein profiling. On the basis of protein profiles, the above 18 isolates were classified into seven groups. The total number of bands ranged from 5 to 13. Within group, the isolates had very little difference in banding pattern both for position and intensities of bands, however, between group difference was observed.

Keywords: spore print, single spore isolates, protein profiling, *Agaricus bisporus*

INTRODUCTION

Mushrooms are delicious, nutritionally rich, medicinally important and non-conventional source of human food. Mushroom production is regarded as the second most important commercial microbial technology, next only to yeast, for large scale profitable bioconversion of lignocellulosic wastes from agro-industry. Presently, mushrooms are being cultivated in about 100 countries. In India, *Agaricus bisporus* (button mushroom) contributes about 80-85 per cent of the total annual production of mushrooms. The yield and quality of the mushroom is determined by three factors, the genetic makeup of the mushroom strain, the environmental conditions in which the mushroom is grown and the quality of nutrition. In India, introduction of exotic strains of button mushroom and their selection on the basis of their evaluation is a continuing process. The present investigation was undertaken to develop improved strains of *A. bisporus* through single spore isolates (SSIs) and genetic characterization of developed SSIs using SDS – PAGE protein profiling.

MATERIALS AND METHODS

On the basis of yield and quality performance, four strains of *Agaricus bisporus* viz., S-11, MS-39, NCS-100 and NCS-101 were selected as parent for the studies.

Collection of spore print

A well formed and healthy sporophore with veil still intact but tightly stretched was selected for the spore print. Sporophore was surface sterilized and mounted on a sterilized wire stand and placed over the bottom plate of a sterilized petriplate and covered with a sterilized bell jar. After a thick deposit of spore mass, the lid was replaced and sealed with a parafilm strip, then stored in a refrigerator for further use.

Preparation of spore suspension

A loopful of spores from spore print was transferred aseptically to 10 ml of sterilized distilled water in a test tube. The suspension was shaken thoroughly and 1 ml of this suspension was transferred to another tube containing 9 ml of sterilized distilled water. Likewise the serial dilution of spore suspension was made four or five times, till 1 ml of the suspension contain 20-25 spores.

Plating for spore germination and isolation of SSIs

For spore's germination, 1 ml of spore suspension was added to 9 ml of sterilized and melted wheat agar media and poured into sterilized petriplate. Then the plates were rotated clockwise and anti-clockwise direction to mix and spread the spores in the media. The plates were then allowed to solidify. After that the plates were inverted and seeded with living mycelia in the lid plate. Then these plates were kept under incubation for spore germination. After a week of incubation, the lids of Petri plates containing living mycelia were substituted with another set of sterilized lids. The plates were then observed under high resolution Inverted Microscope. The individual germinating spores were marked and individual spore were transferred to the slants having malt extract agar media. The isolated spores were then incubated at 25±1 °C temperature. After 5-6 days of incubation, the SSIs start growing and mycelium was visible.

Spawn preparation and yield evaluation

Selected SSIs were then taken to spawn preparation, for that, the wheat grain spawn was prepared using the standard methodology. The isolates were cultivated in the winter season from October to March and cultivation trials were conducted under natural climatic conditions of temperature 15-20 °C and relative humidity (75-90%) at Mushroom Research and Training Center, Pantnagar using standard cultivation technology on the compost prepared by short method of composting.

Protein Profiling

Identification of different isolates of *A. bisporus* was carried out using protein profile, Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). For doing the estimation, average sized fruit bodies were collected and washed with distilled water. After that the protein profiling was done using standard protocol applied for SDS-PAGE method and the results obtained were described.

RESULTS AND DISCUSSION

Development of Single Spore Isolates

120 SSIs of strain S-11, 80 SSIs of strain MS-39, 100 SSIs of strain NCS-100 and 110 SSIs of strain NCS-101 were developed and were designated as SSIs A, B, C & D, respectively, for convenience for further use (Table 1). All the isolates were then tested for their yielding ability and fruiting test. None of the isolates were found sterile for the use in hybrid development. All the SSIs developed (410) from 4 strains of *A. bisporus* were further divided into three categories on the basis of their yield performance as (i) below parent, (ii) at par with the parent and (iii) above parent. The number of SSIs grouped in these categories was 180, 75 and 155 respectively (Table 1). [1] tested yield performance of SSIs from 3 strains of *A. bisporus* and the evaluation of various SSIs indicated that maximum numbers of isolates were below or at par in yield with the parents, however, few number of isolates out yielded the parent strains.

