ORIGINAL RESEARCH ARTICLE

Strategies for improvement in cultivation practices of oyster mushrooms in North Bengal, India

ABSTRACT

Mushrooms are being used as food and medicine since time immemorial and it constitutes an ideal source to reduce body weight. The different wastes are used to cultivate the mushroom. In West Bengal, huge amount of different agricultural wastes is produced annually, and are of no uses. These wastes could be used as source of food i.e. substrate for mushroom cultivation. However, the most common methods of oyster mushroom were found in sterilized or partial sterilized paddy straw. To make this mushroom cultivation more profitable and popular, different types of agro-wastes, crop residues and by-products can be used with conventional polybag method. Oyster mushroom was cultivated on different substrates viz. paddy straw, wheat straw, sugarcane bagasse and combination of different straw using to find out the suitable substrate. Different substrates significantly affected the number of primordia and fruiting bodies, and the amount of fresh weight or yield of mushroom. The highest number of fruiting bodies, the amount of fresh weight and the yield was obtained with rice straw in combination with wheat straw in all flushes followed by rice straw and the lowest with wheat straw. The biological efficiency was also higher in rice with wheat straw. The N, P and K content in straw was found higher in rice straw. Molecularly, the *Pleurotus* shows that maximum similarity of the sequence with *Pleurotus ostreatus*. A 95 % coverage of the sequence resulted in 92.55% similarity with fungal strain.

Key words: Oyster mushroom, Substrates, Yield, Bioefficiency, Nutrient content, ITS Sequencing

INTRODUCTION

Improving food safety and food security is imperative to adequately feed a growing population that is expected to exceed 9 billion people in this globe by 2050. Agriculture sector in India occupies a key position in the economy and provides employment to more than 60% of the working population of India and also contributes 15.4 % in GDP. The food and nutritional security of growing population is a great challenge in India, which looks for new crop as a source of food and nutrition. In this context, mushroom cultivation provides unique opportunity to take advantage of underutilized resources and produce high quality food from different wastes. Mushrooms can be grown even by landless people, that too on waste material and could be a source for high proteinaceous food (Ambili and Nitiya, 2014). Use of mushrooms as food and neutraceutical have been known since time immemorial, as it is evident from the description in old epics Vedas and Bible. Earlier civilization had also valued mushrooms for delicacy and therapeutic value. The mushroom cultivation has grown up in almost all the parts of the world and during last decades, the world mushroom production achieved the growth rate of about 10%. In India, owing to varied agro-climate abundance of farm waste, different types of mushrooms are cultivated throughout the country (Shah *et al*., 2004).

Among the different mushrooms, oyster mushrooms are one of the most popular edible mushrooms in South Asia and its cultivation is becoming increasingly popular in India with the growing

awareness of people about their food and health. Beside this, the sub-tropical and tropical climate in India favours the growth of this mushroom which thrives better in a wide range of temperature and moisture. Among the different *Pleurotus* spp., *P. ostreatus* has received increasing attention for applications in bio-bleaching and the catalysis of difficult chemical conversions in the paper industry, textile dye decolourization, and detoxification of environmental pollutants (Park *et al.*, 2014).
With this background information, a study was undertaken to identify the most suitable oyster mushroom and its growth in different substrates available in this region.

MATERIALS AND METHODS

Studies on cultivation of oyster mushroom (*Pleurotus* spp.) on different agro-wastes were experimented in Research Laboratory, Department of Plant Pathology, Uttar Banga Krishi Viswavidyalaya, Pundibari, Coochbehar, West Bengal.

The substrates used for *Pleurotus* spp. cultivation were rice straw, wheat straw and sugarcane bagasse. They are used either as a whole substrate or in combination substrates. Six (6) treatments were used (RS - rice straw, SB - sugarcane bagasse, WS - wheat straw, PW - polygonum weed).

