

OPTIMUM VEGETATIVE GROWTH CONDITIONS AND GENETIC DIVERSITY IN DIFFERENT STRAINS OF *PLEUROTUS SALMONEASTRAMINEUS* L.J.N. VASSILZEVA

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Abstract

This experiment was undertaken to depict the favourable condition for mycelial growth, molecular identification and phylogenetic relationship of the selected strains of *Pleurotus salmoneostramineus*. Suitable temperature and pH were obtained at 25°C and 6, respectively. Mushroom complete, glucose peptone and yeast malt extract culture media were favorable, while Hennerberg and Hoppkins were unfavorable. Dextrin was the best and xylose was the less effective carbon sources. Inorganic nitrogen sources were less effective for the mycelial growth of *P. salmoneostramineus*. The sequences of internal transcribed spacer (ITS) region of selected strains revealed that the total length ranged from 614 to 663 bp. The size of the ITS1 and ITS2 regions varied among the strains. Sequence analysis showed that 5.8S of rDNA sequences were identical. Phylogenetic tree of the ITS region sequences indicated that strains of *P. salmoneostramineus* belong to same cluster. The reciprocal homologies of the ITS region sequences ranged from 98 to 100%. The strains of *P. salmoneostramineus* were also analyzed by random amplification of polymorphic DNA (RAPD) with 20 arbitrary primers. RAPD results suggested that tested strains of *P. salmoneostramineus* were genetically similar with some variations, thus it could be concluded that RAPD and ITS techniques were well competent for detecting the genetic diversity of all tested strains of *P. salmoneostramineus*.

Introduction

Pleurotus salmoneostramineus L.J.N. Vassilzeva commonly known as pink oyster mushroom grows on dead deciduous plants (Murakami and Takemaru 1990). It is becoming popular due to attractive color, sustainable yield, delicious test, and unique texture. *P. salmoneostramineus* is rich in protein, lipids, fiber, carbohydrates, vitamins and contains an abundant amount of essential amino acids (Hossain *et al.* 2007, Yoon *et al.* 2012).

Pink oyster mushroom has complicated morphological variations of basidiospores, resulting in taxonomic confusion and difficulties in delimiting species boundaries (Venturella 2000). It has a tetrapolar system of sexual compatibility and a well defined haplo dikaryotic life cycle. Mycelium cultivation is enhanced by different environmental and nutritional factors as well as propagation of mycelium is an earlier and essential step to cultivate fruiting bodies of mushrooms.

Investigation of genetic diversity is necessary to distinguish genotypes of *P. salmoneostramineus* when seeking traits of interest and to identify strains with high yield potential. Various molecular genetics tools have been introduced for the verification of fungi, such as restriction fragment length polymorphism (RFLP), RAPD, and small subunit ribosomal DNA (SSU rDNA), and ITS sequence analyses. Recent molecular phylogenetic studies have demonstrated that the ITS region of genomic DNA is very useful for assessing phylogenetic relationships at lower taxonomic levels (Park *et al.* 2004, Tuchwell *et al.* 2005, Cho *et al.* 2010).

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Genetic analysis on the mushroom species has shown that RAPD was superior to rDNA sequence-based methods when distinguishing strains within species. RAPD was particularly successful when applied for verifying mushroom strains from various hosts with a wide range of geographical origins (Alam *et al.* 2010). Hence, the present study was undertaken to investigate molecular identification and genomic polymorphism among the selected strains of *P. salmoneostramineus*. Experiments also explored the physical and chemical conditions for the mycelial growth.

Materials and Methods

Five cultivated strains of *Pleurotus salmoneostramineus* such as IUM-1794, IUM-2130, IUM-3705, IUM-3975 and IUM-4146 were obtained from culture collection of DNA Bank of Mushrooms (CCDBM), Division of Life Sciences, Incheon National University, Korea and acquired accession number Incheon University Mushroom (IUM), which was collected from the different ecological regions of Korea, Taiwan and Thailand (Table 1). Experiment was conducted in the laboratory of mycology and plant pathology, Department of Botany, Jahangirnagar University.

Table 1. List of IUM and NCBI GenBank strains used for the studies on molecular phylogenetic relationships.