Table 1. Performance of single spore isolates from four strains of *A. bisporus*

Strain	Number of Isolates yielding			
	Below parent	**At par**	**Above parent**	**Total**
S-11 (A)	43	16	61	120
MS-39 (B)	27	23	30	80
NCS-100 (C)	51	18	31	100
NCS-101 (D)	59	18	33	110
Total	**180**	**75**	**155**	**410**

Screening of high yielding SSIs

The isolates selected on the basis of their yield from 6 weeks of harvesting period were tested for their performance in respect of yield first and then for quality parameters. Those, which yielded higher or at par with their parental strains were

selected for further tests and categorized as low, high and very high yielders with respect to their yield in a period of 6 weeks harvest. On the basis of higher yields 5 SSIs of A, 2 SSIs of B, 5 SSIs of C and 6 SSIs of D were selected (Table 2). Various workers have concluded that single spore isolates had variation in the cultures and in yield than that of parent strains and an increase in yield up to 21 percent was recorded in one of the single spore isolates of the parental strain S-11 by Kumar & Munjal [2, 4, 5]. The single spore isolates were found to show considerable difference in growth, morphological characters and yield [5-8]. Bhandal & Mehta [9] and Mehta *et al*. [10] isolated for promising single spore isolates with regards to mycelial characteristics, spawn run, yield and sporophore characters which resulted in release of two single spore isolates, NCS-100 and NCS-101 for commercial cultivation.

Table 2. Yield performance of different high yielding *A. bisporus* SSIs

Isolates	Yield kg/q compost			
	Crop I		Crop II	
	Numbers	Weight	Number	Weight
C-6	1725	16.08	1423	17.21
C-13	1816	18.26	1460	16.77
C-15	1630	18.75	1540	19.71
A-27	2030	19.80	1799	21.48
A-89	2482	23.37	1916	23.40
A-61	2000	19.84	1655	20.22
B-8	1798	17.66	1582	20.90
B-49	1955	19.92	1760	22.49
D-47	1975	20.03	1425	16.65
D-54	2204	22.55	1410	19.38
C-68	1945	20.31	1790	22.40
D-20	2060	20.55	1827	19.97
D-2	2720	23.58	1952	22.50
A-46	2190	20.59	1985	20.80
D-48	2240	23.09	1904	22.59
C-47	2710	23.37	1806	17.82
D-63	1230	10.61	2695	20.04
A-67	1490	15.59	1895	20.55
Check I(A)	1635	14.97	1354	16.75
Check II(B)	1440	15.05	1520	16.68
Check III(C)	1116	13.39	1213	14.26
Check IV(D)	1380	15.68	1233	13.64
CD at 5%	40.5	0.62	35.44	0.75

Protein profiling analysis

The total protein was analyzed by SDS-PAGE. Banding pattern obtained on gel was utilized for checking zymogram, representing the relative position and intensities of bands. The whole zymogram was divided into three zones (A,B and C) as three different band intensities were obtained viz., dark, medium dark and light bands (Table 3, Plate 1). Total number of protein bands ranged from five to thirteen spread over three zones. On the basis of total number of bands and their intensity, isolates were classified into seven groups. There were two isolates in five bands group, four in six bands group, three in nine bands group, one in ten bands group and one isolate was grouped in thirteen bands group (Plate 1). In five band groups, two isolates A-61 and D-63 were present. D-63 had bands spread in all the three zones while A-61 had no band in zone A. In A-61 most of the bands were present in zone C while in D-63 bands were uniformly distributed in all the

Table 3. Banding pattern of pileus of different *A. bisporus* SSIs

Isolate	Total number of bands in different zones				Intensity of bands		
	Total no.	A	B	C	Dark	Medium	Light
A-61	5	0	1	4	1	4	0
D-63	5	1	2	2	5	0	0
C-15	6	3	1	2	2	4	0
A-27	5	3	1	2	2	2	2
D-20	6	1	1	4	0	2	4
D-2	6	2	2	2	5	0	1
C-13	7	2	2	3	0	2	5
B-8	7	3	0	4	0	0	7
C-68	7	3	1	3	0	3	4
A-67	7	2	1	4	7	0	0
D-18	8	2	1	5	6	0	2
A-89	8	3	1	4	1	0	7
D-47	8	2	2	4	1	6	1
B-49	9	2	3	4	3	3	3
D-54	9	2	3	4	0	4	5
C-47	9	3	1	5	9	0	0
A-47	10	3	2	5	8	2	0
C-6	13	6	4	3	2	1	10