T1=500g (RS)

T2=437.5g (RS) + 62.5g (SB)

T3=375g (RS) + 125g (SB)

T4=437.5g (RS) + 62.5g (WS)

T5=375g (RS) + 125g (WS)

T6=500g (WS)

Heltay and Zavodi, (1960) observed that the importance of rice straw used as substrate not only for *Volvariella* but it is also used as an important component of synthetic *Pleurotus* compost containing 41% cellulose, 13% lignin, 0.8% total Nitrogen, 0.25% phosphorus pentoxide, 0.3% , 6% SiO2, pH 6.9, C:N 58:1. Analysis on chemical composition of wheat straw which consists of cellulose (34-40%), hemicellulose (20-25%), and lignin (20%) (Rodriguez *et al*., 2012). Rice and wheat straws and cotton wastes are recommended the best productive substrates among the other substrates (Kimenju, 2009). Park *et al*. (1975) performed rice and wheat straw as substrates, and the yield on rice straw was slightly higher as compare to wheat straw.

Data collection

Data was collected from sprouting till maturity of the mushroom and recorded periodically during the growing season as 1st, 2ndand 3rd flush. Days of spawn running, primordial initiation, number of fruiting bodies and fresh weight of mushroom were recorded. The observation on stipe length (cm) and pileus width (cm) which are considered as the main constituent of fruiting bodies were also collected. The total yield, moisture content and dry weight of each substrate were recorded from all the treatments of each substrate.

Moisture and dry matter

Twenty gram (20g) of freshly harvested fruit body was taken as a sample for calculating the amount of dry matter and was kept in a hot air oven for drying at 100-105□C. The process of heating and cooling was repeated till a constant weight was achieved. The percent moisture and dry matter were determined with the help of laboratory oven, from the differences between fresh and dry weights of the samples by using the following equation (Rahman *et al*. 2012).

$$\text{Moisture \%} = \frac{w1-w2}{weight\ of\ sample} \times 100$$

Dry mater % = 100 – Moisture %

W1 = Fresh weight

W2 = Oven dry weight

2.3. Biological efficiency

Khan, (2017) concluded that *Pleurotus ostreatus* obtain highest yield in the first harvest and subsequently reduces in second and third flush. The highest number of pin-head and fruiting bodies of oyster mushroom was found in sterilized paddy (Al Amin, 2004). According to the report, (Kimenju *et al*., 2009; Iqbal *et al*., 2016; Khare *et al*., 2010). cultivation of oyster mushroom in paddy straw produce the maximum yield to influence its growth, yield and composition.

The BE of mushroom was calculated from the equation (Chang and Miles, 1989).

$$\text{BE} = \frac{fresh\ weight\ of\ mushroom\ (g)}{dry\ weight\ of\ substrate(g)} \times 100$$

Effect of substrates on media at different temperature

The prepared media of different substrates are autoclaved at 121□C for 15-20 minutes at 15p.s.i. The radial growths of mycelium were recorded periodically during 3rd, 5th, 7th, 9th days after inoculation of oyster mushroom (*Pleurotus* spp.) at 20□C, 25□C and 30□C. Kong, (2004) reported that *P. ostreatus, P. florida, P.sajor-caju* achieve their optimum growth at 25℃, while *P. cornucopiae and P.cystidiosus* reach their growth at the temperature 25-35℃ temperature. The optimum temperature for the mycelia growth of *P. ostreatus* has been recorded at 21-26℃ (Block *et al*., 1960).

Determination of N, P and K content of substrates

Nitrogen in culture broth was determined by KEL PLUS nitrogen estimation system (Jackson, 1973), phosphorous and potassium was estimated by Vanado molybdo phosphoric yellow colour method, Flame photometry respectively. (Vogel, 1961).

Quantitative estimation of protein in fresh and dry mushroom

Total protein of mushroom was estimated following Lowry's method (Lowry et al., 1951)**.** Oyster mushrooms contribute a high nutritional value of protein (25-50%), sugars (17-47%), cellulose (7-38%) and minerals (Stanley *et al*., 2011).

ITS sequencing of Pleurotus

Pleurotus ostreatus culture was used for ITS sequencing. The DNA was extracted with fungal DNA isolation kit (Qiagen). The DNA was checked for its quality and sent to sequencing company with the set of primers.

Chart1: Primer used in the study

Primer Name	Primer sequence (5'-3')	rRNA operation binding site
ITS1	*CTTGGTCATTT AGAGGAAGTAA*	Small subunit Gardes and Bruns, (1993).
ITS 4	TCCTCCGCTTA TTGATATGC	Large subunit (White et al., 1990).

RESULTS AND DISCUSSION

Relative performance of *Pleurotus* spp.