Oyster mushrooms	IUM strains and sources of collections	NCBI GenBank strains
<i>P. salmoneostramineus</i>	Korea (IUM-1794, 3705 and 3975), Taiwan, IUM-4146 and Thailand, IUM-2130	AY-265843, AY-265844 and AY-636057
<i>P. citrinopileatus</i>	Bangladesh (IUM-4311 and 4312), China (IUM-0557 and 2652), Korea (IUM-3967 and 4502) and Taiwan (IUM-1307 and 4152)	AB-115043, AY-696301 and DQ-77889
<i>P. eryngii</i>	China (IUM-3487, 3501, 3865 and 3920), Japan (IUM-2253 and 2263), Korea (IUM-1550, 2106, 3140 and 3939), and Taiwan (IUM-2126, 2137, 2143 and 2161)	AY-581425, EF-514246, EU-395845
<i>P. nebrodensis</i>	China (IUM-1381, 1495, 2210, 2235, 3061, 3424, 3514, 3914 and 3918)	AY-581429, AY-581430 and AY-581431
<i>P. ostreatus</i>	China (IUM-1932, 2013 and 2036), Korea (IUM-1316, 1319, 1395 and 1721), and Taiwan (IUM-2679, 4143, 4162 and 4171)	AB-115051, AF-423120 and DQ-077884

Six different temperatures such as 10, 15, 20, 25, 30, 35°C and pH 4, 5, 6, 7, 8 and 9 were used to find out the optimum temperature for the mycelial growth of *P. salmoneostramineus*. The measurement of mycelial growth was performed according to the methods described by Alam *et al.* (2010). Ten different culture media (Czapek dox, glucose peptone, glucose tryptone, Hamada, Hennerberg, Hoppkins, Lilly, mushroom complete, potato dextrose agar and yeast malt extract) were used to investigate the mycelial growth of *P. salmoneostramineus*. The compositions of culture media were prepared according to the method described by Alam *et al.* (2009). Carbon and nitrogen sources experiment was performed on the basal medium supplemented with each of ten carbon sources (Dextrin, fructose, galactose, glucose, lactose, maltose, mannose, sorbitol, sucrose and xylose) and ten nitrogen sources (Ammonium phosphate, arginine, ammonium acetate, calcium nitrate, glycine, histidine, methionine, potassium nitrate and urea).

Fresh mycelia of the selected strains of *P. salmoneostramineus* were collected from the 10 days old culture on potato dextrose agar (PDA) medium and were frozen with liquid nitrogen and genomic DNA was extracted according to the procedure of Lee and Taylor (1990).

Genomic DNA was amplified by the RAPD technique (Williams *et al.* 1990) in which 20 sorts of arbitrary 10-base oligonucleotide primers was used to produce amplified fragments. RAPD-PCR reaction was performed using a thermal cycler with an initial denaturation stage of 5 min at 94°C, followed by 35 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 36°C, extension for 2 min at 72°C and a final extension for 7 min at 72°C. RAPD products were electrophoresed on 1.4% agarose gel in 1×TAE buffer for 1.15 hr at 100v, with a 1kb DNA ladder as a size marker and then stained while agitated in an EtBr solution (0.5% µg/ml). The stained gels were visualized and photographed using a UV transilluminator. RAPD bands were recorded as present (1) or absent (0) to generate the data matrix. The similarity coefficients (S) were calculated between isolates across bands for all primers using the formula $S = 2N_{xy} / (N_x + N_y)$, where N_x and N_y are the number of bands shared by the two strains (Nei and Li 1979).

Results and Discussion

Effects of temperature for the vegetative growth of *P. salmoneostramineus* presented in Table 2 shows that the highest average mycelial growth (86.82 mm) was at 25°C and lowest (9.33 mm) at 10°C. Therefore, experimental results indicated that 25°C was the optimum temperature for the mycelial growth of *P. salmoneostramineus*. This experimental finding is comparable to the previous study of Alam *et al.* (2010), who reported that 25°C is the optimum temperature for the mycelial growth of oyster mushrooms.

Table 2. Effect of temperature on the mycelial growth in different strains of *Pleurotus salmoneostramineus*.

