**DOI: https://doi.org/10.36036/MR.33.2.2024.161474**
***Mushroom Research* 33 (2) : 135-143, 2024**

# Morphological and molecular characterization of native isolates of Jew's ear mushroom (*Auricularia auricula-judae*) from Southern Kerala and their suitability in different culture media

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## ABSTRACT

*Auricularia auricula-judae* (Jew's ear mushroom), an edible and medicinal mushroom, is widely distributed in Kerala, but is not cultivated or used by the common people. Surveys were conducted in 3 different Agro Ecological Units of southern Kerala to explore the native isolates of Jew's ear mushroom and to study their morphological, microscopic and phylogenetic characters. The suitability of different media for their *in-vitro* culture was also studied. Nucleotide-level sequencing of the isolates using LSU primers confirmed all the collections to be *A. auricula*. All the sequences were deposited in the NCBI gene bank and accession numbers of each culture were obtained. Cultural studies showed that carrot agar medium was the best suited medium for the mycelial growth of Jew's ear mushroom.

**Keywords:** Jew's Ear mushroom, *Auricularia auricula-judae,* Keral, phylogenetic characters, *in-vitro* culture

Jew's ear is a macrofungus that belongs to the order *Auriculariales*. Basidiocarps (fruiting bodies) of this mushroom are brown in colour, viscous and shaped like a human ear. It has a gelatinous and velvety texture. It is edible but is not well recognized in the culinary world. They thrive on wood, particularly older trees. The common term "Judas's ear" was given, followed by the correction "Jew's ear" because of the notion that Judas Iscariot hanged himself from an elder tree. According to folklore, the ears were the resurrected spirit of Juda, all of which were left to remind him of his suicide. Several herbalists have explored *Auricularia auricula-judae* as a medicinal mushroom (Harding, 2008). This species has long been assumed to occur worldwide, but it varies in colour, texture, habitat and microscopic traits. Lowy (1952) regarded it as a temperate species and suspected it to occur in the tropics as well. It has a mild flavour. Wood ear mushrooms are popular for their ability to add texture to soups (Chinese hot and sour soup), stir-fry and use as salad (Verma and Verma, 2017). *A. auricula* polysaccharides (AAP) were reported to be having high antioxidant activity (Fan *et al.,* 2006), high anticoagulant activity (Yoon *et al.,* 2003) and even greater immunostimulatory effect (Kong *et al.,* 2020).

Although, Jew's ear mushroom is highly edible and highly nutritious, its cultivation and consumption in Kerala is not very sustainable due to lack of awareness about this mushroom among the common people. Natural isolates of the fungus can be studied

Recieved on 29-11-2024; Accepted on 04-12-2024
https://epubs.icar.org.in/index.php/MR/article/view/161474

and used for cultivation if shown to be superior in favourable traits. In light of this, this study was carried out to evaluate the morphological, microscopic and phylogenetic characteristics of locally available isolates of Jew's ear mushroom and to interpret their suitability for cultivation in different parts of Kerala.

## MATERIALS AND METHODS

### Collection and observation of native isolates of *A. auricula*

Surveys were conducted, and native isolates of Jew's ear mushrooms were collected from different locations of AEU 8 (Southern Laterites), AEU 9 (South Central Laterites), and AEU 12 (Southern and Central Foothills) of Kerala, *viz*. Kadakkal, Anchal, Kulathuppuzha, Navayikkulam, Vellayani and Nilamel during the North-east and South-west monsoon seasons of 2021-2022. Morphological characters such as weight of sporocarp, colour and texture of the sporocarp, shape of pileus, pileus diameter, stipe length and thickness were recorded in the collected isolates. Microscopic observations of spore, basidia, cystidia and mycelia were also made using stereo microscope.

### Molecular analysis of collected isolates

For confirming the collected isolates of wood ear mushrooms from different locations to be *A. auricula* at species level, molecular characterization of each isolate was done by DNA sequencing using LSU primers. A phylogenetic tree was also constructed using Tree View software.