Among the three different isolates of *Pleurotus* in rice straw substrate, there is a variation of yield and biological efficiency. The highest yield (847g) was obtained in Isolate 1 and no statistical differences between Isolate 2 and 3 were recorded. Regarding spawn run, there are no significant differences (Table 1). Based on the observation, *Pleurotus* 1 isolate was selected for further experimentation.

Effect of different substrates on mycelial growth of *Pleurotus* Isolate 1

In the next step, the effect of different substrates viz. sugarcane baggase, mustard straw, polygonum weed, rice straw and wheat straw on the mycelial growth of *Pleurotus* 1 isolate were carried out at three different temperature levels (20^0C, 25^0C and 30^0C). The substrates were dried, powdered and agar was mixed. After sterilization, the substrate agar media was pour into petri-plates and subsequently inoculated with *Pleurotus* Isolate 1. The observation of mycelial growth in mm were recorded at 3^{rd}, 5^{th}, 7^{th} and 9^{th} day (Left to Right) after inoculation and presented in Table-2.

From Table 2 indicates that there is a strong variation of mycelial growth of *Pleurotus* spp. among the substrates at different temperature levels. Irrespective of the substrates, higher growth was observed at 25^0C and lowest growth at 30^0C. At 25^0C, the highest growth was recorded in wheat straw followed by rice straw; the lowest growth was recorded with polygonum weed (Fig. 1), though the differences are significant (at 5%). Based on the performance of different substrates, rice straw, wheat straw and sugarcane bagasse were selected for their efficacy on yield parameters of *Pleurotus* spp.

Effect of different substrates on yield performance of oyster mushroom

Most organic matters containing cellulose, hemicelluloses and lignin can be used as mushroom substrate i.e. rice straw, wheat straw, sugarcane bagasse, mustard straw, banana leaves, saw dust, waste paper etc. As per information available the amount of nutrition requirements differs according to mushroom species and types of substrates used. This study was designed to identify the suitable substrate for *Pleurotus* spp. available in this region. Accordingly, six (6) different treatments consist of either single substrate or in combinations were selected and inoculation was done as described in

methodology. The yield and yield attributing characters of mushroom obtained from different substrates are presented in Table -3.

The substrate used in this study showed variation in spawn run, fruiting body formation, cap diameter, stipe length and fresh weight (Table 3). Among the different substrate, wheat straw (29%) required lesser time for spawn run (14 days) followed by rice + wheat straw (14.5%) and longer duration was required for rice straw in combination with sugarcane bagasse (18 days). However, primordial formation was fastest in rice straw with wheat straw (14.5%) and lowest in wheat straw.

The stipe length and cap diameter of *Pleurotus* mushroom was measured in an average up to two harvests and observed significant difference among the substrate. The highest stipe length was obtained in rice straw with wheat straw (3.2 cm) followed by T5 (3.1) and lastly in wheat straw (2.4 cm). Likewise, cap diameter was also found highest from T4 i.e. 7.40 cm followed by T5 (7.27 cm) under similar environment and cultural practices among the substrates (Fig. 2).

Table 1: Relative performance of three different species of *Pleurotus* spp.

***Pleurotus* spp. (Isolate)**	**Spawn run (days)**	**Yield (g) of 1st harvest**	**Yield (g) of 2nd harvest**	**Yield (g) of 3rd harvest**	**Total fresh yield (g/bag)**	**Biological Efficiency (%)**
*Pleurotus*1	18	613	211	23	847	179.22
*Pleurotus*2	17	523	123	31	677	167.8
*Pleurotus*3	19	534	129	35	698	169.24
CD at 5%	NS	36.17	32.19	12.28	36.88	8.79

Substrates	**Mycelial growth in mm at 20□C**				**Mycelial growth in mm at 25□C**				**Mycelial growth in mm at 30□C**			
	3rd day	5th day	7th day	9th day	3rd day	5th day	7th day	9th day	3rd day	5th day	7th day	9th day
SB	9	19	60	70	7	38	64	82.5	16.5	35	50	55
PW	8	35	62.5	69	22	36	60	80.5	11	26.5	57.5	70
RS	14.5	30	48	79.5	13	42.5	68	83.0	19	28.5	33.5	41.5

WS	13.5	29	74.5	85.5	11	40	72.5	84.5	20.5	33.5	37.0	39.5
MS	15	30.5	43	71.5	27	49.5	79.5	81.5	22.5	35.0	44.5	56.0
CD at 5%	1.35	2.56	4.66	2.18	1.01	2.34	2.89	2.11	1.23	1.9	2.11	2.78