Strains	Mycelial growth (mm)*					
	10°C	15°C	20°C	25°C	30°C	35°C
IUM-1794	9.08 ± 1.0	45.67 ± 1.4	62.50 ± 1.0	87.00 ± 0.0	63.92 ± 5.3	24.67 ± 1.1
IUM-2130	8.83 ± 0.8	48.58 ± 4.4	66.67 ± 0.8	87.00 ± 0.0	76.67 ± 0.4	65.42 ± 6.5
IUM-3705	6.25 ± 0.5	35.58 ± 1.4	67.42 ± 0.5	86.08 ± 0.5	67.00 ± 0.0	22.42 ± 6.2
IUM-3975	10.58 ± 1.4	41.58 ± 5.1	58.83 ± 1.4	87.00 ± 0.0	75.83 ± 0.6	75.83 ± 0.6
IUM-4146	11.92 ± 1.2	36.33 ± 3.5	70.92 ± 1.2	87.00 ± 0.0	66.25 ± 0.3	41.08 ± 5.3
MEAN	9.33 ± 0.9	41.55 ± 3.2	65.21 ± 0.9	86.82 ± 0.1	69.93 ± 1.3	45.88 ± 3.9

*Mean of 3 replications.

pH value at the range of 4 - 9 was studied to find out the favourable culture conditions for the mycelial growth. The highest radial growth of mycelium was found at pH 6 (Table 3). This result is in agreement with the data collection reported by Hur (2008) who studied that the cultural characteristics and log-mediated cultivation of *Phellius linteus* and found that the pH value 6 was the best. Present results indicated that *P. salmoneostramineus* grew well at acidic or neutral conditions.

The results of effects of culture media on the mycelial growth in different strains of *P. salmoneostramineus*, indicated that mushroom complete, glucose peptone and yeast-malt extract media were the most favorable, while Hennerberg and Hoppkins were the unfavorable culture media for the vegetative growth of *P. salmoneostramineus* (Table 4). Hur (2008) reported that the

excellent mycelial growth of *P. linteus* was in mushroom complete medium. This result is more or less similar to the present findings.

Table 3. Effect of pH on the mycelial growth in different strains of *Pleurotus salmoneostramineus*.

Strains	Mycelial growth (mm)*					
	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9
IUM-1794	44.17 ± 4.5	76.25 ± 0.4	87.00 ± 0.0	81.17 ± 1.4	76.78 ± 0.4	57.33 ± 2.5
IUM-2130	61.75 ± 2.2	76.50 ± 0.4	86.83 ± 0.3	82.58 ± 0.3	76.67 ± 0.4	67.00 ± 0.0
IUM-3705	49.33 ± 1.6	66.58 ± 0.3	86.83 ± 0.3	77.42 ± 5.0	74.92 ± 0.2	59.25 ± 0.9
IUM-3975	34.75 ± 1.2	65.92 ± 0.3	87.00 ± 0.0	83.78 ± 2.8	74.33 ± 2.0	52.89 ± 1.6
IUM-4146	35.25 ± 0.7	56.92 ± 0.2	86.75 ± 0.5	79.67 ± 4.2	77.00 ± 0.0	37.22 ± 0.7
MEAN	44.45 ± 2.1	68.43 ± 0.3	86.88 ± 0.2	80.92 ± 2.7	75.94 ± 0.6	54.74 ± 1.1

*Mean of 3 replications.

The results of the effect of carbon sources on the mycelia growth in different strains of *P. salmoneostramineus* have been presented in Table 5 indicated that dextrin was the best, while xylose was the most unfavourable carbon sources for the mycelial propagation of *P. salmoneostramineus*. This result is similar to that of Shim *et al.* (2005). Ten different nitrogen sources were assayed and the results showed that arginine was the best, whereas lowest mycelial growth was recorded in histidine (Table 6). These experimental findings are comparable to the previous studies on oyster mushrooms (Alam *et al.* 2010). Shim *et al.* (2005) reported that glycine was the most favorable nitrogen source, this result is not similar to the present findings. They also clarified that histidine was the most unfavorable nitrogen sources for the mycelial growth of *Macrolepiota procera*, which is similar to the present result. Results suggested that carbon and organic nitrogen sources are effective for mycelial propagation of mushrooms.

To study the molecular identification and genetic variation of selected strains of *P. salmoneostramineus*, the ITS region was amplified using ITS1 and ITS4 primers and sequenced. The results indicated that a length polymorphism at the sequence level ranged from 614 to 663 bp. The size of the ITS1 and ITS2 regions varied among the strains from 200 to 244 and 177 to 203 bp, respectively. Total C + G and A + T contents of ITS region varied from 39.5 to 40.9 and 58.5 to 60.5%. Sequence analysis showed that the 5.8S rDNA sequence was identical (154 bp) for all of the tested strains of *P. salmoneostramineus* (Table 7).