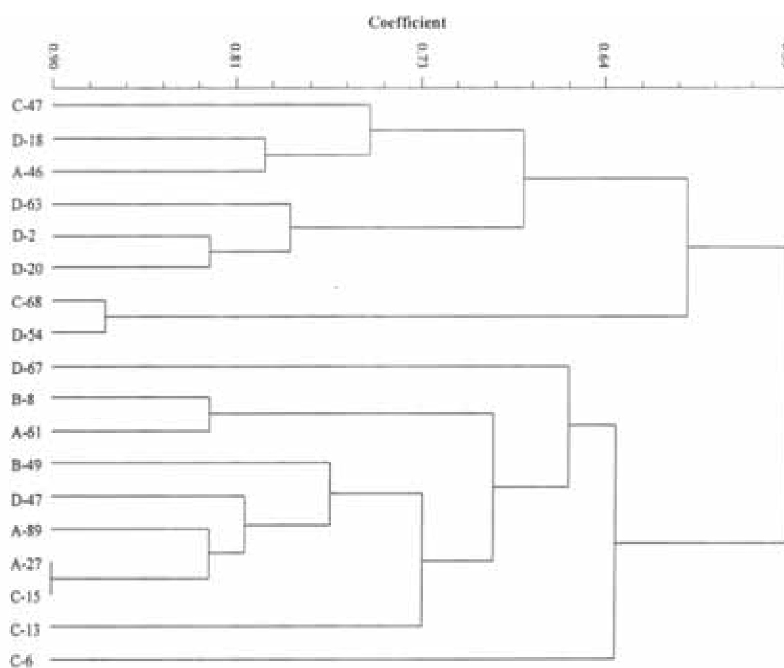

Figure 1. Combined Dendogram

three zones. Intensities wise both the isolates had mostly dark and medium intensity bands (Plate 1). In six band group, out of four isolates, two C-15 and A-27 had similar relative position of bands in zone A and zone C. Remaining two isolates had different banding pattern with D-20 having one band each in zone A and B and four bands in zone C while D-2 had two bands in each zone. C-15 had no light bands while D-20 had no dark band. D-2 had maximum number of five dark bands.