### DNA isolation and quality check

DNA from collected isolates were isolated using 100 mg of the mycelium. The mycelium was homogenized using liquid nitrogen and DNA was isolated using Nucleospin DNA extraction kit (Macherey-Nagel) following the protocols given by the manufacturer. Agarose gel electrophoresis (0.8 per cent) was used to check the quality of the extracted DNA. 5 µl of DNA was mixed with 1 µl of 6X gel-loading buffer. The gels were observed using a UV transilluminator (Genei) and an image was captured under UV light utilizing a Gel documentation system (Bio-Rad).

### PCR Analysis

For the identification of the collected samples, isolated DNA was used to amplify the large subunit ribosomal RNA (LSU) using universal primers LRoR (forward) (ACCCGCTGAACTTAAGC) (Rehner and Samuels, 1994) and LR7 (reverse) (TACTACCACCAAGATCT) (Vilgalys and Hester, 1990). For the amplification Phire Tissue Direct PCR Master (Thermo Scientific) was used. The reaction mixture (11 µl) contained master mix 5 µl, LRoR primer 0.25 µl, LR7 primer 0.25 µl, nuclease free water 4 µl and 1 µl DNA. The PCR used step 1- 98°C for 30 sec, step 2- 98°C for 5 sec, step 3- 54°C for 10 sec, step 4- 72°C for 15 sec, step 5- 72°C for 60 sec and hold at 4°C. Step 2 to step 4 was repeated 40 cycles for amplification.

For the removal of unwanted primers and dNTPs from the PCR product, 5 µl of PCR product was mixed with 0.5µl of ExoSAP-IT and incubated for 15 minutes at 37! followed by enzyme inactivation at 85°C for 5 minutes.

### DNA Sequencing

Sequencing of DNA was done in a PCR thermal cycler (Gene Amp PCR System 9700, Applied Bio-systems) by using the Big Dye Terminator v3.1 Cycle sequencing Kit (2010). The PCR mix used included 5X sequencing buffer (1.9 µl), forward Primer (0.3 µl), reverse primer (0.3 µl), sequencing mix (0.2 µl), nuclease free water (6.6 µl) and Exosap treated PCR

product (1 µl). Sequencing reaction included initial denaturation at 96°C for 2 min followed by 30 cycles of 96°C for 30 sec and 50°C for 40 sec and final elongation at 60°C for 4 min. The PCR product was cleaned up by mixing D/W (5 µl), 125mM EDTA (0.1 µl), 3M sodium acetate (pH 4.6) (1 µl) and ethanol (44 µl). The mixture was vortexed and incubated for 30 minutes at room temperature and spun at 3700 rpm for 30 minutes. Supernatant was decanted and the pellet was washed twice with 50 µl 70 %. Ethanol followed by centrifugation at 3700 rpm for 20 minutes. The pellet was air dried (White *et al.,* 1990) and sequenced in an ABI 3500 DNA Analyzer (Applied Biosystems).

### Sequence Analysis

The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Alignment and editing of the obtained sequences were carried out using Geneious Pro v5.1 (Drummond *et al.,* 2010).

### Effect of different culture media on the mycelial growth of *A. auricula*

Culturing of the collected isolates was done using standard tissue culture technique. The suitability of various culture media for the *in-vitro* culture of *A. auricula* was assessed using six different media, *viz.* Potato Dextrose Agar (PDA), Yeast Extract Agar (YEA), Malt Extract Agar (MEA), Carrot Agar (CA), Yeast Pepton Mannitol Agar (YPM) and Oat Meal Agar (OMA). Radial mycelial growth and the nature of mycelial growth of the mushroom in each media were recorded at periodic intervals.

## RESULTS AND DISCUSSION

Six native isolates of wood ear mushroom were collected during the survey from Kadakkal (C1), Anchal (C2), Kulathuppuzha (C3), Navayikkulam (C4), Vellayani (C5) and Nilamel (C6) from fallen wood logs of different trees such as Rubber, Teak, Mahagoni, Jack etc. The study was conducted during May, June, October and November months of 2021-22. All the isolates obtained from the survey were lignicolous and gregarious. Almost all the collections were obtained from old woods of fallen trees (Table 1).

