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Infra-red Spectra of Different Species of Cultivated Oyster Mushrooms: Possible Tool for Identifying Bioactive Compounds and Establishing Taxonomic Linkage

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ABSTRACT

Mushrooms are macrofungi that serve as a vegetarian source of protein along with various bio-active molecules of primary health importance. The activity of the bio-active molecules range from antioxidant, immunomodulation, hepatoprotection. Cultivated oyster mushrooms are also rich in these components which may be estimated quantitatively by skill intensive 'destructive' chemical techniques. Infra Red spectroscopy provides a non-destructive user friendly technique to quickly assess the presence of bio-active compounds in mushroom species to be used as a quality control measure as this non-destructive tool can be used to segregate mushroom harvest according to availability of bioactive compounds. IR spectra based strain classification and taxa delimitation of mushroom samples are also attempted vis a vis DNA sequence based phylogeny analysis of the same, but no correlation is observed between the two types of phylogeny analysis.

Keywords: IR spectroscopy; Mushroom; Bioactive compound, Macrofungi

1. INTRODUCTION

Mushrooms are fruiting body of macrofungi which are a good source of proteins minerals[1] and non conventional sugars like mannitol and trehalose[2]. Mushroom is a low calorie food, which is particularly beneficial to diabetic and hypertensive patients[3] due to the presence of unconventional sugars and high content of potassium. Moreover mushroom is a vegetarian source of protein which is particularly important with respect to food habits of Indians. Edible mushrooms are now termed as culinary-medical mushrooms as they possess therapeutic effects against pathologic condition[4]. Mushrooms are rich in bioactive compounds polysaccharides called glucans and disaccharides like trehalose. Glucans are glucose based polymers having glycosidic linkages (eg. β-1,3). Mushroom β-D-glucans act as immunomodulators[5], particularly those from *Pleurotus sajor-caju* (β-1,3-glucan) have anti-diabetic activity[6] . Trehalose is a disaccharide found in oyster mushrooms that help in arresting hepatic steatosis and thus provides a therapeutic option for non alcoholic fatty liver disease (NAFLD)[7]. Glucan rich fractions are extracted from mushrooms for therapeutic purposes and prebiotic activity[8]. Likewise, trehalose rich fractions extracted from mushrooms are also used for different therapeutic purpose[9]. The aim of this paper is to give an account of the different bio-active molecules found in cultivated edible oyster mushrooms and to seek for probable correlation among taxonomic relations (derived from IR spectra *vs* DNA sequence) between them, if any. The authors have explored the scope to use IR spectroscopy to segregate mushroom harvests according to availability of bioactive compounds so as to ensure greater profits on extraction of compounds from them. For this purpose, sugars, β-1,3-glucan and chitin (polymer of N-acetyl D-glucosamine) found in cell wall of fungus and chitosan which is deacetylated derivatives of chitin, present in oyster mushrooms are taken as experimental analytes. Chitin and chitosan reportedly decreases the physiological cholesterol pool. The sugar concentrations in the mushrooms are quantified by HPLC and the signature IR spectra of mushrooms are compared to that of standard sugars. The proximity between the signature IR profiles of standard sugars and those in mushroom samples may be useful in assessing the presence or absence of that particular sugar (analyte) within a particular mushroom sample. Similar IR spectra matching strategy is also applied for glucan, chitin and chitosan between mushroom samples and respective standards.

2. MATERIALS AND METHODS

2.1 Oyster Mushroom Cultivation

Oyster mushrooms were cultivated in mushroom cultivation unit of Defence Research Laboratory, Tezpur, India. The following strains were cultivated; *Hypsizygus ulmarius* (Hu), *Pleurotus cornonucopiae* (Pc), *Pleurotus ostreatus* (Po), *Pleurotus flabellatus* (Pf), *Pleurotus sajor-caju* (Psc) and *Pleurotus platypus* (Pp). The fruiting bodies were harvested,

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sundried and ground to powder. The saccharide, chitin and chitosan standards were procured from M/s Himedia, India and β-1,3-glucan standard was procured from M/s Sigma-Aldrich, St. Louis, USA.

