Profile of the extracellular lignocellulolytic enzymes activities as a tool to select the promising strains of *Volvariella volvacea* (Bull. ex Fr.) sing.

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Abstract Out of 26 strains of *Volvariella volvacea* used, 18 were of ‘typical’ type and possessed all the characteristics of a normal *V. volvacea* mycelium, while the rest 4 ‘atypical’ type strains showed completely distinct mycelial growth characteristics. The remaining 4 strains grew very slowly and exhibited growth characteristics of single spore isolates of *V. volvacea*. Strains varied in their extracellular lignocellulolytic activities and strains; OE-274, OE-272 and OE-210 with high ligninase enzymes (laccase and polyphenol oxidase) activities, gave highest mushroom yield on pasteurized paddy straw substrate. On the composted paddy straw substrate, additional two strains, OE-213 and OE-215 with lower activities of ligninases also gave higher mushroom yield. Mushrooms were harvested 3 to 4 d early from the composted substrate than on the pasteurized substrate. Activities of endoglucanase, laccase and polyphenol oxidase were found to be more crucial for mushroom yield on pasteurized substrate, while xylanase and β -glucosidase were more important for composted substrate. Strains also varied in their fruiting bodies quality and the substrate used for mushroom cultivation also affected the fruiting body quality. The superior yielding strains varied in shape, size, weight, colour and contents of sodium and potassium in their fruiting bodies; while contents of carbon, calcium and protein did not vary much with the strains.

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Introduction

The paddy straw mushroom, *Volvariella volvacea*, is known for its unique aroma and texture and is cultivated mainly in South-East Asian countries [1]. This mushroom ranks sixth in the global production of cultivated mushrooms [2] and the estimated production in 2003 was approximately 6, 00, 000 tonnes, accounting for about 5% of the total world production [3]. It is a fast growing mushroom compared with most other cultivated mushrooms [1]. The biological efficiency of a mushroom species is mainly attributed to its hydrolytic enzymes system [4] and the biological efficiency is very low in paddy straw mushroom (about 10% to 15 % on rice straw) in comparison to other popularly cultivated mushrooms [5]. The activities of hydrolytic enzymes responsible for mycelial colonization (cellulases) and sporophore formation (laccase) [4, 6] have been found to vary in different strains of *V. volvacea* [6, 7]. The selection of a strain based upon its capacity to produce degradative enzymes can be one of the strategies for selecting a promising strain. The present study aimed at using the mycelial growth characteristics and enzymes activities of different strains as a tool for selecting a better performing strain for commercial mushroom cultivation.

Materials and methods

Strains and their morphological characterization: A total of 26 strains were obtained from National Mushroom

Culture Bank of the National Research Centre for Mushroom, Solan. All the strains were grown on Malt Extract Agar in petridishes and sterilized pounded paddy straw substrate in 250 mL conical flasks at $32 \pm 2^\circ\text{C}$ for 8 d. Dried and pounded paddy straw (10 g) was first filled in 250 mL flasks in triplicate for each strain and wetted overnight. The flasks were placed in inverted position on the next day to get rid of excess water, retain about 70 per cent initial moisture and then plugged with non-absorbent cotton, sterilized at 20psi for 1.30 h in an autoclave. Each flask and petridish was inoculated with a mycelial disc of 6 mm dia and incubated at $32 \pm 2^\circ\text{C}$ for 8 d. The strains were observed for mycelial growth rate, type of growth and formation of aerial hyphae as well as intensity of chlamydospores [8].

Enzyme assay: Only 'typical' *V. volvacea* strains (vigorously growing mycelia with abundant aerial and horizontal hyphae, mycelia usually thick at the margins of agar plate) were used for enzymes assays. Production potential for extracellular lignocellulolytic enzymes of 20 strains was studied by growing them on sterilized paddy straw substrate with 70 % moisture in flasks. Three replications were kept for each strain. The enzymes were extracted from the mycelium-colonized substrate in 50 mL phosphate buffer (0.1 M) of pH 7.0 by keeping the buffer mixed substrate at 40°C for 30 min in an incubator shaker maintained at 100 rpm. The extract was filtered through glass microfibre filter (GF-C) fitted in a three-piece filter funnel (Whatman) and then stored at 4°C for further use. The enzyme assay was carried out in triplicate for all the enzymes and data were subjected to statistical analysis.