Morphological characters like colour, shape, texture and dimensions of pileus and stipe of the collected mushrooms from different locations are listed in table 2. Maximum sporocarp weight was observed in the Nilamel isolate (8.5 g). Average fruiting body weight ranged from 4.2 to 8.5 g. Dimensions of pileus and stipe were also larger in the case of isolates obtained from Nilamel region of AEU 9. Compared to other mushrooms, the stipe of wood ear mushroom was found to be rudimentary, length ranged from 0.2-0.6 cm. The texture of the sporocarp varied from soft and leathery to velvety in nature. The colour of the fruiting bodies also varied from light brown to dark

**Table 1.** Details of native isolates of *Auricularia* under natural conditions

| No. | Location | Habit | Habitat | Latitude and longitude | Substrate | Collection period |
|---|---|---|---|---|---|---|
| C-1 | Kadakkal | Gregarious | Lignicolous | 8.81°N; 76.91°E | Rubber, teak wood | Oct 2021 |
| C-2 | Anchal | Gregarious | Lignicolous | 8.90°N; 76.93°E | Rubber wood | Oct 2021 |
| C-3 | Kulathuppuzha | Gregarious | Lignicolous | 8.90°N; 77.05°E | Mahagony, teak wood | Nov 2021 |
| C-4 | Navayikkulam | Gregarious | Lignicolous | 8.77°N; 76.78°E | Mango, Cashew tree | May 2022 |
| C-5 | Vellayani | Gregarious | Lignicolous | 8.43°N; 76.99°E | Jack, Rubber wood | Oct 21-June 22 |
| C-6 | Nilamel | Gregarious | Lignicolous | 8.82°N; 76.88°E | Wild jack, teak wood | Oct 21-June 22 |

**Table 2.** Morphological characters of Jew's ear mushroom collected from different locations

| No. | Colour of pileus and stipe | Shape of pileus | Wt. of sporocarp (g) | Dia of pileus (cm) | Texture of stipe and pileus | Size of stipe (l x b) (cm²) |
|---|---|---|---|---|---|---|
| C1 | Dark brown | Ear shaped with curved margin | 7.4 | 5.6 | Soft and leathery | 0.4 x 0.3 |
| C2 | Brown | Round to ear shaped | 4.5 | 3.4 | Leathery | 0.2 x 0.2 |
| C3 | Brown | Margin incurved and ear shaped | 5.7 | 2.6 | Soft texture | 0.2 0 x .2 |
| C4 | Dark brown | Ear shaped | 6.5 | 4.6 | Velvety | 0.4 x 0.2 |
| C5 | Light brown | Round to ear shaped | 4.2 | 5.8 | Leathery | 0.3 x 0.2 |
| C6 | Dark brown | Ear shaped | 8.5 | 6.3 | Soft and velvety | 0.6 x 0.5 |

brown. Pilei of all the isolates were ear shaped with some having incurved margins (C1 and C3). Due to its dark brown colour, ear shaped pileus, soft and velvety texture, Nilamel isolate (C6) was found to be superior in case of morphological characters (Table 2). Sporocarps of all growth stages, from primordia to fully mature ones were obtained from all the locations. Primordia were club- shaped rather than the typical ear shape of the mature fruiting bodies of *Auricularia* (Fig. 1).