2.2 IR Spectra

For transmission infrared spectroscopy, powdered fruiting body was milled with 1 % potassium bromide and pressed into a pellet. FT-IR spectra was recorded on a Bruker instrument (model: Alpha II) from 500 cm^{-1} - 4000 cm^{-1} having resolution limit of 4 cm^{-1} and 20 scans per second. Similarly spectra of standards were also determined in a similar fashion. IR spectrum of *Pleurotus flabellatus* was recorded on a Perkin Elmer Frontier FT-IR at Sophisticated Analytical Instrumentation Centre (SAIC), Tezpur University under similar experimental conditions.

2.3 HPLC Analysis

For HPLC analysis of saccharide content in mushroom, method of Jagannath[10], *et al*. has been followed. Extraction and clean up of dried mushroom powder (1g) was done with 80% ethanol (40 ml) at 80 °C for 30 min. The suspension was subjected to centrifugation at (15,000g; 10 min). Concentration of supernatant was done at 60 °C under low pressure condition and defatting with ethyl ether (10 ml) was done thrice in regular interval. Post concentration of the supernatant at 40°C, the residue was dissolved in water and volume made up to 5 ml. The samples were put to centrifugation (8000g; 20 min) followed by filtering the supernatant via 0.45 μ syringe filter. The extracts were passed via alumina Sep-Pak C-18 cartridge (M/s Waters Corporation, Milford, MA, USA) before subjecting them to HPLC analysis. HPLC system consisted of a HPLC pump (Waters 515), pump control module II, Rheodyne sample injector (4 μm), Carbohydrate High Performance Column (4.6 x 250 mm) and a Waters 2414 Refractive Index Detector. 10 μl extract was injected into the column maintained at 30 °C. The isocratic mobile phase was acetonitrile : water mixture (80:20, v/v) with a flow rate of 1.25 ml/min. Standard sugars were used for calibration. Identification of sugar was made by comparing the relative retention times of sample peaks with standards. Sugar concentration was calculated based on peak area measurements. Results were expressed in g/100 g dry weight, calculated by internal normalisation of the chromatographic peak area.

2.4 ITS based Phylogeny Analysis

Phylogenetic tree of mushroom species was prepared from DNA sequence derived from secondary sourced data from Pubmed. DNA sequence of internal transcribed space region (ITS) with accession numbers (MF459667.1 *Pleurotus flabellatus*, AY540329.1 *Pleurotus sajor-caju* strain S049, KY962504.1 *Pleurotus cornucopiae* isolate P88, MF459668.1 *Hypsizygus ulmarius* and NR_163515.1 *Pleurotus ostreatus* TENN 53662; ITS sequence of *Pleurotus platypus* was not available) were downloaded from the National Centre for Biotechnology Information (NCBI) data base using BLASTN. Sequence alignment was done using MUltiple Sequence Comparison by Log-Expectation (MUSCLE). Evolutionary history was inferred using the Neighbour-Joining method in MEGA X.0[11,12]. The bootstrap consensus tree (500 replicates) is taken to represent the evolutionary history of the taxa in question[13]. The evolutionary distances were computed using the Kimura-2 parameter method[14] (Fig. 1).

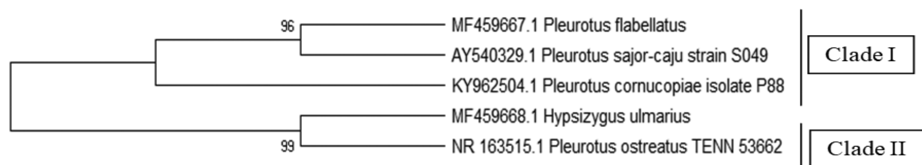

Figure 1. A Neighbour-Joining unrooted phylogenetic tree showing evolutionary relationships of the ITS gene sequences.

2.5 IR-based Phylogeny Analysis

The IR data based phylogenetic tree was prepared by the following method; post smoothing and baseline-correction of spectral data, a spectral range for each strain were exported as Excel files. The wavenumber values were further processed through SPSS Statistics ver. 20 (IBM) software package by applying Ward/Euclidean distance methods[15] as shown in Fig. 2.