The cellulases were measured according to the method of Mandels *et al* [9] as modified by Sandhu and Kalra [10]. The reaction mixture for exoglucanase (FPase, EC 3.2.1.91) comprised of eight filter paper (Whatman No. 1) discs of 0.6 cm dia in 0.5 mL acetate buffer of pH 5.0 and 0.25 mL of the enzyme source. The reaction mixture was incubated at 40°C for 4 h and the reducing sugars released were measured by Nelson Somogyi method [11]. The endoglucanase (CMCase, EC 3.2.1.4) activity was measured following the above method, replacing filter paper discs with 0.5 mL of 5 mg mL^{-1} Carboxy methyl cellulose. Xylanase (EC 3.2.1.8) was assayed at 40°C following a method described by Reese and Mandels [12]. β -glucosidase (EC 3.2.1.21) assay was carried out with a reaction mixture containing 1.5 mL of *p*-nitrophenyl β -D-glucopyranoside (60.26 mg dissolved in 100 mL acetate buffer, pH 5.0) and 0.5 mL of the enzyme extract after incubating the reaction mixture at 45°C for 4 h. The reaction was terminated by adding 2 mL of 1 M Na_2CO_3 and the absorbance was read at 420 nm.

Laccase (EC 1.10.3.2) was assayed by adding 0.3 mL enzyme source to 2.5 mL of 30 μM Guaiacol in phosphate buffer (0.1 M) of pH 6.0 and ΔA was read at 470 nm after incubating the reaction mixture for 30 min at room temperature against zero time control. Polyphenol oxidase (EC 1.10.3.1) was assayed using Catechol as substrate in place of Guaiacol. One unit of laccase and polyphenol oxidase activity was calculated as change in absorbance by 0.001 $\text{min}^{-1} \text{mL}^{-1}$ of enzyme source at 25°C , while that of β -glucosidase as $\mu\text{mol } p\text{-nitrophenol released h}^{-1} \text{mL}^{-1}$ of enzyme source, and FPase, CMCase and xylanase as the $\mu\text{mol glucose released h}^{-1} \text{mL}^{-1}$ of enzyme source.

Crop raising: Crop for evaluating the parent strains for their yield potential and quality was raised following the method of Verma [13], where in steam pasteurized paddy straw bundles of $30 \times 10\text{cm}^2$ size were used for bed preparation. Eight bundles were used for preparing one layer of bed and each bed was prepared with 5 such layers consisting of total 40 bundles. Five replications were kept for each treatment. The strains were also evaluated for mushroom yield by using composted paddy straw-based substrate prepared by the method of Quimio [14]. Substrate was prepared by using paddy straw, chicken manure, rice bran and lime as the basal ingredients (paddy straw – 1000kg, Lime – 10kg, Chicken manure – 100kg and Rice bran – 10kg). The fresh mushroom yield obtained was converted into biological efficiency (kg q^{-1} dry substrate) by using equation $\text{BE (\%)} = \text{Fresh mushroom yield in kg/Quantity of dry substrate used} \times 100$.

Mushroom quality: Different quality parameters of fruiting bodies viz., size, shape, color, weight, pileus opening and contents of potassium, sodium, calcium, carbon, phosphorous and protein (nitrogen) were recorded. The size, shape, color and pileus opening were recorded based upon the visual observations, while whiteness was recorded as reflectance in per cent using pre-calibrated Elico Reflectometer. Fruiting body weight was recorded by weighing 10 randomly selected unopened fruiting bodies and calculating per unit fruiting body weight in g. The contents of calcium, potassium and sodium were determined by using Ion Meter (Thermo Orion model 720A+) in ppm g^{-1} of dry mushroom sample. The instrument was calibrated using 10, 100, 1000 ppm standard solutions of calcium ion in CaCO_3 , potassium in KCl, sodium in sodium chloride. The ion concentration was measured by dipping the electrode in the solution containing 1 g composite mushroom fruiting body sample and measuring the potential against a constant reference potential using Nernst equation i.e. $E = E_0 + S \log(A)$ (*S=2.3 $\times RT / N \times F$, *Slope is directly proportional to changes in mV decade $^{-1}$ and is calculated directly by the equipment),

where in, E = measured electrode potential; E_0 = reference potential (a constant); A = ion level in solution; N = number of ions; S = electrode slope; R = gas constant T = temp in Kelvin; F = Faraday's constant. The total available carbon was determined by the method of Walkley and Black [15], while phosphorous by the method of Allen [16]. The nitrogen content was determined by Micro-Kjeldhal method using Kel-Plus Nitrogen estimation unit from Pelican Equipments, Chennai (Tamil Nadu).