Phylogenetic tree based on the nucleotide sequences of the ITS regions in 62 different IUM and NCBI GenBank strains of *P. citrinopileatus*, *P. eryngii*, *P. nebrodensis*, *P. ostreatus* and *P. salmoneostramineus* was constructed by neighbor joining method. The phylogenetic tree was separated into 11 groups (Fig. 1). Eight different including IUM and NCBI GenBank strains of *P. salmoneostramineus* also belong to single cluster. IUM-3975 (Korea) strain is similar to NCBI GenBank strains. A maximum similarity was observed between IUM-4146 (Taiwan) and IUM-1794 (Korea), and IUM-3705 (Korea) and IUM-2130 (Tailand). Reciprocal homologies of the ITS region sequences ranged from 98 to 100%. The sequencing data of the selected IUM strains of *P. citrinopileatus*, *P. eryngii*, *P. nebrodensis*, *P. ostreatus* and NCBI GenBank strain were used as control strain for the comparative studies on phylogenetic relationships with the selected strain of *P. salmoneostramineus*. The results indicated that all the individual species of oyster mushrooms belongs to single major cluster. The results also indicated that most of the individual species of NCBI Genbank strains were very similar to IUM tested strains.

Table 4. Effect of culture media on the mycelial growth in different strains of *Pleurotus salmoneostramineus*.

Strains	Mycelial growth (mm)*											
	CZA	GLP	GLT	HAM	HEN	HOP	LIL	MUC	PDA	YEM		
IUM-1794	42.58 ± 2.6	86.67 ± 0.4	86.25 ± 0.6	80.42 ± 0.6	34.42 ± 1.3	42.92 ± 3.2	68.75 ± 1.6	87.00 ± 0.0	80.58 ± 1.2	87.00 ± 0.0		
IUM-2130	42.08 ± 1.1	87.00 ± 0.0	85.08 ± 1.5	83.17 ± 2.3	33.08 ± 2.4	35.33 ± 0.5	71.67 ± 1.2	87.00 ± 0.0	76.89 ± 3.0	84.33 ± 1.8		
IUM-3705	39.33 ± 2.7	86.08 ± 0.6	83.50 ± 1.6	81.08 ± 1.0	25.92 ± 1.8	30.33 ± 0.8	64.83 ± 4.0	84.92 ± 1.0	80.17 ± 1.0	87.00 ± 0.0		
IUM-3975	40.17 ± 3.2	86.50 ± 0.4	85.89 ± 1.2	81.67 ± 0.7	38.67 ± 1.4	42.75 ± 3.0	69.75 ± 0.3	87.00 ± 0.0	82.50 ± 3.6	86.58 ± 0.8		
IUM-4146	38.92 ± 0.7	86.58 ± 0.5	81.92 ± 1.9	77.92 ± 1.4	28.00 ± 4.5	33.75 ± 0.8	59.00 ± 3.6	87.00 ± 0.0	86.75 ± 0.5	87.00 ± 0.0		
Mean	40.61 ± 2.6	86.57 ± 0.4	84.42 ± 1.4	80.85 ± 1.2	32.02 ± 2.3	37.02 ± 1.7	67.6 ± 2.2	86.58 ± 0.2	81.38 ± 1.9	86.38 ± 0.5		

*Mean of 3 replications. CZA- czapek dox, GLP- glucose peptone, GLT- glucose tryptone, HAM- hamada, HEN- hennerberg, HOP- hopppkins, LIL- lily, MUC- mushroom complete, PDA- potato dextrose agar and YEM- yeast-malt extract.

Table 5. Effect of carbon sources on the mycelial growth in different strains of *Pleurotus salmoneostramineus*.