3. RESULTS

3.1 IR Spectral Band Delineation

Galactose and trehalose were the sugars found in all the mushroom samples. Other than these two sugars, fructose and sucrose were found in Hu and Pc respectively. The concentrations of different sugars in different mushroom samples are given in Table 1. Table 2 enumerates the IR signature

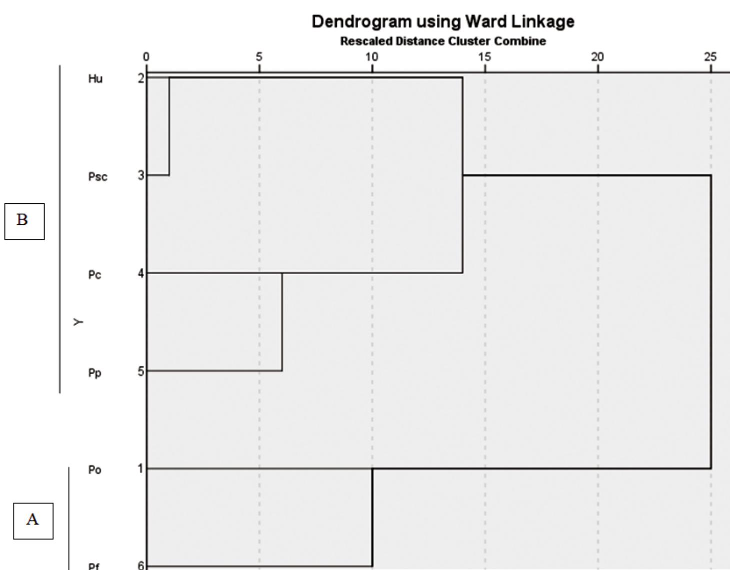

Figure 2. Comparison of assigned bands (cm^{-1}) from the IR spectra of different *Pleurotus* species.

* Dendrogram illustrating the grouping of 06 *Pleurotus* taxa after ward linkage analysis and rescaled distance clustering in the 1750-800 cm^{-1} region. Po and Pf are placed in group A while Pp, Pc, Psc and Hu are placed in group B.

Table 1. Concentration of sugar in different mushroom samples (Mean ± SD, n =3).

Name of mushroom	Name of sugar	Concentration of sugar (g/100 g dry weight)
Pleurotus sajor-caju	Galactose	3.38 ± 0.40
	Trehalose	19.32 ± 0.98
Pleurotus ostreatus	Galactose	3.82 ± 0.36
	Trehalose	12.46 ± 1.0
Hypsizygus ulmarius	Fructose	0.574 ± 0.22
	Galactose	2.52 ± 0.11
	Trehalose	18.60 ± 0.88
Pleurotus platypus	Galactose	1.42 ± 0.04
	Trehalose	7.00 ± 0.20
Pleurotus flabellatus	Galactose	3.54 ± 0.03
	Trehalose	5.801 ± 0.12
Pleurotus cornonucopiae	Galactose	1.96 ± 0.02
	Trehalose	4.99 ± 0.11
	Sucrose	0.58 ± 0.04

of the standard compounds like trehalose dihydrate, β-1,3-glucan, chitin and chitosan. The assigned bands of different oyster mushroom species are compared to that of the standards [as shown in Table 3] in their closest approximate values in cm^{-1}. Mushrooms are rich in glucans and a group of fungal cell walls are made up of β-1,3-glucan. For β-1,3-glucan, the band at 890 cm^{-1} 'glucan band' [β anomer; C-H deformation] is the signature band[16]. This glucan band is found too in chitin and chitosan. The bands between 750-950 cm^{-1} interval correspond to glucans [α or β anomer, C_1-H deformation band][17]. A band at 1747 cm^{-1} (attributed as Amide I band) assigned to cell membrane phospholipids. 1747 cm^{-1} band may either be sharp or appear as a broad shoulder on water bending band, depending upon the moisture levels in the sample[18]. Another two signature bands for glucan are 1642 cm^{-1} (O-H-O bending of bound water) and 1558 cm^{-1} (Amide II band). The origin of the Amide bands (I and II) are as follows, the Amide I band is because of carbonyl stretching vibrations while Amide II is chiefly due to NH bending vibrations[19]. 806cm^{-1} and 952 cm^{-1} are confirmatory bands for Mannan [α (1-6) linked backbone with α (1-3) and α (1-2) linked branches] type glucan and chitin (N-acetylglucosamine based polymer) respectively[20]. Spectra

Table 2. Comparison of assigned bands (cm^{-1}) from the IR spectra of standard compounds of trehalose dihydrate, β-1,3-glucan, chitin and chitosan.