Results and discussion

Morphological characteristics: The morphological characteristics of 26 strains have been depicted in Table 1. On

malt extract agar, strains showed variations in their radial mycelial growth and formation of aerial hyphae as well as chlamydospores. Fourteen strains showed radial growth of 90 to 100 mm in 8 d of incubation, while 8 strains grew up to only 60–70 mm. Rest 4 strains grew very slowly and showed growth of 11 to 35 mm. Most of the fast growing strains formed thin or thick fluffy growth with high level of aerial hyphae and no chlamydospores formation upto 8th d of growth. However, some exceptions were noticed, where in the fast growing strains, OE-272 and OE-274 with radial growth of 90 mm formed quite low level of aerial hyphae. Most of the strains showed characteristics of a 'typical' *V. volvacea* strain (vigorously growing mycelia with abundant aerial and horizontal hyphae, mycelia usually thick at the margins of agar plate) [8], excepting 4 strains (OE-49,

Table 1. Morphological characteristics of different strains of *Volvariella volvacea* grown on different cultural media under *in vitro* conditions.

Strain	Growth characteristics							
	Malt extract agar				Pounded paddy straw			
	a	b	c	d	a	**	b	c
OE-272	90 mm(Tn)	+	–	T	Complete	+	–	–
OE-273	95 mm(Tk)	+++	–	T	Complete	++	±	++
OE-274	90 mm(Tn)	+	–	T	Complete	±±	±	+
OE-12	65 mm(TnF)	++	–	T	Complete	++	±	+
OE-29	65 mm(Tk&Tn)	+	–	T	Complete	++	+	–
OE-49	67 mm(Tk)	–	–	A(D)	Not used			
OE-55	60 mm(TkS)	–	–	T	Complete	+	–	–
OE-91	95 mm(Very Tk)	+	–	A(D)	No growth	–	–	–
OE-112	87 mm(TnF)	+++	–	T	Complete	+	–	–
OE-117	100 mm(TnF)	+++	–	T	Complete	+++	+	–
OE-139	95 mm(Tk)	++	–	T	Complete	++	+	+
OE-140	90 mm(Tk)	++	–	T	Complete	+++	+	–
OE-143	11 mm(Tk)	–	–	T(SSIs)	No growth	–	–	–
OE-144	63 mm(TkS)	±	++	T	60%	+	–	–
OE-145	35 mm(Tk)	–	–	T(SSIs)	90%	+	–	–
OE-146	63 mm(Tk)	–	–	A(D)	Not used			
OE-209	90 mm(Tn)	+++	–	T	Complete	+	–	–
OE-210	95 mm(Tk)	++	–	T *	90%	++	–	–
OE-211	95 mm(Tn)	++	–	T*	Complete	+	–	–
OE-212	100 mm(Uniform)	+++	–	T*	Complete	+++	–	–
OE-213	65 mm(Tn)	+	–	T	Complete	+++	–	+
OE-214	100 mm(Tk)	++++	–	T*	Complete	+++	+	++
OE-215	100 mm(HF)	6+	–	T*	Complete	+++	+	+
OE-224	62 mm(Tk)	–	–	A(D)	Not used			
OE-225	25 mm(Tk)	–	–	T(SSIs)	No growth	–	–	–
OE-226	Very thin growth	–	–	T(SSIs)	No growth	–	–	–

a-Radial mycelial growth b-Aerial hyphae c-Chlamydospore intensity d-Strain type T-Typical A-Atypical Tn-Thin Tk-Thick TnF-Thin fluffy TkS-Thick strandy HF-Highly fluffy *-Best with respect to all parameters ** Visual growth - to 6+ -Absent to very high

Table 2. Extracellular lignocellulolytic enzymes activities profile of different strains of *Volvariella volvacea* grown on paddy straw substrate.

