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MOLECULAR IDENTIFICATION AND ANTIBACTERIAL ACTIVITY OF MACROFUNGUS TO *Trametes sanguineus* (L.)

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Aim. The molecular identification of *Pycnoporus sanguieneus*, a previously morphologically recognised mushroom, was done to see the antibacterial activity against pathogenic bacteria *Staphylococcus aureus and Salmonella typhi*.

Methods. A fragment of the D2 region of 28S rDNA was amplified by PCR, sequenced, and BLAST was performed using the consensus sequence. The maximum identity score was used to build a phylogenetic tree. Agar well diffusion was used to study the antibacterial activity.

Results. Sequencing of a 700 base pair PCR amplicon was carried and a 616 base pair of D2 region of large subunit gene was generated. The 100 blast hits on the D2 region of LSU gene showed similarity to *Trametes sanguineus* voucher PRSC95 (GenBank Accession Number: JN164795.1) based on nucleotide homology and phylogenetic analysis. Antibacterial screening revealed that the crude extract had higher activity on *Staphylococcus aureus*, with a 3 mm to 13 mm zone of inhibition and a 100 µg minimum inhibitory concentration, compared to *Salmonella typhi*. *Salmonella typhi* had a 5 mm to 15 mm zone of inhibition and a 200 µg minimum inhibitory concentration.

Conclusion. According to the obtained result, the morphologically identified mushroom *Pycnoporus sanguies* can be referred to as *Trametes sanguineus*, and it can be used to make antibacterial agents.

Key words: Diseases; mushroom; identification; pathogenic; *Tramates*.

Mushrooms, which are classified as macrofungi with a unique achlorophyllus fruiting body, are members of the Fungi kingdom. A conservative estimate of 1.5 million fungus species has been made, while the real range is 2.2 to 3.8 million [1]. Agaricomycetes is a clade of the Basidiomycota division that contains around 21,000 identified species, accounting for one-fifth of all known fungus. This class includes macrofungi that generate basidiocarps that range in size from small cups a few millimetres wide to massive polypores [2, 3].

Pycnoporus sanguineus is a member of the polyporaceae family of the agaricomycetes order and produces annually basidiocarps. The pileus is dimidiate, flabelliform, reddish orange in colour, zonal with an acute border, and sterile from smooth to wavy. Hymenophore is reddish orange hue and deep round pores [4].

Mushrooms are not only a great source of nourishment, but they also have incredible therapeutic properties. Polysaccharides, lipopolysaccharides, proteins, peptides, glycoproteins, nucleosides, triterpenoids, lectins, and their derivatives are among the bioactive chemicals found in mushrooms [5]. Bioactive metabolites with immunostimulatory, cardiovascular, hepato protecting, antiinflammatory, antidiabetic, antioxidant, and antimicrobial activities accumulate in fruiting bodies and mycelia [5]. Sometimes different species belongs to same family are morphologically so identical that they cannot be differentiate on the basis of morphological characteristics. Thus molecular characterization of species is

the most accurate method for identification of organism [6].

In the present study molecular identification of morphologically similar to macro fungus *Pycnoporus sanguineus* was done to screen its antibacterial activity against pathogenic bacteria *Salmonella typhi*, *Staphyllococcus aureus*.

Materials and Methods

Collection of macrofungus and culture of mycelia

Fresh fruiting bodies of macrofungus were collected from Kaziranga National Park in Assam (26°30′N-26°45′N to 93°08′E-93°36′E) and a voucher specimen (PS No. 834M) was deposited at Plant Identification & Preservation Division of the Department of Botany, Gauhati University, Assam, India and some fruiting bodies were brought to Department of Zoology, Ranchi University, Ranchi. A small portion of fruiting bodies was placed in potato dextrose agar media (dextrose- 20g; potato extract-4g; agar-15g; chloramphenicol-25mg) for mycelia culture.