Study on microscopic characters of *A. auricula* (Table 3) revealed that they possess septate hyphae with clamp connections. Basidia of all the cultures were found to be club/cylindrical in shape with 3 transverse septa and the basidiospores were sausage-shaped (Fig 2). Cystidia was absent in all the collections. Hairs present on the upper surface of the fruiting bodies (abhymenial hairs) were hyaline with pointed to round tips. Dimensions of basidia and mycelia (Fig. 3) were found to be larger in the case

**Fig. 1.** Sporocarps of *Auricularia* collected from different locations during monsoon seasons 2021-22; C1-Kadakkal, C2-Anchal, C3-Kulathuppuzha, C4-Navayikkulam, C5-Vellayani, C6-Nilamel

**Table 3.** Microscopic observations of local isolates of Jew's ear mushroom (*Values are range of 10 observations)

| Collections | Basidia (µm)* | Shape of basidia | Basidiospores (µm)* | Spore shape | Mycelial width (µm)* |
|---|---|---|---|---|---|
| C1 | 59.5-65 x 5.6-6.8 | Cylindrical | 12.15-13.56 x 4.55-5.2 | Allantoid | 0.80-2.76 |
| C2 | 58-63 x 4.8-5.5 | Cylindrical | 12.34-14.67 x 5.6-6 | Allantoid | 0.83-2.52 |
| C3 | 55-61.5 x 4.2-5 | Cylindrical | 14.4-15.8 x 6-6.4 | Allantoid | 0.82-2.61 |
| C4 | 57.6-62 x 5-5.5 | Cylindrical | 12.4-15.2 x 5.7-6.25 | Allantoid | 0.74-2.55 |
| C5 | 54.6-62 x 4.7-5.4 | Cylindrical | 14.15-15 x 5.92- 6.52 | Allantoid | 0.72-2.63 |
| C6 | 60-65 x 5.3-6 | Cylindrical | 13.65-15.4 x 6.15-6.46 | Allantoid | 1.04-2.74 |