<i>V. volvacea</i> strain	Enzyme activity					
	Exoglucanase	Endoglucanase	Xylanase	β -glucosidase	Laccase	Polyphenol oxidase
OE-272	0.70	1.19	2.05	0.71	1.75	5.23
OE-273	0.50	0.05	1.41	1.03	1.12	1.38
OE-274	0.46	0.81	1.02	1.18	1.13	1.20
OE-12	0.55	0.28	1.28	1.50	1.32	1.98
OE-29	0.81	1.15	1.54	2.27	3.28	2.42
OE-55	0.28	1.07	ND	1.06	1.13	ND
OE-112	0.63	0.74	1.48	0.52	0.68	0.98
OE-117	0.54	0.64	1.88	2.55	ND	1.80
OE-139	1.23	1.23	0.65	1.38	ND	5.02
OE-140	0.93	0.82	1.71	0.82	1.63	ND
OE-143	1.38	1.57	1.49	1.06	3.33	2.50
OE-144	0.40	0.60	1.80	2.23	1.12	1.38
OE-145	0.13	1.49	0.97	2.59	3.23	4.38
OE-209	ND	1.16	1.92	0.09	ND	0.75
OE-210	0.54	1.24	0.80	2.50	1.88	6.50
OE-211	0.02	0.74	1.43	2.10	0.55	1.23
OE-212	0.37	1.18	1.20	1.10	ND	1.45
OE-213	0.46	0.46	1.95	2.04	1.66	0.93
OE-214	1.27	1.23	1.80	2.22	ND	2.82
OE-215	1.00	0.61	2.24	1.31	0.32	0.68

Exoglucanase, Endoglucanase and Xylanase activities- μmol glucose released $\text{h}^{-1}\text{mL}^{-1}$ Laccase and polyphenol oxidase-change by absorbance of $0.001 \text{ min}^{-1}\text{mL}^{-1}$ and β -glucosidase- μmol pNP released $\text{h}^{-1} \text{mL}^{-1}$ ND-not detected

Table 3. Mycelial colonization of substrate, first harvest and mushroom yield in different strains of *V. volvacea* grown on pasteurized paddy straw bundles.

<i>V. volvacea</i> strain	Mycelial colonization		First harvest (days post-spawning)	Mushroom yield (kg q^{-1} dry substrate)
	Type of growth (visual)	Mycelial density (visual)		
OE-272	fluffy	+++++	21.4 $\pm$ 0.68	10.49
OE-274	fluffy	+++++	20.6 $\pm$ 1.25	12.36
OE-12	thin	++	23.0 $\pm$ 0.94	5.35
OE-29	thin fluffy	+++	No pinning	No yield
OE-55	fluffy	+++++	25.33 $\pm$ 3.66	2.23
OE-139	thin	++++	21.4 $\pm$ 0.45	5.36
OE-140	fluffy	+++	21.8 $\pm$ 0.42	7.03
OE-209	thin	+++	22.0 $\pm$ 0.35	7.05
OE-210	thin	++++	24.2 $\pm$ 2.48	10.46
OE-213	thin	++++	22.0 $\pm$ 1.00	7.42
OE-214	thin	+++	25.8 $\pm$ 1.85	4.66
OE-215	cottony	+++++	22.6 $\pm$ 0.57	5.03
CD (0.05%)			1.69	1.16

++ to +++++ – Very thin to highly dense mycelial growth

OE-91, OE-146 and OE-224) which showed quite ‘atypical’ characteristics. The remaining 4 strains (OE-143, OE-145, OE-225 and OE-226) showed characteristics of single spore isolates of *V. volvacea* [6, 8]. In the present study, absence of chlamydospores in all ‘typical’ strains on 8th d of growth is in accordance to the observation of Chang *et al.* [8], who also reported absence of chlamydospores on freshly grown cultures. The ‘typical’ type strains completely colonized the paddy straw substrate within 8 d of growth and formed scanty aerial hyphae. Only 7 strains (OE-273, OE-274, OE-12, OE-139, OE-213, OE-214 and OE-215) formed chlamydospores showing no relationship with the rate of mycelial growth, as some of the slow growing strains (OE-12, OE-212) also formed chlamydospores. The variations in morphological characteristics in strains of *V. volvacea* and changes in their morphological growth characteristics at different stages of growth have also been reported earlier [8]. In molecular characterization of different accessions of mushroom germplasm at the National Research Centre for Mushroom, Solan, Singh *et al.* [17] also found variations among different strains of *V. volvacea* and reported that some of the strains were actually not of *V. volvacea*, which were initially deposited as paddy straw mushroom strains.