Table 2: Mycelium growth of *Pleurotus* Isolate 1 in different substrates at different temperature levels

Table 3: Effect of different substrates on oyster mushroom *(pleurotus* spp.*)* production

Treatment	Spawn run (days)	Primordial formation (days)	Fruiting body formation (days)	No. of fruiting bodies bag^{-1}	Stipe length (cm)	Cap diameter (cm)
T1	16.33	25.00	28.67	58.33	3.0	7.17
T2	18.00	26.33	30.67	49.67	2.9	6.77
T3	17.00	25.67	29.33	52.67	2.7	7.07
T4	15.00	23.33	26.33	59.00	3.2	7.40
T5	15.67	23.67	26.67	54.67	3.1	7.27
T6	14.00	27.67	31.33	37.67	2.4	6.63
CD at 5%	3.30	4.06	4.05	9.05	0.506	0.78

Treatment	Yield of 1st harvest (gm)	Yield of 2nd harvest (gm)	Yield of 3rd harvest (gm)	Total fresh yield (gm bag^{-1})	Dry wt. (20g fresh wt.)	B.E (%)	Moisture content (%)
T1	656.67	191.00	66.33	914.00	2.63	182.80	87
T2	588.67	168.67	48.33	805.67	2.83	161.13	86
T3	591.67	174.00	50.00	813.67	2.37	162.73	88
T4	664.67	197.33	76.67	938.67	2.80	187.73	86
T5	621.67	181.33	52.00	855.00	3.00	171.00	85
T6	368.33	80.33	20.33	469.00	2.53	93.80	87
CD at 5%	78.94	52.49	43.13	95.5	0.979	19.10	4.75

Table 4: Amount of N, P and K in the substrate

Treatments	Nitrogen (%)	Phosphorous (%)	Potassium (%)
RS	1.01	0.27	1.90
RS + WS	1.02	0.19	1.55
CD at 5%	0.002	0.0234	0.035

Table 5: Effect of substrate on protein content of mushroom

Treatment	Protein content (g 100g^{-1} fresh wt.)	Yield of mushroom (500 g bag) in Kg
T1	31.24	1.256
T2	29.68	1.012
T3	28.76	0.987
T4	31.78	1.345
T5	30.98	1.298
T6	32.36	0.876
CD at 5%	1.256	0.0257

Table 6: Cost of materials (10 kg)

Treatment	Cost of spawn	Polypro-pylene cost	Covering plastic (Rs.30 m^{-1})	Rope	Fuel	Straw cost (Rs.3 kg^{-1})	Labour cost	Total cost
T1	150.0	6.00	10.00	5.00	10.00	30.0	125.0	336.0
T2	150.0	6.00	10.00	5.00	10.00	40.0	125.0	346.0
T3	150.0	6.00	10.00	5.00	10.00	50.0	125.0	356.0
T4	150.0	6.00	10.00	5.00	10.00	50.0	125.0	356.0
T5	150.0	6.00	10.00	5.00	10.00	55.0	125.0	361.0
T6	150.0	6.00	10.00	5.00	10.00	60.0	125.0	366.0

Table 7: Benefit from selling of mushroom

Treatment	Total yield (kg)	Rate (Rs. Kg^{-1})	Total sale (Rs.)	Total cost (Rs.)	Benefit (Rs.)
T1	18.28	60.00	1096.80	336.0	760.80
T2	16.10	60.00	966.0	346.0	620.0
T3	16.26	60.00	975.60	356.0	619.0
T4	18.76	60.00	1125.60	356.0	769.6
T5	17.10	60.00	1026.0	361.0	665.0
T6	9.38	60.00	562.80	366.0	196.8