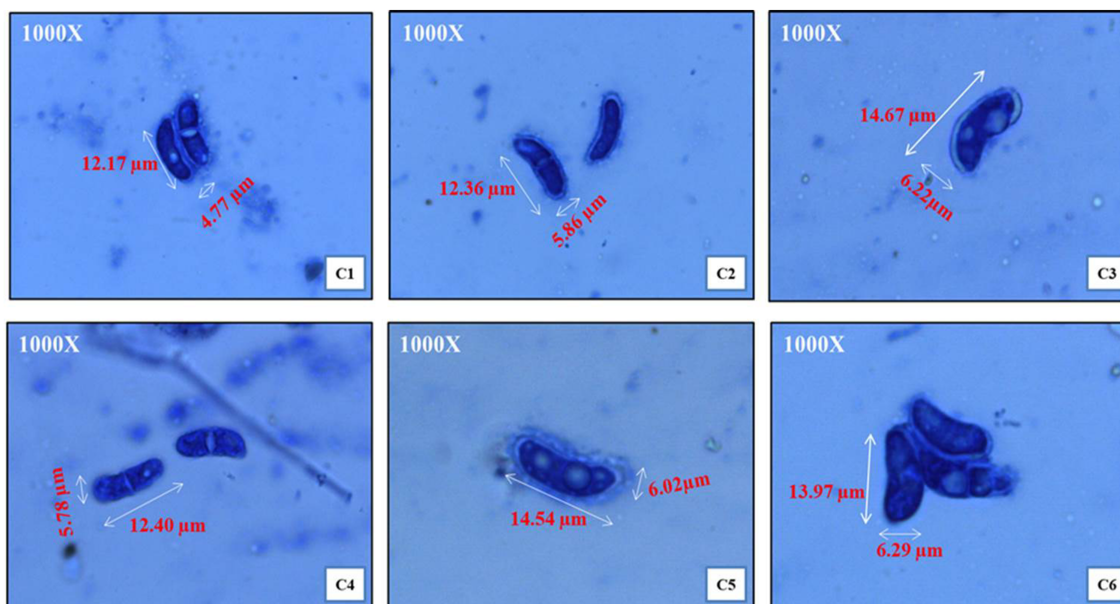


**Fig. 2.** Allantoid shape basidiospores of native isolates of Jew's ear mushroom

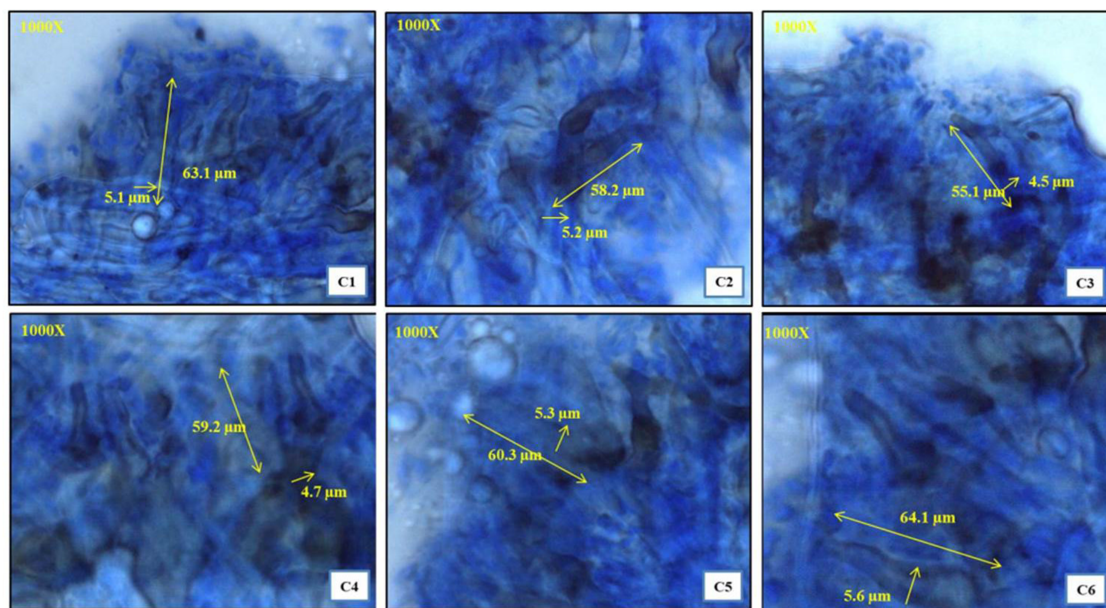


**Fig. 3.** Basidia of *Auricularia* collected from different locations

of C6 (60-65 x 5.3-6 and 1.04-2.74 respectively) and spore size was larger in the case of C3 (14.4-15.8 x 6-6.4 µm).

DNA sequencing of the collected mushrooms and mega blast analysis of the nucleotide sequence confirmed that all the isolates were *A. auricula (*synonym*: A. fibrillifera)* with a per cent identity of 98.27, 98.92, 99.19, 98.92, 99.34 and 98.97 respectively for C1, C2, C3, C4, C5 and C6 (Table 4), (Fig. 4).

Study on the suitability of different culture media for the mycelial growth of *A. auricula* revealed that out of all the six media used in the experiment, Carrot Agar media is the best one. It took 9 days for the completion of mycelial growth in petri dish (9 cm) followed by Oat Meal Agar and Malt Extract Agar (Fig. 5). The least suitable media was found to be Yeast Extract Agar which took around 15 days for the completion of growth in petri dish with the lowest growth rate of 0.79 cm per day. Characteristic brown pigmentation at the centre of white fluffy mycelial

**Table 4.** Results of species level identification of the native isolates by genome sequencing

| Collections | Species | Percentage identity | Gene bank accession number |
|---|---|---|---|
| C1 | *A. auricula-judae* | 98.97 | OP626091 |
| C2 | *A. auricula-judae* | 98.92 | OP626190 |
| C3 | *A. auricula-judae* | 99.19 | OP626750 |
| C4 | *A. auricula-judae* | 98.92 | OP627013 |
| C5 | *A. auricula-judae* | 99.34 | OP627083 |
| C6 | *A. auricula-judae* | 98.97 | OP627103 |