Isolation of DNA

The DNA was isolated from the cultured mycelia by using and by following the protocol of the Nucleo-pore gDNA fungal/ bacterial mini kit (Genetix Biotech Asia Pvt. Ltd.). 200 mg of fungal mycelia was taken in 1X phosphate buffer saline then placed in a thrashing bead tube. The thrashing bead tube was vortex for 5 minutes and then microcentrifuge at 10000 Xg for one minute. 400 µL of supernatant was transfer to a FB shredder column placed in a collection tube and then centrifuge at 7000 Xg for 1 minute. Filtrate from above step and 200 µL of DNA binding buffer FBB. 800 µL of the mixture was transfer to a FB spin column contain in collection tube and centrifuged at 10000 g for 1 minute. The collected flow was discarded, and the procedure was repeated. In a fresh collection tube, 200 µL of pre-washing buffer was introduced to the FB spin column and centrifuged for 1 minute at 10000 g. 500 µL FBW wash buffer was added to the FB spin column, and it was centrifuged for 1 minute at 10000×g. The FB spin column was put in a new 1.5 micro centrifuge tube, and 100 µL of elution buffer was poured to the centre of the column matrix without wetting the rim. The column was allowed to stand for 1 minute at room temperature before centrifuging at 10000×g for 1 minute to elute pure DNA. A single band of high-molecular weight DNA was seen on a 1.0 percent agarose gel, which was used to assess quality [7].

PCR amplification of D-2 region

The portion of D2 region of 28S rDNA was amplified by PCR from the above isolated genomic DNA using primer-1: NL-1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and primer-2: NL-4 (5′-GGTCCGTGTTTCAAGACGG-3′) [8]. PCR amplification were performed in final volume of 25µL containing 2.5µL 10X assay buffer with $MgCl_2$, 0.19 µL of Taq DNA polymerase (3U/ µl), 2.0 µL dNTPs (10 mM), 1µL primer-1 (10 pM/µL), 1µL primer-2 (10 pM/µL), 16.3 µL deionized water and 2.5µL template DNA (25 ng/µL). The PCR was performed in Bio-Rad's S1000 thermal cycler using the following cycling parameters- Cycle 1: Denaturation (94 °C) 5 Minutes; Cycle 2 — 30: Denaturation (94 °C) 1 minute, primer annealing (45 °C) 1 minute, primer extension (72 °C) 2 minutes; Cycle 32: primer extension (72 °C) 7 minute; and a final single cycle at 72 °C for 5 minutes.

Following amplification, the PCR product was loaded onto a 1% agarose gel with 2 µL of tracking dye in 1X TBE buffer containing 0.5 µg/mL of ethidium bromide, which was produced in 1X TBE buffer containing 0.5 µg/mL of ethidium bromide. The amplified products were electrophoresed at 100 V for 3 to 3.5 hours with cooling in between. After separation, the gel was photographed using a digital camera and a UV trans-illuminator.

Sequencing of PCR amplicon

The PCR amplicon was purified following the instruction of Thermo Fisher Scientific's PureLink™ quick gel extraction ad PCR purification combo kit. With the forward primer (5′-GCGAGTCGTGTTGCTTGATAGTGCAG-3′) and reverse primer (5′-TTGGTCCGTGTTTCAAGACGGG-3′) the purified PCR amplicon was further processed for forward and reverse DNA sequencing reaction [9]. The sequencing was done on an ABI 3730xL genetic analyzer using and following protocol of BigDye® Terminator v3.1 cycle sequencing kit (Applied Biosystem). Sequences generated were analyzed using the aligner software (MegAlign Pro) to generate a consensus sequence of D2 region of large sub unit gene.

Construction of phylogenetic tree and distance matrix

The D2 portion of the 28S rDNA sequence was utilised to perform BLAST against the NCBI genbank database's nrdatabase. The phylogenetic tree was built using MEGA 6.0 programme and fifteen sequences were chosen based on highest identity score. The Neighbor-Joining technique was used to infer the evolutionary relation [10]. The evolutionary relation of the species studied is represented by a bootstrap consensus tree derived from 1000 repetitions [11]. The Kimura 2-parameter technique was used to calculate evolutionary distances [12]. All positions with gaps or incomplete data were removed. The final dataset included 540 locations. MEGA6.0 was used to perform evolutionary studies [13].