Trehalose dihydrate	β-1,3-glucan	Chitin	Chitosan
3494 (anti stretching H_2O,w)	------	------	------
2948 (C-H stretching, w)	2924 (C-H stretching; Pyranose ring)	------	------
2933 (C-H stretching, w)	------	------	2926 (C-H stretching; Pyranose ring, s)
2902 (CH_2 stretching, w)	------	------	------
2879 (C-H stretching, w)	------	------	------
2351 (C-H stretching, w)	------	------	------
1684 (Deformation H_2O)	------	1624 (sharp, vs)	1664 (Amide I band)
1443 (Deformation CH_2,m)	------	1552 (Amide II Band)	1586 (NH_2 deformation)
1353 (Rocking C-H, m)	1367 (Bending + O-H bending)	1369 (Bending + O-H bending)	1378 (Bending + O-H bending)
1329 (Rocking C-H, w)	1313 (CH_2 deformation + C-O-H bending)	------	------
1237 [C.F.]	1257 [C.F.]	1264 [C.F.]	1250 [C.F.]
1125 [C.F.]	1197 [C.F.]	1203 [C.F.]	1147 (C-H stretching; Pyranose ring) [C.F.]
------	1150 [C.F.]	1150 (C-H stretching; Pyranose ring) [C.F.]	1056 [C.F.]
------	1100 [C.F.]	1116 [C.F.]	1022 [C.F.]
1078 (Torsion CO-H, m) [C.F.]	1044 [C.F.]	1074 [C.F.]	------
995 (C-O stretching, v)	886 (Glucan band β anomer; C-H deformation)	890 (Glucan band β anomer; C-H deformation)	888 (Glucan band β anomer; C-H deformation)
953 (C-O stretching, w)	------	------	------
695 (Torsion CO-H, w)	------	------	------
539 (Torsion CO-H, w)	------	------	------

(s = strong, m = medium, w = weak, vs = very strong and v = variable intensity, anti = antisymmetric and sym = symmetric movements) C.F.= Carbohydrate Fingerprint [generally 1000-1200 cm^{-1} is taken as the carbohydrate fingerprint zone[9].

Table 3. Comparison of assigned bands (cm^{-1}) from the IR spectra of different strains of oyster mushrooms. The band data given in parentheses corresponds to the bands of the standard compounds that are in alignment with bands of the oyster mushroom samples. Bands of oyster mushroom samples without parentheses could not be assigned.

Pleurotus ostreatus (Po)	*Hypsizygus ulmarius* (Hu)	*Pleurotus sajor-caju* (Psc)	*Pleurotus cornonucopiae* (Pc)	*Pleurotus platypus* (Pp)	*Pleurotus flabellatus* (Pf)
----------	3393	3393	----------	------	3400
3271 [3265 vs, vbr O-H str, chitin]	---------	--------	3271 [3265 vs, vbr O-H str, chitin]	3273 [3265 vs, vbr O-H str, chitin]	------
2926 [C-H str pyranose ring; glucan or chitosan]	2930 [2933m, C-H str: chitin]	2926 [2925, C-H str of pyranose ring: chitosan or glucan]	2919 [2925, C-H str of pyranose ring: chitosan or glucan]	2919 [2925, C-H str of pyranose ring: chitosan or glucan]	2923 [2925, C-H str of pyranose ring: chitosan or glucan]
2852 [2855, CH2 str pyranose ring; glucan]	-------	--------	2850 [2855, CH2 str pyranose ring; glucan]	2859 [2855, CH2 str pyranose ring; glucan]	------
2356	2355	-------	2358	-------	-------
2344	------	--------	2340	-------	--------
--------	--------	-------	-------	2315	--------
--------	-------	1740 [1747, Amide I band corresponding to membrane phospholipids]	1747 [1747, Amide I band corresponding to membrane phospholipids]	1743 [1747, Amide I band corresponding to membrane phospholipids]	---------
-------	--------	---------	-----------	---------	1657 [1656m, Amide I band; chitosan]
------	1648 [1642m, br,+ O-H-O bending of bound water in glucan]	1642 [1642m,br, O-H-O bending of bound water in glucan]	--------	1637 [1642m,br, O-H-O bending of bound water in glucan]	---------
1631 [1628 vs, sh; chitin]	---------	---------	--------	--------	---------
----------	----------	---------	1570	--------	-----------
1555 [1558, Amide II band; glucan]	1560 [1560vs Amide II band; chitin]	1550w [1558w, Amide II band; glucan]	1555 [1558w, Amide II band; glucan]	--------	-----------
--------	---------	---------	---------	1546	-----------
--------	1399 [1391m C-H str pyranose ring;chitin]	---------	---------	----------	--------
--------	--------	1380 [1381m, + O-H bending; chitosan]	--------	------------	--------
1373 [1376, O-H bending, glucan]	----------	----------	---------	---------	1376 [1376, O-H bending, glucan]
-------	---------	----------	1367	1369	--------
1311 [1314, C-O-H bending + CH_2 deformation; chitin]	--------	---------	1311 [1314, C-O-H bending + CH_2 deformation; chitin]	1313 [1314, C-O-H bending + CH_2 deformation; chitin]	--------