Table 8: BLAST search of the sequence with existing database at NCBI

Alignments | Download | GenBank | Graphics | Distance tree of results

Description	Max score	Total score	Query cover	E value	Perc. ident
Pleurotus ostreatus voucher Pingqu2006 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; a	691	691	95%	0.0	92.55%
Pleurotus sapidus small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal trans	689	689	95%	0.0	92.52%
Eukaryota small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete seque	689	689	95%	0.0	92.53%
Eukaryota small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete seque	689	689	95%	0.0	92.53%
Eukaryota small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete seque	689	689	95%	0.0	92.53%
Pleurotus ostreatus strain 55 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	689	689	95%	0.0	92.52%
Pleurotus ostreatus strain 69 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	689	689	95%	0.0	92.52%
Pleurotus ostreatus strain 82 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	689	689	95%	0.0	92.52%
Pleurotus ostreatus strain 79 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	689	689	95%	0.0	92.52%
Pleurotus ostreatus strain 58 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	689	689	95%	0.0	92.52%

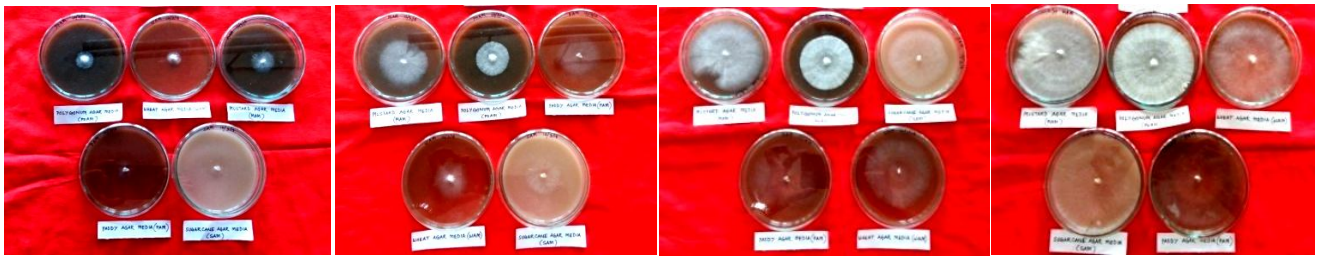

Figure 1. a) Mycelial growth of *Pleurotus*Isolate 1 in different substrates at 20□ C

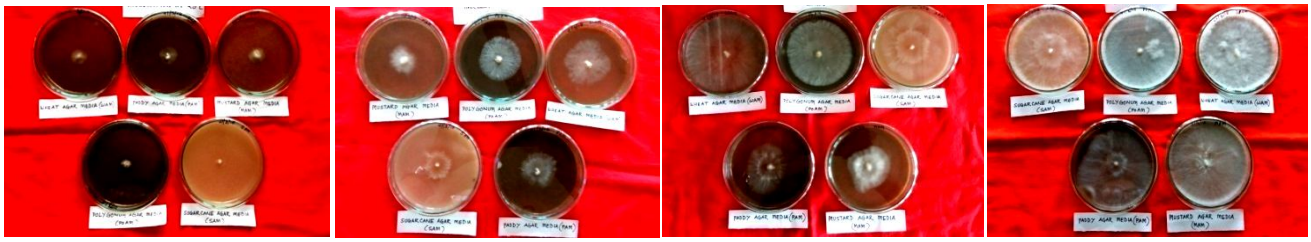

Figure 1. b) Mycelial growth of *Pleurotus*Isolate 1in different substrates at 25□ C

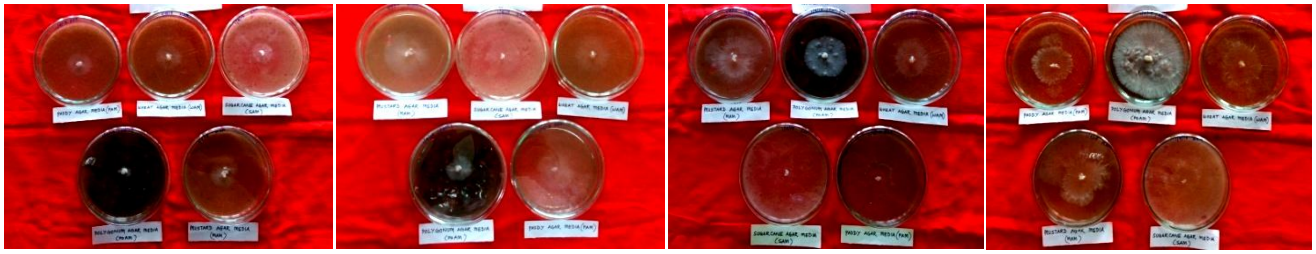

Figure 1. c) Mycelial growth of *Pleurotus* Isolate 1 in different subatrates at 30□ C

T1 **T2** **T3** **T4** **T5** **T6**

Figure 2. Mushroom production in different substrates

Regarding yield of mushroom, highest yield obtained in rice straw with wheat (938g) which is closely followed by rice straw alone (914g) and lowest yield in wheat straw (469g) (Fig. 3). The dry weight was higher in rice + wheat straw followed by rice + sugarcane bagasse. The biological efficiency was highest in rice + wheat straw. The moisture content was varied from 85-87%.