Preparation of extract

To avoid microbial contamination, the fresh fruiting body of macrofungus was first rinsed with distilled water before being rinsed with 100% ethyl alcohol (99.8%). The mushrooms were pulverised and sieved after six to seven days of drying in the shade at room temperature. Aqueous extraction was performed on 50 g of fine powder using the Soxhlet extraction chamber and 300 mL methanol in a boiling flask as the extraction solvent. For appropriate dehydration, the extract was filtered, concentrated, and dried in a rotary flash evaporator set at 45 °C, and the dried extract was kept in airtight containers at room temperature for further research [14].

Mycochemical screening

The aqueous extract of the macrofungus was screened to determine the presence of different compounds using reagents such as molisch's reagent, anthrone reagent, bradford's reagent, dragendorff's reagent, H_2SO_4, folin-ciocalteau reagent, HCL, NaOH following the protocol of Dandapat et al. [15]. Tests were done in triplicates. Results were interpreted as: (+) if chemicals are present in trace amount and (–) if chemicals are absent.

Pathogenic bacteria

Salmonella typhi MTCC-3216 and *Staphylococcus aureus* MTCC-3160 used during the present experiment were procured from Microbial Type Culture Collection and Gene Bank (MTCC), CSIR-Institute of Microbial Technology, Chandigarh, India.

Agar well diffusion assay for antibacterial screening

Agar well diffusion assay is broadly used to study the antibacterial activity of crude extract obtained from plant materials or fungi [17]. The culture media includes 10 g peptone, 10 g NaCl and 5 g yeast extract, 20 g agar in 1000 mL of distilled water. Bacterial stock cultures were inoculated in broth medium for growth at 37 °C for 18 hours. The agar plates with wells were made and each plate was inoculated with 18 hours old cultures (100 L, 10^{-4} cfu) that were equally distributed throughout the plate. The wells were filled with varying concentrations of extracts after 20 minutes. Gentamycin treated control wells were also made. The diameter of the inhibitory zone was measured after 24 hours of incubation at 37°C.

Results

The cultured mycelia (Fig. 2) from the collected macrofungus (Fig. 1, ***A*** and ***B***) showed morphological similarity with the macrofungus *Pycnoporus sanguineus*. The pileus of fungus showed morphological characters such as sessile, thin, semicircular, flabelliform, reddish orange colour, zoned with acute margin etc.

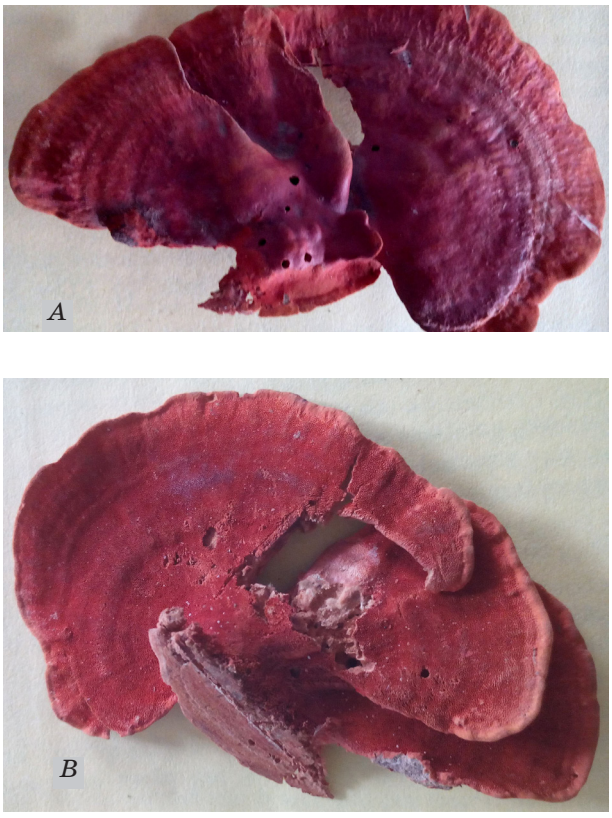

Fig. 1. ***Pycnoporus sanguineus:*** *A* — Dorsal view of basidiocarp of collected macrofungus; *B* —Ventarl view