Strains	Mycelial growth (mm)*											
	Dex	Fru	Gal	Glu	Lac	Mal	Man	Sor	Suc	Xyl		
IUM-1794	72.10 ± 5.5	70.20 ± 8.5	21.30 ± 1.6	35.60 ± 2.0	49.30 ± 3.7	50.40 ± 3.7	66.30 ± 6.4	42.90 ± 6.0	39.70 ± 2.2	13.30 ± 2.1		
IUM-2130	65.80 ± 2.9	71.30 ± 8.0	13.00 ± 0.5	32.80 ± 1.2	35.40 ± 1.7	45.30 ± 2.1	74.30 ± 3.8	37.80 ± 2.6	61.00 ± 3.3	8.30 ± 0.8		
IUM-3705	71.40 ± 3.3	50.00 ± 4.0	13.50 ± 2.1	30.10 ± 2.4	35.30 ± 0.3	47.70 ± 5.0	53.30 ± 10.6	40.50 ± 1.5	38.10 ± 1.9	8.60 ± 1.5		
IUM-3975	76.30 ± 6.4	63.80 ± 5.0	22.80 ± 0.3	45.80 ± 4.4	32.80 ± 1.3	61.40 ± 5.0	71.00 ± 3.8	42.80 ± 3.9	64.80 ± 4.5	10.70 ± 0.6		
IUM-4146	62.40 ± 3.7	42.60 ± 6.5	19.90 ± 3.1	24.50 ± 0.8	24.10 ± 0.8	52.60 ± 14.6	34.70 ± 1.9	40.50 ± 3.5	58.50 ± 9.6	6.10 ± 0.2		
Mean	69.6 ± 4.4	59.57 ± 6.3	18.10 ± 1.5	33.76 ± 2.2	35.38 ± 1.6	51.48 ± 6.1	54.52 ± 5.3	40.9 ± 3.5	52.42 ± 4.3	9.4 ± 1.0		

*Mean of 3 replications, Dex- dextrin, Fru- fructose, Gal- galactose, Glu- glucose, Lac- lactose, Mal- maltose, Man- mannose, Sor- sorbitol, Suc- sucrose and Xyl- xylose.

Table 6. Effect of nitrogen sources on the mycelial growth in different strains of *Pleurotus salmoneostramineus*.

Strains	Mycelial growth (mm)*									
	Amp	Arg	Ama	Can	Gly	His	Met	Pon	Ure	
IUM-1794	31.25 ± 2.1	61.89 ± 2.9	53.75 ± 1.6	21.92 ± 5.4	42.25 ± 4.4	21.25 ± 1.3	25.00 ± 0.6	37.33 ± 0.7	47.58 ± 5.0	
IUM-2130	32.67 ± 0.3	54.25 ± 12.7	55.92 ± 1.8	21.00 ± 0.5	37.08 ± 2.4	18.83 ± 0.8	21.67 ± 0.3	34.22 ± 1.5	38.50 ± 5.0	
IUM-3705	26.83 ± 1.3	48.08 ± 10.8	52.33 ± 3.2	24.42 ± 3.8	36.92 ± 5.7	17.58 ± 1.0	22.58 ± 0.3	34.25 ± 1.9	33.92 ± 1.3	
IUM-3975	42.50 ± 2.1	65.58 ± 1.5	51.33 ± 0.7	41.08 ± 3.8	43.08 ± 2.8	20.58 ± 1.0	25.08 ± 0.8	31.50 ± 1.1	66.08 ± 3.8	
IUM-4146	34.75 ± 1.5	58.17 ± 3.3	53.33 ± 2.3	33.00 ± 1.1	42.25 ± 1.7	18.50 ± 1.0	16.42 ± 0.3	33.00 ± 1.1	47.50 ± 1.0	
MEAN	33.6 ± 1.5	57.59 ± 6.3	53.33 ± 1.9	28.28 ± 2.9	40.31 ± 3.7	19.35 ± 1.0	22.15 ± 0.5	34.06 ± 1.3	46.72 ± 3.2	

*Mean of 5 replications. Amp- ammonium phosphate, Arg- arginine, Ama- ammonium acetate, Can- calcium nitrate, Gly- glycine, His- histidine, Met- methionine, Pon- potassium nitrate and Ure- urea.

Table 7. Nucleotide distribution, ITS1, 5.8S, and ITS2 of rDNA sequences in different strains of *P. salmoneostramineus*.