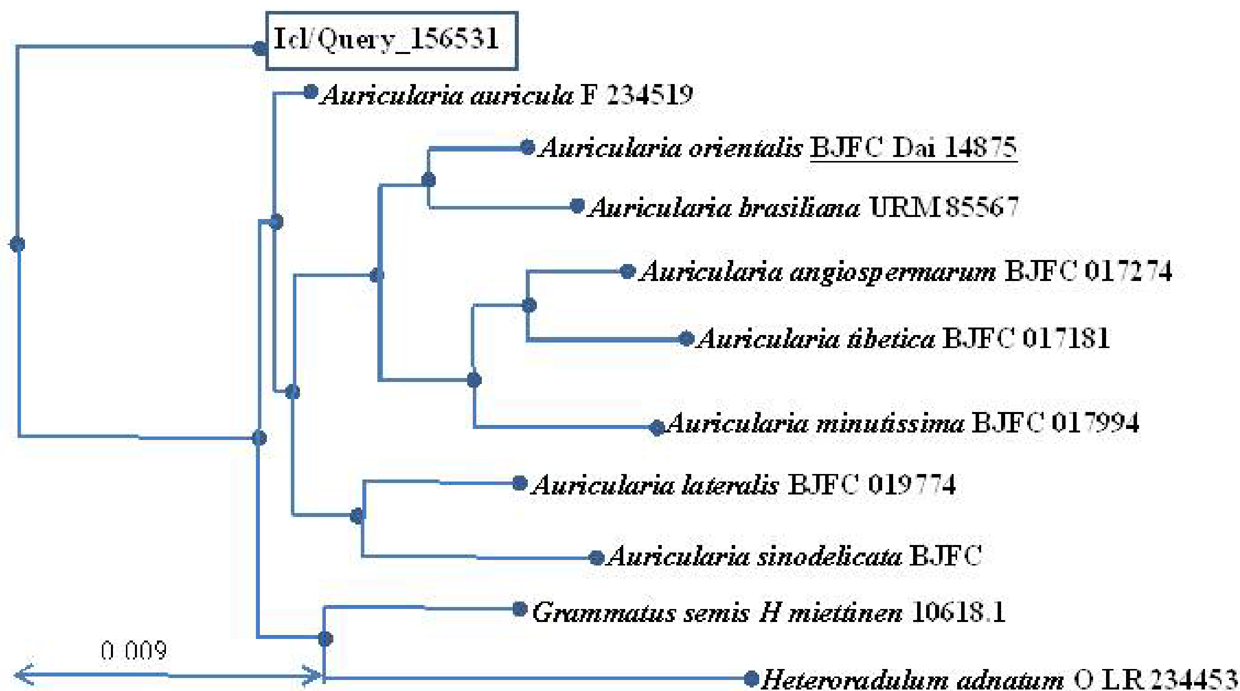


**Fig. 4.** Phylogenetic tree constructed using NCBI BLAST-Tree view software

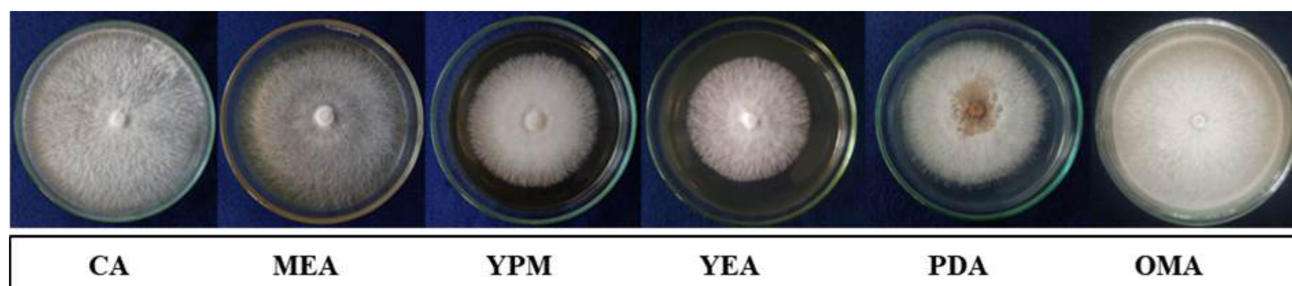


**Fig. 5.** Culture plates showing mycelial growth of *A. auricula* in different media on 9th day

**Table 5.** Mycelial growth of *A. auricula* on different culture media

| Culture media | Radial mycelial growth on 9th day (cm) | Growth rate per day (cm) | No. of days taken for complete growth in petri dish (9cm) | Nature of mycelial growth (9th day) |
|---|---|---|---|---|
| Potato Dextrose Agar | 6.17[d] | 0.98 | 12 | White cottony growth with brown pigmentation at centre |
| Oat Meal Agar | 7.14[c] | 1.1 | 12 | White, thick and cottony growth |
| Yeast Extract Agar | 5.35[f] | 0.79 | 15 | White, moderately thick cottony growth |
| Yeast Peptone Mannitol Agar | 6.03[e] | 0.84 | 14 | White, thick fluffy growth with smooth margin |
| Carrot Agar | 9.00[a] | 1.27 | 9 | White thin growth with smooth margin |
| Malt Extract Agar | 8.44[b] | 1.12 | 10 | Light white thin growth with smooth margin |

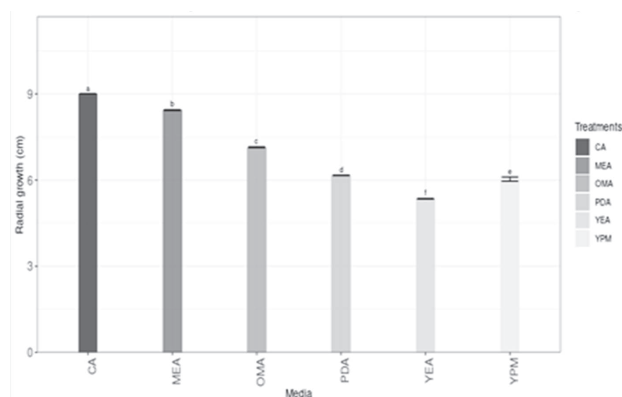


**Fig. 6.** Effect of different media on mycelial growth of A. auricula on 9th day

growth was observed when the mushroom culture was grown in PDA media on 5th day itself, whereas pigmentation of mycelia in other media started only after 15 days of inoculation (Table 5), (Fig. 6).