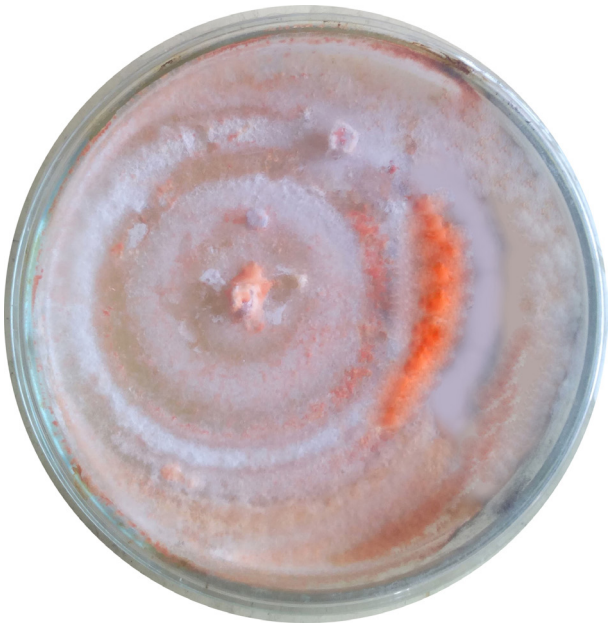

Fig. 2. **Cultured mycelia from collected macrofungi**

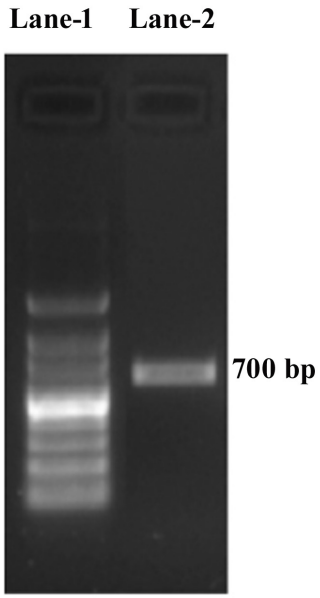

Fig. 3. **1% agarose gel showing 700 bp amplicon (D2 region) of 28S rDNA**

Table 1. **Nucleotide sequence of D2 region of PCR amplicon of mushroom PS No. 834M**