1248 [carbohydrate fingerprint zone, 1246, PO_2 antisymm str]	1247 [carbohydrate fingerprint zone, 1246, PO_2 antisymm str]	1246 [carbohydrate fingerprint zone, 1246, PO_2 antisymm str]	1239 [carbohydrate fingerprint zone]	1241 [carbohydrate fingerprint zone]	1250 [carbohydrate fingerprint zone, 1246, PO_2 antisymm str]
1199 [carbohydrate fingerprint zone]	------	--------	1199 [carbohydrate fingerprint zone]	-------	1204 [carbohydrate fingerprint zone]
1150 [carbohydrate fingerprint zone]	------	--------	1150 [carbohydrate fingerprint zone]	1150 [carbohydrate fingerprint zone]	1151 [carbohydrate fingerprint zone]
------	------	-------	-------	--------	1074 [carbohydrate fingerprint zone; 1078vs; glucan + 1074s, br; chitin]
------	-------	--------	--------	--------	1043 [carbohydrate fingerprint zone; 1042vs, glucan]
1033 [carbohydrate fingerprint zone; confirms presence of chitin or chitosan]	1033 [carbohydrate fingerprint zone, confirms presence of chitin or chitosan]	1033 [carbohydrate fingerprint zone, confirms presence of chitin or chitosan]	1038 [carbohydrate fingerprint zone]	1027 [carbohydrate fingerprint zone]	------
807 [806; confirmatory band for Mannan type glucan]	-------	----------	805 [806; confirmatory band for Mannan type glucan]	--------	---------

vs = very strong, vbr = vibration, str = stretching, sh= sharp, br= broad.

in the range of 1000-1200 cm^{-1} serves as the carbohydrate fingerprint region and may serve as an indicator of mushroom genus[21].

3.2 Phylogenetic Tree Construction based on IR Data

For IR based phylogeny delineation, the following spectral points and/or regions (cm^{-1}) were selected [1740-1750, 1657, 1650-1640, 1631, 1570, 1569-1560, 1546, 1399, 1380, 1379-1370, 1369-1360, 1320-1310, 1250-1239, 1205-1195, 1151-1150, 1074, 1043, 1035-1025 and 807-805] and dendrogram prepared thereafter (Fig. 3). Group B includes the strains, Pp, Pc, Psc and Hu which are subdivided into two separate clusters. Group A consists of strains Po and Pf. Due to differential IR response in the 807-805 cm^{-1} region, Pc and Po are likely to be linked although they fall under two separate groups. In the wavenumber range 1250-1239 cm^{-1}, all the species record closely spaced characteristic "carbohydrate fingerprint" IR signal, which provides somewhat unique chemical identification mark for different mushroom species belonging to genus *Pleurotus*.