## DISCUSSION

The fruiting bodies of the mushrooms, collected from different agroecological units, were cartilaginous and jelly, ranging from light to dark brown in colour and resembling the human ear in shape, akin to what was stated in pertinent literature regarding *A. auricula-judae* (Odamtten *et al.,* 2021). The occurrence of the mushroom in wood logs of different trees suggests a list of host plants supporting the growth and development of *A. auricula-judae* in Kerala, most of which are softwood trees. Due to its dark brown colour, ear shaped pileus, soft and velvety texture, Nilamel isolate (C6) was found to be superior over other isolates. Microscopic observations of the basidia, basidiospores and mycelia of the

collected mushrooms, as given in Table 3 fairly agree with the reported data in the pertinent literature by Mohanan (2011) and NCBI (2020). Genome sequencing of 28 sRNA using LSU primers confirms the collected isolates to be *A. auricula* at the molecular level and the phylogenetic tree constructed using BLAST Tree view software provides a list of closely related species of the mushroom. *In-vitro* culture of the mushroom using different media suggested carrot agar media as the best one, providing faster mycelial growth. Carrot agar media, as per the reports, is already proven to be an enhancer of sporulation and mycelial growth in different fungi (Leslie and Summerell, 2006). Characteristic Fig. 4: Culture plates showing mycelial growth of *A. auricula* in different media on 9th day. {CACarrot brown coloured pigmentation in mycelial growth might be due to the production of melanin by the fungus (Sun *et al.,* 2016).