Data obtained with DF primer (573bp)	CTGCGAGTGAAGCGGGAAAAGCTCAAATTTAAAATCTGGCGGTCTTTGGCCGTCC-GAGTTGTAGTCTGGAGAAGTGCTTTCCGCGCTGGACCGTGTATAAGTCTCTTGGAA-CAGAGCGTCATAGAGGGTGAGAATCCCGTCTTTGACACGGACTACCAGTGCTTT-GTGATGCGCTCTCAAAGAGTCGCGTTGTTTGGGAATGCAGCGCAAAATGGGTGGT-GAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGT-GAGGGAAAGATGAAAAGCACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCT-GAAAGGGAAACGCTTGAAGTCAGTCGCGTTGTCCGGGACTCAGCCTTGCTTCGGCTTG-GTGCACTTTCCGGATGACGGGCCAGCATCGATTTTGACCGCTGGAAAAGGGCTGGAG-GAATGTGGCACCTTTTGGTGTGTTATAGCCTCCAGTCGCATACGGCGGTTGGGATC-GAGGAACGCAGCACGCCTTACGGCGGGGGTTCGCCCACCTTCGTGCTTAGGATGCTG-GCATAATGGCTTTAAA
Data obtained with DR primer (572bp)	CCCCCGCCGTAAGGCGTGCTGCGTTCCTCGATCCCAACCGCCGTATGCGACTGGAGGC-TATAACACACCAAAAGGTGCCACATTCCTCCAGCCCTTTTCCAGCGGTCAAAATCGAT-GCTGGCCCGTCATCCGGAAAGTGCACCAAGCCGAAGCAAGGCTGAGTCCCGGACAAC-GCGACTGACTTCAAGCGTTTCCCTTTCAGCAATTTCACGTACTGTTTAACTCTCTTTC-CAAAGTGCTTTTCATCTTTCCCTCACGGTACTTGTTCGCTATCGGTCTCTCGC-CAATATTTAGCTTTAGATGGAATTCACCACCCATTTTGCGCTGCATTCCCAAA-CAACGCGACTCTTTGAGAGCGCATCACAAAGCACTGGTAGTCCGTGTCAAAGAC-GGGATTCTCACCCTCTATGACGCTCTGTTCCAAGAGACTTATACACGGTCCAGCGCG-GAAAGCACTTCTCCAGACTACAACTCGGACGGCCAAAGACCGCCAGATTTTAAATTT-GAGCTTTTCCCGCTTCACTCGCAGTTACTAGGGGAATCCTTGTTAGTTTCTTTTCCTC-CGCTTATTG
Consensus Sequence (616bp)	CAATAAGCGGAGGAAAAGAAACTAACAAGGATTCCCCTAGTAACTGCGAGT-GAAGCGGGAAAAGCTCAAATTTAAAATCTGGCGGTCTTTGGCCGTCCGAGTTG-TAGTCTGGAGAAGTGCTTTCCGCGCTGGACCGTGTATAAGTCTCTTGGAACAGAGCGT-CATAGAGGGTGAGAATCCCGTCTTTGACACGGACTACCAGTGCTTTGTGATGCGCTCT-CAAAGAGTCGCGTTGTTTGGGAATGCAGCGCAAAATGGGTGGTGAATTCCATCTA-AAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGAT-GAAAAGCACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAAC-GCTTGAAGTCAGTCGCGTTGTCCGGGACTCAGCCTTGCTTCGGCTTGGTGCACTTTC-CGGATGACGGGCCAGCATCGATTTTGACCGCTGGAAAAGGGCTGGAGGAATGTG-GCACCTTTTGGTGTGTTATAGCCTCCAGTCGCATACGGCGGTTGGGATCGAGGAACG-CAGCACGCCTTACGGCGGGGGTTCGCCCACCTTCGTGCTTAGGATGCTGGCATAATG-GCTTTAAA

In the present study, PCR amplicon of D2 region of the 28S rDNA obtained from the mycelia of morphologically identified mushroom (PS No. 834M) was amplified with DF and DR primers. The size of the amplified DNA amplicon is approximately 700 bp presented in Figure 3 [Lane 1: 1 Kb DNA Ladder, Lane 2: 700 bp amplicon (D2 region) of *28S* rDNA]. On sequencing a 700 bp amplicon of the D2 region, a forward sequence of 573 bp, a reverse sequence of 572 bp, and a consensus sequence of 616bp were found (Table 1).

The 28S rDNA gene of the macrofungus PS No. 834M shared 100 percent identity with four macrofungi species namely *Trametes sanguinea* voucher Cui8015 (Accession: KC848413.1), *Trametes sanguinea* voucher PRSC95 (Accession: JN164795.1), *Trametes sanguinea* voucher MEL: 2382815 (Accession: KP012695.1) and *Trametes sanguinea* voucher MEL: 2382627 (Accession: KP012989.1) but among them *Trametes sanguinea* voucher PRSC95 and *Trametes sanguinea* voucher Cui8015 showed 1077 manimum score (Table 2).