Figure 3. Relationship between fresh yield and biological efficiency in different treatmentss

Relationship between colonization duration and first harvest duration was assessed and presented in Fig. 2. A positive linear relationship was observed between first colonization and first harvest. The result shows that first harvest depends on first colonization duration and more than 26% (R^2= 0.2589) in the first harvest duration may be explained by variation of first colonization duration.

Figure 4. Correlation between spawn run period and days to fruiting body formation in different treatment

Estimation of N, P, K content in selected straw

The above studies indicate that the nature of substrate has a positive effect on the yield of oyster mushroom, so it was considered to assay the nitrogen, phosphorus and potassium content in selected effective substrate viz. Rice straw alone and Rice + wheat straw combination. The standard protocols were followed which was described in methodology and the results are presented in Table 4.

Figure 5. Diagrammatic relationship between substrate and N, P and K content

Table 4 shows that the variation in nitrogen content is negligible whereas the phosphorus and potassium content were higher in rice straw than rice + wheat straw combination. The trend was also graphically presented in Fig. 5.

Impact of substrate on protein content and yield of mushrooms

Mushroom is known for its high protein content. As earlier studies indicate that different substrates have strong influence on the yield parameters, it was considered to investigate the relationship between the various substrates and the protein content of *Pleurotus*. Accordingly, the fresh mushroom was harvested from 1st harvesting date and the protein value was estimated as per methods described earlier. The result is presented in Table 5.

A variation in protein content and nature of substrate was recorded. The highest amount of protein was recorded in wheat straw followed by rice + wheat straw mixture (Table 5) which indicates that wheat straw is best substrate for quality protein mushroom though the yield is low.

Economic analysis

The economic analysis were also carried out in different substrate by calculating the cost of materials and production and presented in Table 6 and Table 7.

The results show that higher economic benefit can be obtained from use of rice straw as substrate for cultivation of *Pleurotus* followed by rice straw + wheat straw. However, Cost benefit ratio is higher in rice straw. The low yield in wheat straw might be due to high moisture content and quality of straw.

Sequencing of *Pleurotus* spp.

To identify the species of *Pleurotus,* the sequence of the fungus was undertaken. The fungal DNA was isolated with DNA isolation kit and assessed for its quality. The DNA was used in PCR to amplify the ITS region using ITS 1 and ITS 4 primers. The 400-900 bp amplification was gel eluted and the product was sequenced by Sanger's method of DNA sequencing. The sequencing results were assembled. The 506 base pairs sequence was derived from sequencing machine and the sequence was subjected to BLAST search in NCBI data base (SOP NO. CRO – 01).