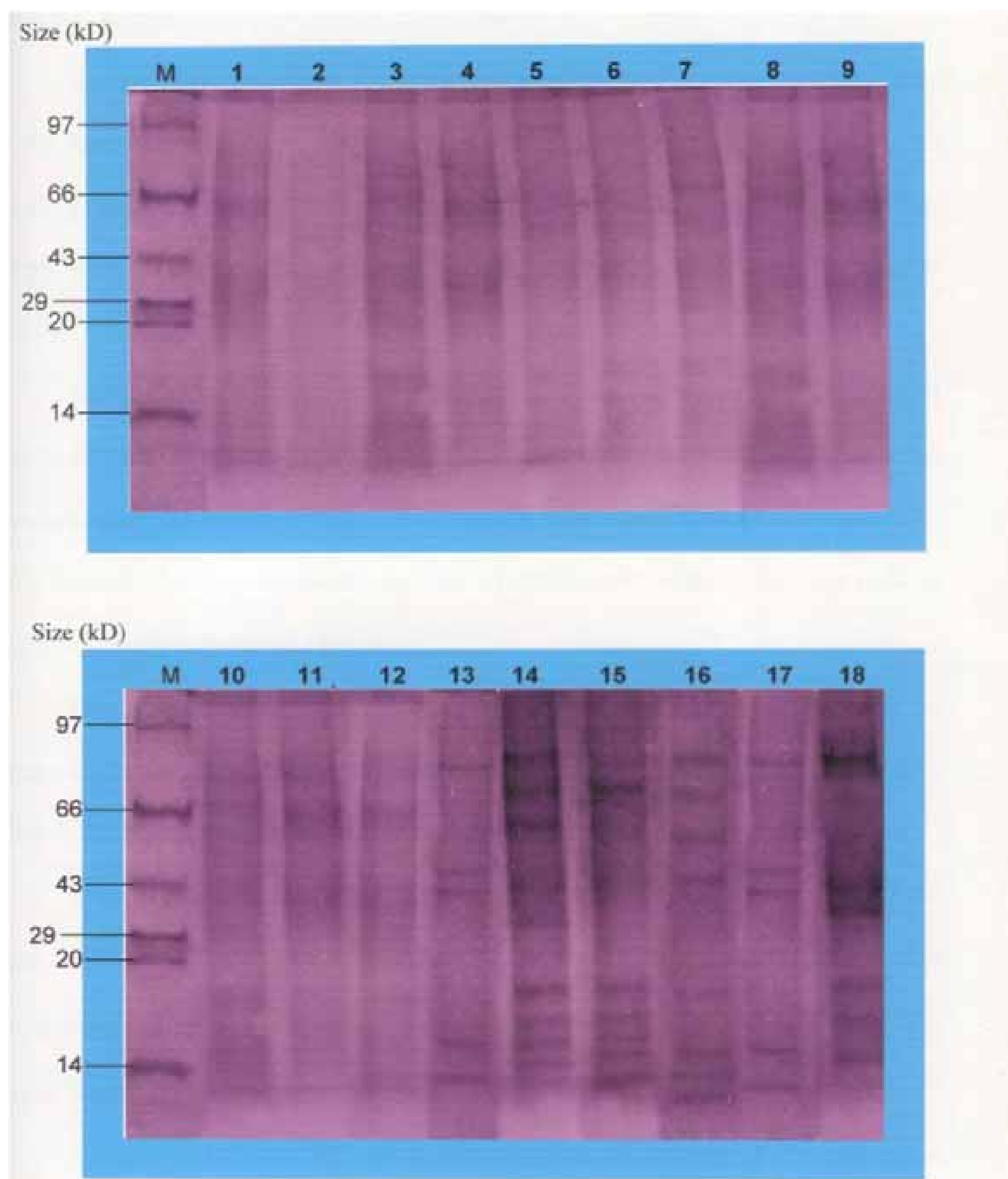

Plate 1: Protein profiling of fruit bodies of different isolates of *A.bisporus*
1=C-6, 2=C-13, 3=C-15, 4=A-27, 5=A-89, 6=A-61, 7=B-8, 8=B-49, 9=D-47,10=D-54, 11=C-68, 12=D-20, 13=D-2, 14=A-46, 15=D-18, 16=C-47, 17=D-63, 18=A-67 And M=Marker

In seven band group, there were four isolates out of which except B-8 all the isolates had bands in all the three zones with most of the bands being clusters in zone A and zone C. Intensity wise, except A-67 which had all dark bands, no isolates had dark band. B-8 had all light bands. In eight bands group, all the three isolates had band in each zone and most of the bands distributed in zone A and zone C. In terms of relative position of bands spread over different zones, it was seen that overall C zone was similar for all the member of this group, while variation in position of bands were observed in zone A and B. There were nine bands in three isolates (B-49, D-54 and A-47) but B-49 and D-54 had equal number of bands in all the three zones although the relative positioning of bands were different. Intensity wise, C-47 had all the bands with dark intensity (having maximum number of dark bands) while other two isolates had all the three intensity of bands. One isolate (A-47) had ten bands and isolates C-6 had a maximum of 13 bands in it. C-6 was the isolate which had maximum number of bands (six) in zone A. This isolate also had maximum number of light intensity bands (Ten). Overall it was observed that

in zone A, total number of bands varied from zero to six. A-61, being the only isolate having no bands in zone A, most of the isolates were having band of 2-3 which varied between and within groups. The bands lying in the zone were medium to light in intensities. The range of bands in zone B was found to be in the range of 1-4 in this zone. 50 percent isolates had one band while other had 2-3 bands. Only C-6 had four bands. All types of bands intensities were present in this zone. In zone C the range of bands were in the range of 2-5. As far as intensity was concerned, it was observed that almost half of the isolates had either zero or one dark band. Six isolates had five dark bands. Medium intensity bands were present in all the zones but most of them were accumulated in zone A and B. Light intensity bands were mostly present in zone A. A full range of zero to ten light bands was present. Maximum numbers of bands were present in C-6 (Plate 1).

CONCLUSION

Isolation of single spores is one of the techniques widely used for the genetic improvement in various species of mushrooms. But on an average about 50 per cent of the isolates are not performing superior than the parents in terms of yield and quality. Amongst the superior isolates we can select the best isolate as one of the strain for cultivation purposes but there is need to check it further for the quality characters. These SSIs may vary genetically among themselves. Therefore, protein profiling is another way to know the genetic diversity. On the basis of protein profiles, all the 18 isolates were classified into seven groups. The total number of bands ranged from 5-13 and spread over three zones (A, B and C). One isolate was in 13 band group while two isolates were in 5 band group, four in 6 bands group, three in 9 bands group, four in 7 bands group, three in 9 bands group and one in 10 bands group. Within group, the isolates had very little difference in banding pattern both for position and intensities of bands, however between group difference was observed. Irrespective to group, isolates varied more for high molecular weight protein band as compared to low molecular weight proteins.

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