Strains	Nucleotide distribution (bp)						Sequence information (bp)			
	A	C	G	T	G+C	A+T	ITS1	5.8S	ITS2	Length
IUM-1794	153	98	154	220	40.3	59.7	221	154	177	625
IUM-2130	150	122	129	215	40.7	59.3	200	154	196	616
IUM-3705	145	126	125	218	40.9	59.1	220	154	199	614
IUM-3975	169	132	130	232	39.5	60.5	244	154	203	663
IUM-4146	149	115	142	213	41.5	58.5	233	154	181	619

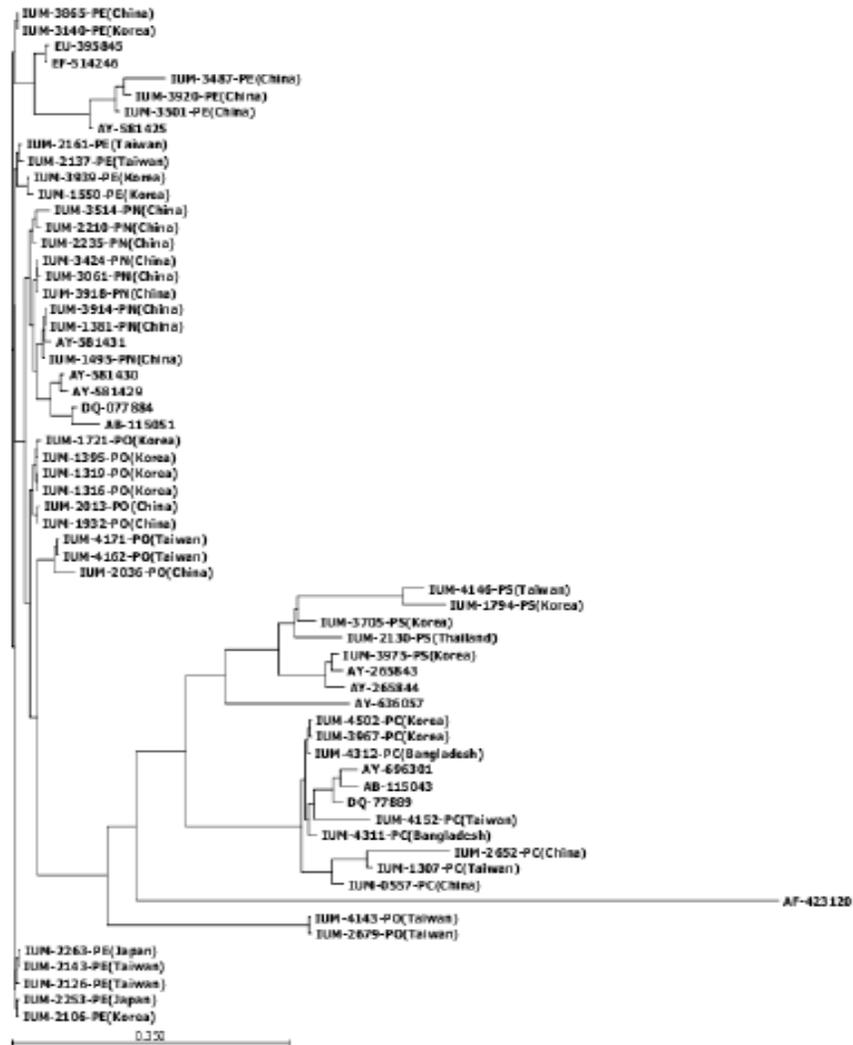


Fig. 1. Phylogenetic tree of sixty-two strains of *Pleurotus* spp. Based on the nucleotide sequences of the ITS regions using neighbor joining method with 1,000 bootstrapping. PC- *P. citrinopileatus*, PE- *P. eryngii*, PN- *P. nebrodensis*, PO- *P. ostreatus* and PS- *P. salmoneostramineus*.

The ITS region is relatively short and can be easily amplified by PCR using universal single primer pairs. Genetic distance exhibited high level of similarity with identical ITS sequences. The size variation was caused by differences in the number of nucleotides, revealing that these strains are clearly distinguishable from each other based on the ecological distribution, substitution, and insertion or deletion polymorphisms of the base position (Ro *et al.* 2007). White *et al.* (1990) reported that ITS sequences are genetically constant or show little variation within species, but vary between species in a genus. The genetic diversity detected within groups is probably due to an efficient gene flow and to a high genetic compatibility within the strains tested. These results are supported and comparable to the study made by Bruns *et al.* (1991) and Zervakis *et al.* (2001).