3.3 Phylogenetic Tree Construction based on DNA Sequence Data

For DNA sequence based phylogeny delineation, ITS sequences of all the mushroom species (except *Pleurotus platypus*, due to non availability of its' ITS sequence in NCBI) were subjected to multiple sequence alignment and an unrooted phylogenetic tree consisting of two main branches was obtained. The phylogenetic relatedness among the species studied was deduced from the tree image. The species *Pleurotus flabellatus* was found to be closely related to *Pleurotus sajor-caju* strain (confidence value 96). Similarly *Hypsizygus ulmarius* is closely related to *Pleurotus ostreatus* (confidence value 99). The species *Pleurotus cornucopiae* is distantly related to both *Pleurotus flabellatus* and *Pleurotus sajor-caju* strain. It has been observed form the tree image that the species can be categorised into two distinct clades, Clade I comprising of the species *Pleurotus flabellatus*, *Pleurotus sajor-caju* strain and *Pleurotus cornucopiae* while Clade II comprises of the species, *Hypsizygus ulmarius* and *Pleurotus ostreatus*.

4. DISCUSSION

From the above results it is evident that IR spectra can be used to qualitatively assess the presence of particular classes of bioactive molecules in mushroom samples. 'Glucan band' at 890 cm^{-1} is a signature identification band for β-1,3-glucan, while 806 cm^{-1} and 952 cm^{-1} are confirmatory bands for Mannan type glucan and chitin. Amide I band (1747 cm^{-1}) is a signature band for cell membrane phospholipids. 'Carbohydrate Fingerprint' region at 1000-1200 cm^{-1} serves as indicator of mushroom genus. Thus IR spectra provide us with a suitable non-destructive tool to segregate mushroom harvest according to presence or absence of certain bio-molecules. But

no correlation exists between IR spectra derived taxonomic relation and that derived from DNA sequence. The different groupings and linkages formed in these two types of taxonomic relations (ie. IR spectra derived and DNA sequence derived) are entirely different from each other and do not bear any correlation whatsoever with each other.

5. CONCLUSIONS

It may be concluded that IR spectral data may serve as a 'non-destructive' tool to characterise and/or classify different mushroom species. Hence this procedure can be used as a quality control measure to screen mushroom harvests rich in particular bio-active compounds.