The phylogenetic tree (Fig. 5) was generated from the aligned sequences of *Trametes* species of macrofungi obtained from NCBI GenBank (Table 2). The phylogenetic tree shows the gene of D2 region of 28S rDNA of *Trametes sanguine* (PS No. 834M) showed very relation with *Trametes sanguine* voucher PRSC95 as well as with the other *Trametes sanguine* vouchers. A similar result also found in the genetic distance matrics (Table 3) of the *Trametes sanguine* vouchers with 100% maximum ident. The culture mycelia of macrofungi, which was labeled as PS No. 834M was showing highest similarity to *Trametes sanguine* voucher PRSC95 (Gen Bank accession number: JN164795.1) based on nucleotide homoginety and phylogenetic analysis.

Mycochemicals includes wide range of primary as well as secondary metabolites. In the present study the mycochemical screening of macrofungus *Trametes sanguine* showed the presence of bioactive metabolites such as carbohydrate,lipid, protein, tannin, saponin, tannin,flavonoid, phenol,alkaloid etc (Table 4).

Table 2. **BLAST result profiles of the 28S rDNA gene of D2 region of the mushroom and consensus sequence producing significant alignments**

Accession	Description	Max score	Total score	Query coverage, %	E value	Max ident, %
KF513171.1	*Trametes sanguine*a strain MX5	1127	1127	100.0	0.0	99.0
LN774882.1	*Trametes cinnabarina*	1120	1120	100.0	0.0	99.0
AB733320.1	*Trametes cinnabarina*	1110	1110	100.0	0.0	99.0
HF937258.1	*Trametes cinnabarina*	1099	1099	100.0	0.0	99.0
AF261536.1	*Trametes cinnabarin*a strain DAOM72065	1090	1090	100.0	0.0	99.0
KC848413.1	*Trametes sanguine*a voucher Cui8015	1077	1077	94.0	0.0	100.0
JN164795.1	*Trametes sanguine*a voucher PRSC95	1077	1077	94.0	0.0	100.0
KP012695.1	*Trametes sanguine*a voucher MEL:2382815	1064	1064	93.0	0.0	100.0
KP012989.1	*Trametes sanguine*a voucher MEL:2382627	1064	1064	93.0	0.0	100.0
AB733344.1	*Trametes hirsut*a	1062	1062	93.0	0.0	100.0
DQ208417.1	*Trametes versicolo*r strain C-4	1011	1011	100.0	0.0	96.0
DQ208416.1	*Trametes versicolo*r strain C-3	1011	1011	100.0	0.0	96.0
AY684159.1	*Trametes versicolor* isolate AFTOL-ID768	1011	1011	100.0	0.0	96.0
AY515342.1	*Trametes conchifer* strain SFC 99100343	1011	1011	100.0	0.0	96.0
AB733330.1	*Trametes versicolo*r	1009	1009	100.0	0.0	96.0

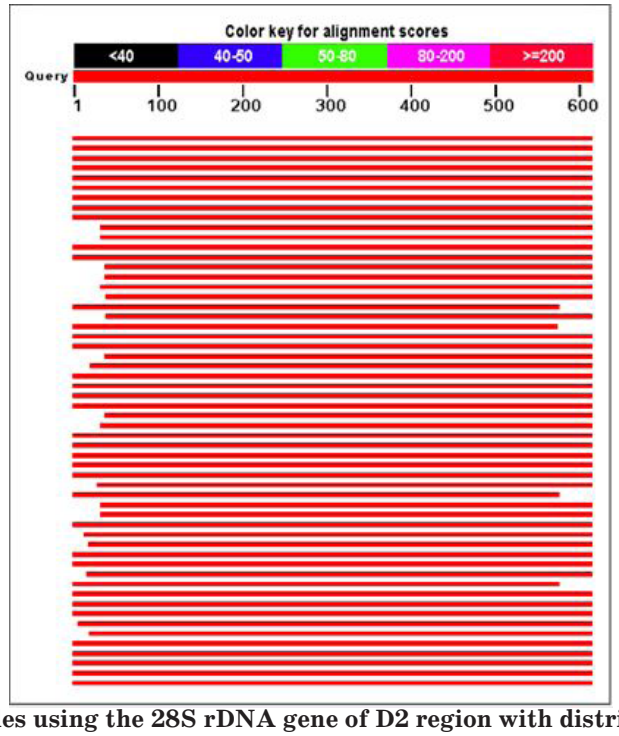

Fig. 4. **BLAST result profiles using the 28S rDNA gene of D2 region with distribution of 100 blast hits on the query sequence**