Twenty sorts' arbitrary 10 base oligonucleotide primers were used to amplify the segments of DNA in five IUM strains of *P. salmoneostramineus*. Among the tested primers, ten primers, namely OPA-1, 2, 3, 4, 7, 8, 9, 10, 11 and 13 were found to be efficient for amplifying the genomic DNA. These efficient primers showed significant band profiles on the tested strains and high possibilities to screening of each strain (Figs. 2, 3 and 4). RAPD-PCR generated distinct multiple products showing considerable variability among the tested strains. The number of amplified bands was variable depending on the primers or the strains. The size of these polymorphic fragments was obtained in the range of 0.2 to 2.3 kb. Polymorphism of DNA bands

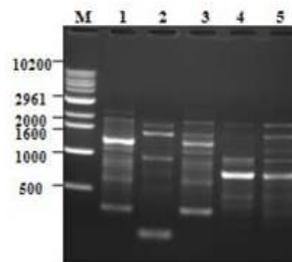


Fig. 2. RAPD profiles in different strains of *P. salmoneostramineus* with OPA-1 primer. M-molecular size marker (1 kb DNA ladder), lane 1- IUM-1794, lane 2- IUM-3705, lane 3- IUM-3975, lane 4- IUM-2130 and lane 5- IUM-4146.

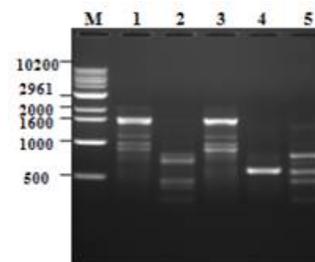


Fig. 3. RAPD profiles in different strains of *P. salmoneostramineus* with OPA-3 primer. M-molecular size marker (1 kb DNA ladder), lane 1- IUM-1794, lane 2- IUM-3705, lane 3- IUM-3975, lane 4- IUM-2130 and lane 5- IUM-4146.

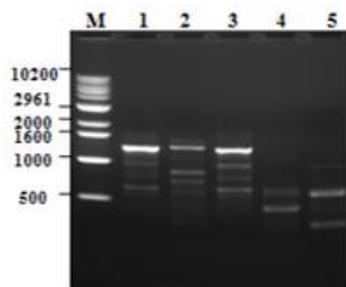


Fig. 4. RAPD profiles in different strains of *P. salmoneostramineus* with OPA-7 primer. M-molecular size marker (1 kb DNA ladder), lane 1- IUM-1794, lane 2- IUM-3705, lane 3- IUM-3975, lane 4- IUM-2130 and lane 5- IUM-4146.

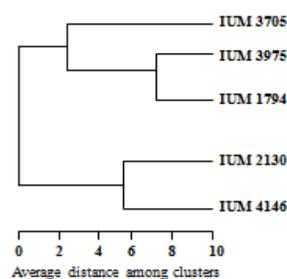


Fig. 5. Dendrogram constructed based on the random amplification of polymorphic DNA markers of *P. salmoneostramineus* strains determined by the average linkage cluster RAPD-PCR generated distinct multiple products showing considerable variability among the tested strains.

showed the same characters in the replication tests. Therefore, if a certain strain is tested for DNA polymorphisms using the same primers, it could be identified whether the strain is the similar or not. To maximize the specificity of polymorphic patterns a combined dendrogram was constructed by using RAPD-PCR amplified bands obtained from the ten RAPD primers. Two putative groups among the 5 strains of *P. salmoneostramineus* were obtained by cluster analysis based on banding patterns and size of amplified products (Fig. 5). Among the five IUM strains, 98-100% similarities were observed between IUM-1794 (Korea), IUM-3975 (Korea), and IUM-3705 (Korea) and IUM-2130 (Thailand) and IUM-4146 (Taiwan). These results are comparable to the findings reported by Alam *et al.* (2010). Results on RAPD analysis were similar from the results obtained by the analysis of ITS region sequences. Strain selection and development of a molecular map of breeding interest would be realistic way to improve yield and quality of pink oyster mushrooms.

RAPD primers are useful tool for clarifying the genetic relationships among strains (Alam *et al.* 2009). Favourable physicochemical conditions for the vegetative growth of *P. salmoneostramineus*, thus the basic information obtained from this study could be useful to make database analysis as well as for farmers and policy makers to make protocol for mass cultivation of mushrooms. Oyster mushroom has complicated morphological variations of basidiospores, resulting in taxonomic confusion and difficulties in delimiting species boundaries. On the basis of the experimental results on molecular characterization it could be concluded that tested strains identified as pink oyster mushroom, which were genetically similar with some variations, RAPD and ITS techniques were well suited for detecting the genetic diversity.

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