REFERENCES

1. Zhang, Y.; Geng, W.; Shen, Y.; Wang, Y. & Dai, Y.C. Edible mushroom cultivation for food security and rural development in China: Bio-innovation, technological dissemination and marketing. *Sustainability,* 2014, **6**, 2961-2973. doi: 10.3390/su6052961
2. Senatore, F.; Dini, A. & Marino, A. Chemical constituents of some *Basidiomycetes*. *J. Sci. Food Agriculture*, 1988, **45**, 337–345. doi: 10.1002/jsfa.2740450408
3. Guillamón, E1.; García-Lafuente, A.; Lozano M.; D'Arrigo, M.; Rostagno, M.A.; Villares, A. & Martínez, J.A. Edible mushrooms: Role in the prevention of cardiovascular diseases. *Fitoterapia*, 2010, **81**(7), 715-723. doi: 10.1016/j.fitote.2010.06.005
4. Cağlarirmak, N. Chemical composition and nutrition value of dried cultivated culinary-medicinal mushrooms from Turkey. *Int. J. Med. Mushrooms*. 2011, **13**(4), 351-6. doi: 10.1615/intjmedmushr.v13.i4.50
5. Kanagasabapathy, G.; Kuppusamy, U.R.; Malek, S.N.A.; Abdulla, M.A.; Chua, K-H. & Sabaratnam ,V. Glucan-rich polysaccharides from *Pleurotus sajor-caju (Fr.) Singer* prevents glucose intolerance, insulin resistance and inflammation in C57BL/6J mice fed a high-fat diet. *BMC Comp. Altern. Med.* , 2012, **12**, 261. doi: 10.1186/1472-6882-12-261
6. Brian J.; DeBosch Monique.; R Heitmeier.; Allyson L. Mayer.; Cassandra B. Higgins.; Jan R. Crowley.; Thomas E. Kraft.; Maggie Chi.; Elizabeth P. Newberry.; Zhouji Chen.; Brian N. Finck.; Nicholas O. Davidson.; Kevin E. Yarasheski.; Paul W. Hruz. & Kelle H. Moley. Trehalose inhibits solute carrier 2A (SLC2A) proteins to induce autophagy and prevent hepatic steatosis, *Sci. Signal*. 2016, **9** (416), ra21. doi: 10.1126/scisignal.aac5472
7. Andriy Synytsya.; Katerina Mickova.; Alla Synystya.; Ivan Jablonsky.; Jiri Spevacek.; Vladimir Erban.; Eliska Kovarikova. & Jana Copikova. Glucans from fruit bodies of cultivated mushrooms Pleurotus ostreatus and Pleurotus eryngii: Structure and potential prebiotic activity. *Carbohydrate Polymers,* 2009, **76**, 548-556. doi: 10.1016/j.carbpol.2008.11.021
8. Yumi Yoshikawa.; Kanji Matsumoto.; Kazuhisa Nagata.; & Toshio Sato. Extraction of Trehalose from thermally-treated Bakers' Yeast. *Biosci. Biotech. Biochem*., 1994, **58**(7), 1226-1230. doi: 10.1271/bbb.58.1226
9. Vlasta Mohacˇek-Grosˇev.; Romano Bozˇac. & Gerwin J. Puppels. Vibrational spectroscopic characterization of wild growing mushrooms and toadstools. *Spectrochimica Acta Part A,* 2001, **57**, 2815–2829. doi: 10.1016/s1386-1425(01)00584-4
10. Jagannath A.; Raju P.S.; & Bawaa A.S. A two step controlled lactic acid fermentation of cabbage for improved chemical and microbiological qualities. *J. Food Quality*, 2012, **35**, 13–20. doi: 10.1111/j.1745-4557.2011.00427.x
11. Saitou, N. & Nei, M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Bio. Evolution* , 1987, **4**, 406-425. doi: 10.1093/oxfordjournals.molbev.a040454
12. Kumar, S.; Stecher, G.; Li, M.; Knyaz, C. & Tamura, K. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Bio. Evolution*, 2018, **35**, 1547-1549. doi: 10.1093/molbev/msy096
13. Felsenstein J. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, 1985, **39**, 783-791. doi: 10.2307/2408678
14. Kimura M. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences*. J. Molecular Evolution*, 1980, **16**, 111-120. doi: 10.1007/bf01731581
15. Zervakis, G.I.; Bekiaris, G.; Tarantilis, P.A. & Pappas, C.S. Rapid strain classification and taxa delimitation within the edible mushroom genus *Pleurotus* through the use of diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy. *Fungal Biology*. 2012, **116**, 715-728. doi: 10.1016/j.funbio.2012.04.006
16. Michell, A.J. & Scurfield, G. An assessment of infrared spectra as indicators of fungal cell wall composition. *Aust. J. Biol. Sci*. 1970, **23**, 345. doi: 10.1071/BI9700345
17. Zhang, P.; Zhang L & S. Cheng. Chemical structure and molecular weights of α- (1→3)-D-Glucan from Lentinus edodes. *Biosci. Biotechnol. Biochem*. 1999, **63**, 1197. doi: 10.1271/bbb.63.1197
18. Gallagher, W. FTIR Analysis of Protein Structure. https://www.chem.uwec.edu/chem455_s05/pages/manuals/FTIR_of_proteins.pdf) (Accessed on 25 April, 2019).
19. Coates, J. & Sanders, A. A universal sample handling system for FT-IR spectroscopy. *Spectrosc. Eur*. 2000**, 12**, 12.
20. Yoshida, I.; Kiho, T.; Usui, S.; Sakushima, M. & Ukai, S. Polysaccharides in fungi. XXXVII. Immunomodulating activities of carboxymethylated derivatives of linear (1-->3)-alpha-D-glucans extracted from the fruiting bodies of Agrocybe cylindracea and Amanita muscaria. *Biol. Pharm. Bull*. 1996, **19**, 114. doi: 10.1248/bpb.19.114

21. Kacˇurakova, M.; Capek, P.; Sasinkova, V.; Wellner, N. & Ebringerova, A. FT-IR study of plant cell wall model compounds: Pectic Polysaccharides and Hemicelluloses. *Carbohydr. Polym*. 2000, **43**, 195. doi: 10.1016/S0144-8617(00)00151-X

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In the current study, he is involved in planning the work and preparing the manuscript.