Enzyme assay: Out of 26 strains, only ‘typical’ types (20) were used for enzyme studies. The exoglucanase activity varied between non-recordable to 1.38U. Similar was the situation with the activities of other enzymes, which varied in different strains (Table 2). Highest exoglucanase

activity was recorded in strain, OE-143, followed by OE-214, OE-139 and OE-215. Highest endoglucanase activity was recorded in the strain, OE-143, followed by 1.49U in OE-145 and 1.24U in OE-210. Highest β -glucosidase activity was recorded in the strain, OE-145, followed by 2.55U in OE-117 and 2.50U in OE-210. Xylanase activity was highest in strain, OE-215, followed by strains, OE-272, OE-213, OE-209, OE-117 and OE-214. Highest activity of laccase was recorded in strain OE-143, followed by strains, OE-29, OE-145, OE-210 and OE-272. Polyphenol oxidase activity was highest in the strain, OE-210, followed by strains, OE-272, OE-139, OE-145 and OE-214. Variations in activities of extracellular enzymes in *V. volvacea* strains along with role of cellulases in mycelial colonization and laccase in sporophore formation have also been reported earlier by other workers [4, 6].

Mushroom yield: Out of 20 strains screened for enzyme assays, 12 strains with varied enzyme activities were used for the yield evaluation trials on pasteurized paddy straw bundles and also on composted paddy straw substrate (Table 3). Mycelial colonization of substrate varied in different strains after 8 d of spawning. On pasteurized paddy straw bundles, strains, OE-274, OE-215, OE-272 and OE-55 showed very thick and fluffy growth, while strains, OE-210, OE-213 and OE-139 completely colonized the substrate but with very light (thin) mycelial growth. Mushrooms were harvested earliest in the strain, OE-274 after 20 d of spawning, followed by strains, OE-272, OE-139

Table 4. Mycelial colonization of substrate, pinning and mushroom yield in different strains of *V. volvacea* grown on composted paddy straw substrate

<i>V. volvacea</i> strain	Mycelial colonization / pinning (visual)		First harvest (days post-spawning)			Mushroom yield (kg q ⁻¹ dry substrate)		
	Mycelial colonization	Pinning pattern	Ist Trial	IIInd Trial	Average	Ist Trial	IIInd Trial	Average
OE-272	+++	+++	15.4±0.87	17.2±0.37	16.30	7.00	4.93	5.96
OE-273	+++++	++	16.6±0.36	17.5±0.29	17.05	5.32	5.71	5.51
OE-274	+++	+++	15.7±0.45	17.6±0.93	16.65	6.89	5.12	6.00
OE-12	+++	+	16.2±0.37	19.2±0.48	17.70	3.28	2.65	2.96
OE-55	++++	-	18.4±0.88	19.7±2.09	19.05	1.62	2.62	2.12
OE-139	+++	+	18.6±0.68	20.0±1.64	19.30	4.26	3.43	3.84
OE-140	+++	+	16.2±0.49	17.6±0.40	16.90	6.42	4.19	5.30
OE-209	++++	++	16.4±0.24	18.6±0.81	17.50	6.10	5.12	5.61
OE-210	+++	++	16.6±1.03	19.0±0.45	17.80	6.00	4.79	5.39
OE-213	+++	++	16.4±0.51	18.2±0.49	17.30	5.80	8.05	6.92
OE-214	+++	+	15.0±0.89	19.2±0.75	17.10	6.10	3.29	4.69
OE-215	++++	+	15.8±0.37	20.0±1.64	17.90	8.28	4.81	6.54
CD (0.05%)			0.68	1.23	-	1.58	0.86	-

Mycelial colonization, +++ to +++++ – Thin to very dense Pinning pattern, – to +++ – Absent to present on full bed.

and OE-140. Highest mushroom yield of 12.36 kg q⁻¹ dry substrate was recorded in the strain, OE-274, followed by 10.49 kg in OE-272 and 10.45 kg in OE-210.