## CONCLUSION

From the study, a detailed understanding of the morphological, microscopic, phylogenetic and cultural (*in-vitro*) characteristics of Jew's ear mushroom was made. Both morphological and molecular characterization confirmed all the collected isolates to be *A. auricula-judae*. Local isolate C6 showed superior character in most aspects and can be used for spawn production and further studies. The lignicolous habit of all the collections suggests sawdust as a suitable substrate for the cultivation of the mushroom. Carrot Agar can be used as the suitable medium for *in vitro* culture.

## ACKNOWLEDGEMENT

Authors are grateful to Kerala Agricultural University for the financial support and the Department of Plant Pathology, College of Agriculture, Vellayani and Rajiv Gandhi Centre for Biotechnology for their technical support.

## REFERENCES

1. Big Dye Terminator v3.1 Cycle sequencing Kit – User Manual. 2010. Applied Biosystems, 72p.

2. Drummond, A.J., B. Ashton, S. Buxton, M. Cheung, A. Cooper, J. Heled, M. Kearse, R. Moir, S. StonesHavas, S. Sturrock, T. Thierer, and A. Wilson. 2010. Geneious v. 5.1, available from 485. http://www.geneious.com.

3. Fan, L., S. Zhang, L. Yu, and L. Ma. 2006. Evaluation of antioxidant property and quality of breads containing *Auricularia auricula* polysaccharide flour. *J Food Chem* **101(3):** 1158-1163.

4. Harding, P., 2008. *Mushroom Miscellany*. 208p. Harper Collins. ISBN 978-0-00-728464-1.

5. Kong, X., W. Duan, D. Li, X. Tang, and Z. Duan. 2020. Effects of polysaccharides from *Auricularia auricula* on the immuno-stimulatory activity and gut microbiota in immunosuppressed mice induced by cyclophosphamide. *Front Immunol* **11:** 595-700.

6. Leslie, J.F. and B.A. Summerell. 2006. *The Fusarium Laboratory Manual*. Blackwell Publishing Ames, IA.

7. Lowy, B. 1952. The genus *Auricularia*. *Mycologia*. **44(5):** 656-92. doi:10.1080/00275514.1952.12024226.

8. Mohanan, C. 2011. *Macrofungi of Kerala*. Kerala Forest Research Institute. 670p. ISBN 978- 81-85041-73-5.

9. NCBI (National Centre for Biotechnological Information). 2020. https:/ncbi.nml.ni.h.gov. Taxonomy ID NCBI: txid29892.

10. Odamtten, G.T., J. Addo, and M. Wiafe-Kwagyan. 2021. Record of medicinal jew's (*Auricularia auricula-judae* (bull.) quél) ear mushroom growing in the Greater Accra region,

Ghana and its possible health values. *Ghana J Sci* **62(2):** 25-35.

11. Rehner, S.A. and G.J. Samuels. 1994. Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. *Mycological Research* **98:** 625-634. https://doi.org/10.1016/S0953-7562(09)80409-7.

12. Sun, S., X. Zhang, S. Sun, L. Zhang, S. Shan, and H. Zhu. 2016. Production of natural melanin by *Auricularia auricula* and study on its molecular structure. *Food Chem* **190:** 801-807.

13. Verma, R.K. and P. Verma. 2017. Diversity of macro-fungi in central India-IV: *Auricularia auricula-judae*, a nutraceutical jelly mushroom. *Van Sangyan* **4(2):** 20-28.

14. Vilgalys, R. and M. Hester. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. *Journal of Bacteriology* **172:** 4238-4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990

15. White, T. J., T. Bruns, S. Lee, and J.W. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In PCR Protocols: A Guide to Methods and Applications, Innis, M.A., Gelfand, D.H., Sninsky, J.J. and White T.J. (eds). pp 315-322 Academic Press, Inc., New York.

16. Yoon, S.J., M.A. Yu, Y.R. Pyun, J.K. Hwang, D.C. Chu, L.R. Juneja, and P.A.S. Mourao. 2003. The nontoxic mushroom *Auricularia auricula* contains a polysaccharide with anticoagulant activity mediated by antithrombin. *J Thromb Res* **112(3):** 151-158.