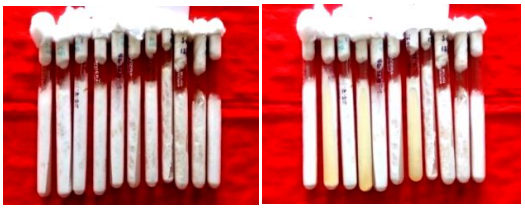

Figure 6. Isolation and pure culture of *Pleurotus* Isolate 1 for sequencing

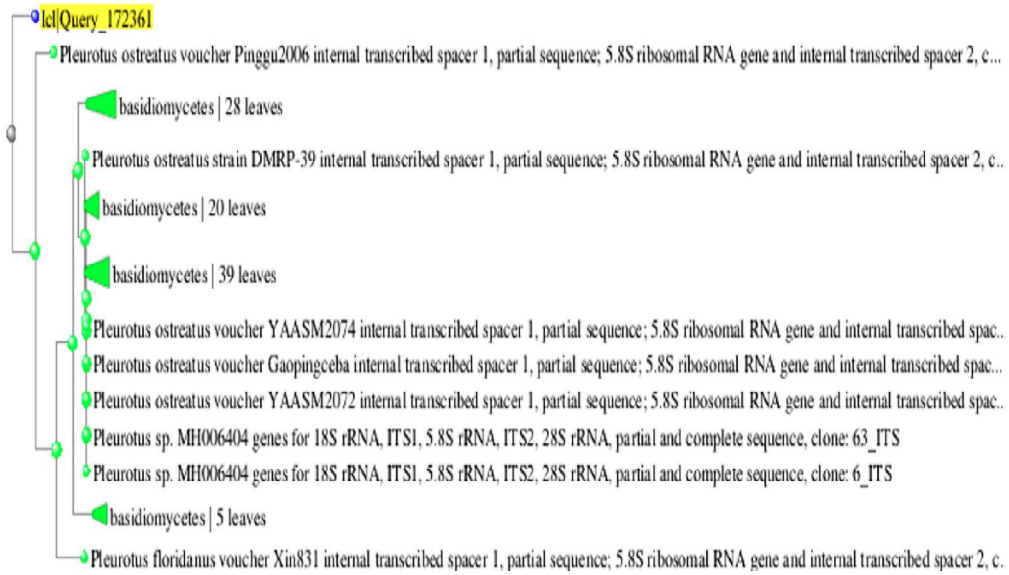

Figure 7 : BLAST results

All the BLAST search results show that maximum similarity of the sequence with *Pleurotus ostreatus* (Table 8). A 95 % coverage of the sequence resulted in 92.55% similarity with fungal strain (Accession No. kx836129).

Oyster mushroom (*Pleurotus* spp.) belongs to the family of Tricholomataceae and is the second widely cultivated mushroom worldwide following the *Agaricus bisporus*. However, Obodai et al. (2003) reported that the oyster mushroom is the third largest commercially produced mushroom in the world market. *Pleurotus* species are popular and widely cultivated throughout the world mostly in Asia, America and Europe because of their simple, low cost production technology and high biological efficiency. Moreover, the interest of oyster mushroom is increasing largely due to its taste, nutrient, and medicinal properties (Sanchez, 2010). *Pleurotus* species can efficiently degrade agricultural wastes and they grow at a wide range of temperatures (Sanchez, 2010). *Pleurotus* species require carbon, nitrogen and inorganic compounds as their nutritional sources. The main nutrients are less nitrogen and more carbon so materials containing cellulose, hemicellulose and lignin (i.e., rice and wheat straw, cotton seed hulls, sawdust, waste paper, leaves, and sugarcane residue) can be used as mushroom substrates (Chang, 1989). Oyster mushroom can grow on a wide variety of substrate. However, the yield and the quality of oyster mushroom depend on the chemical and nutritional content of substrates (Badu *et al.*, 2011).

The present study was conducted in this direction. Among the three different isolates of *Pleurotus* available in the Department of Plant Pathology, Isolate 1 was selected for better yield. The selected *Pleurotus* was tested *in-vitro* in different substrate of locally available materials and observation indicates that 25^0 C is optimum of this mushroom. The effect of substrates on yield parameters indicates that rice and wheat mixture is best. The poor yield in sugarcane bagasse amended substrate and wheat straw is probably due to high sugar and moisture content respectively. These results confirm the earlier observation of (Dubey et al., 2019). The protein content of mushroom, however, was higher in substrate of wheat straw. The sequencing data of selected *Pleurotus* indicates the similarity with *P. ostreatus.*

CONCLUSION

The relative efficacy of three different isolates of *Pleurotus* in rice straw substrate showed Isolate 1 was better in terms of yield and biological efficiency. The *in vitro* studies of five different substrates (rice, wheat, mustard, polygonum, sugarcane bagasse) at three temperature levels (20□ C, 25□ C and 30^0 C) that higher growth was observed at 25^0 C and lowest growth at 30^0 C. At 25^0 C, the highest growth was recorded in wheat straw followed by rice straw; the lowest growth was recorded with mustard straw. The effect of six different substrates viz. Rice straw, wheat straw and sugarcane bagasse and used either as a whole substrates or in combination substrates on yield and other parameters of *Pleurotus* spp. The rice straw mixed with wheat straw was better in terms of yield, biological efficiency as well as economics. This substrate is good with respect to N, P and K content though higher protein content was recorded with wheat straw. The sequencing data confirms the selected strain as *Pleurotus ostreatus.*

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