In the first cultivation trial conducted on composted paddy straw substrate, mycelial colonization of substrate was superior in strains, OE-273 and OE-215, followed by strains, OE-272, OE-210, OE-213 and OE-209. Earliest mushroom pinheads were recorded in strain, OE-272, followed by OE-210, OE-213 and OE-209. Earliest first harvest of mushrooms (post-spawning) was in strain, OE-214 after 15.0 d of spawning, followed by OE-272, OE-274 and OE-215. Highest mushroom yield of 8.28 kg q⁻¹ dry substrate was recorded in strain, OE-215, followed by strains, OE-272, OE-274 and OE-140. In the second trial, earliest harvest was in strain OE-272 after 17 d of spawning, followed by strains, OE-273 and OE-274. Highest mushroom yield of 8.05 kg q⁻¹ dry substrate was recorded in strain, OE-213, followed by strains, OE-273 and OE-274. On the average of two trials, strain, OE-272 took lowest time for first harvest and yielded mushrooms almost at par with other high yielding strains like OE-213, OE-215 and OE-274, which fruited later than strain, OE-272. Yield evaluation trials for selecting a better performing strain or single spore isolate have also been performed earlier by several workers [6, 18–20], but none of them have correlated the morphological and biochemical characteristics of a strain with its yield potential. Strain evaluation trails by using substrate prepared with different methods have not been carried out earlier, except by Garcha *et al* [19], who also compared yield potential of 6 strains on paddy straw substrate prepared by a single method but on different methods of bed preparation.

Mushroom quality: The size, shape, whiteness, colour, weight, pileus-opening and contents of potassium, sodium, calcium, carbon, phosphorous and protein (nitrogen) varied in the fruiting bodies of different strains. Fruiting bodies harvested from two different types of substrates also varied in their qualities. From the pasteurized paddy straw substrate, big size, compact fruiting bodies (50–70 mm long) with oval or round shape were harvested in strains, OE-274, OE-213 and OE-214. Other high yielding strains, OE-272 and OE-210, produced medium size (30–50 mm long), oval shaped fruiting bodies. The colour/whiteness varied in fruiting bodies of different strains and whitest fruiting bodies were harvested from strain, OE-209, followed by OE-12 and OE-55. Average fruiting body weight also varied and the heaviest fruiting bodies were harvested from strain, OE-274, followed by OE-213 and OE-140. Pileus-opening tendency was highest in strains, OE-12, OE-140 and OE-272. The contents of calcium, carbon and phosphorus did not vary much in different strains, but the content of sodium

Table 5. Quality characteristics of fruiting bodies of different strains of *V. voluacea* grown on pasteurized paddy straw bundles.

<i>V. voluacea</i> strain	Fruiting body quality characteristics											
	Size (visual)	Shape (visual)	Whiteness (reflectance %)	Colour (visual)	Weight (g)	Pileus opening (visual)	K (ppm g ⁻¹)	Na (ppm g ⁻¹)	Ca (ppm g ⁻¹)	Carbon (%)	Phosphorus (%)	Protein/ Nitrogen (%)
OE-272	medium	oval	7.40	dirty white	10.25	high	31.6	1.34	7.13	0.41	0.89	18.75/3.0
OE-274	big	oval	12.20	dark black	16.80	nil	62.0	2.03	11.01	0.21	0.95	16.25/2.6
OE-12	medium	round	17.00	black	8.90	high	45.80	1.54	8.57	0.31	0.57	17.5/2.8
OE-55	small	oval	16.40	white	8.63	nil	1729.0	0.84	10.1	0.37	0.61	13.12/2.1
OE-139	medium	roundish oblong	13.20	light black	10.90	medium	1850.0	0.082	8.92	0.38	0.65	17.5/2.8
OE-140	small	oval	12.60	light black	11.00	high	1667.0	0.00	9.08	0.45	0.99	17.5/2.8
OE-209	medium	oval	17.40	dirty white	10.14	nil	1830.0	0.430	7.13	0.59	1.01	18.75/3.0
OE-210	small	oval	12.00	dirty white	9.95	nil	1431.0	0.747	7.42	0.71	0.89	18.125/2.9
OE-213	big	oval	11.60	dark black	11.12	nil	1339.0	0.780	8.39	0.80	1.05	16.81/2.69
OE-214	big	round	14.80	dirty white	10.95	nil	2370.0	0.910	7.85	0.29	1.09	14.25/2.28
OE-215	medium	oval	14.60	dirty white	9.71	nil	2150.0	1.00	7.31	0.59	0.90	20.00/3.2

Small-2 to 3 cm long Medium-3 to 5cm long Big-5 to 7cm long

Table 6. Quality of fruiting bodies obtained from different strains of *V. volvacea* grown on composted paddy straw substrate.

<i>V. volvacea</i> strain	Fruiting body colour (visual)	Fruiting body size (cm)	Toughness (comparative)	Veil opening (visual)	Fruiting body weight (g)
OE-272	+++	3–4	++	+	10.25
OE-273	++++	4–5	++	–	15.48
OE-274	++++	5	+++	–	16.80
OE-12	+++++	4–5	++	+++	8.90
OE-55	++	4–5	++	++	8.63
OE-139	++	5	++++	–	10.90
OE-140	+	5	++++	–	11.00
OE-209	+	4–5	+++	+	10.14
OE-210	++	3–4	+++	–	9.95
OE-213	++	4–5	+++	–	11.12
OE-214	+++	4–5	++	–	10.95
OE-215	+++	3–4	++++	–	9.71
CD (0.05%)		0.66			2.32

Fruiting body colour, + to +++++ – Creamy to dark brown Toughness, ++ to ++++ – Soft to very hard Veil opening, – to +++ – 0 to 60%

was much higher in high yielding strains (OE-274 and OE-272) in comparison to other strains. However, the trend with respect to potassium content was just the reverse and it was much lower in strains, OE-272, OE-274 and OE-12.

From the composted substrate, tough and big size fruiting bodies were harvested from strains, OE-139, OE-140, OE-274 and OE-209. Fruiting bodies from strains, OE-12, OE-273 and OE-274 were whiter than fruiting bodies from other strains. Veil-opening tendency was highest in strains, OE-12, OE-55 and OE-272, forming lightweight soft fruiting bodies. There was no correlation between length of the fruiting body and tendency to open, as comparatively bigger size fruiting bodies of strains, OE-12 and OE-55 opened much more than smaller size fruiting bodies of strain, OE-215. Fruiting bodies obtained from strains, OE-274, OE-273 were heaviest in weight. There are not many references available on comparative analysis of fruiting body quality of different strains of *V. volvacea*, except some studies on its preservation [21–23].

In the present study, the fast growing strains with fluffy mycelial growth and abundant aerial hyphae, namely, OE-272, OE-274 and OE-210, gave higher mushroom yield on the pasteurized paddy straw bundles than other strains. Highest yielding strain, OE-274 was recorded to possess fairly good level of activities of all the cellulases, xylanase and ligninases, while the second best yielding strain, OE-272 also recorded higher activities of PPO and xylanase along with medium level activities of the other enzymes. The third strain, OE-210, also recorded to possess highest activities of PPO and β -glucosidase along with good level of activities of other enzymes. On composted substrate

along with strains, OE-272 and OE-274, two other strains, OE-213 and OE-215 gave higher yield than other strains. All these strains were fast growing, formed abundant aerial hyphae and chlamydospores, and formed early pinning than other strains. In enzymatic studies, strains, OE-213 and OE-215 also showed higher activities of xylanase and β -glucosidase. However, in contrast to correlation of enzymes activities with mushroom yield on pasteurized substrate, here the strain, OE-213 was deficient in exoglucanase activity and strain, OE-215 in laccase activity. This study clearly indicates that different sets of enzyme activities are responsible for mushroom yield on the substrate prepared by different methods; on pasteurized substrate, strains with higher activities of PPO, laccase, xylanase/ β -glucosidase and average activities of exoglucanase and endoglucanase gave superior yield, while on the composted substrate, strains even deficient in cellulases and ligninases gave good yield. The possible role of different sets of enzymes in mushroom yield on different types of substrates can be attributed to the variations in the initial composition of substrates; as in pasteurized substrate, the substrate has to be broken-down with the help of cellulases and ligninases, while the composted substrate harbours other sets of microorganisms with abilities to breakdown the substrate. Different types of substrates need strains with different set of enzymes activities for obtaining optimum yield. The yield performance of a strain on a particular substrate will be regulated both by its mycelial growth, enzymes activities as well as chemical composition of the substrate. Very few studies of this type have been conducted earlier; Chen *et al* [4] and Ahlawat *et al* [6], who reported the role of cellulases in substrate

colonization and laccase in sporophore formation in this mushroom. The present study has provided an insight into the subject, which will help in developing a protocol for selecting potentially superior strains based upon their extra-cellular lignocellulolytic enzymes profile and the chemical composition of the substrates to be used.

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