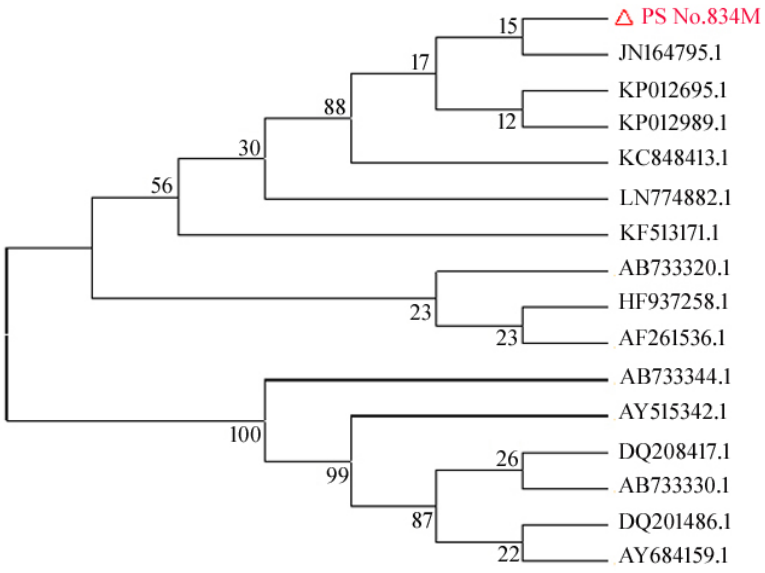

Fig. 5. **Evolutionary relationships among the listed macrofungi**

In the present study *Trametes sanguine* extract showed high antibacterial activity against *S. typhi* and *S. aureus* (Table 5, 6 and Figure 6). The extract showed highest ZOI against *S. typhi* (15 mm) but the high MIC was observed against *S. aureus* (100µg/mL). However the antibiotic Gentamycin showed very high antibacterial activity against both the bacteria but the extract also showed moderate antibacterial activity compared to antibiotic.

Discussion

Only the morphological characters used in the identification and classification are time consuming and often carry inadequate information for exact strain identification.

Table 3. **Distance matrix (genetic distance) among species of the mushroom**

		1	2	3	4	5	6	7	8	9	10	11
PS No. 834M	1		0.0025	0.0025	0.0035	0.0036	0.0044	0.0000	0.0000	0.0000	0.0000	0.0070
KF513171.1	2	0.0037		0.0000	0.0025	0.0025	0.0035	0.0025	0.0025	0.0025	0.0025	0.0066
LN774882.1	3	0.0037	0.0000		0.0025	0.0025	0.0035	0.0025	0.0025	0.0025	0.0025	0.0066
AB733320.1	4	0.0075	0.0037	0.0037		0.0025	0.0035	0.0035	0.0035	0.0035	0.0035	0.0072
HF937258.1	5	0.0075	0.0037	0.0037	0.0037		0.0024	0.0036	0.0036	0.0036	0.0036	0.0067
AF261536.1	6	0.0112	0.0075	0.0075	0.0075	0.0037		0.0044	0.0044	0.0044	0.0044	0.0066
KC848413.1	7	0.0000	0.0037	0.0037	0.0075	0.0075	0.0112		0.0000	0.0000	0.0000	0.0070
JN164795.1	8	0.0000	0.0037	0.0037	0.0075	0.0075	0.0112	0.0000		0.0000	0.0000	0.0070
KP012695.1	9	0.0000	0.0037	0.0037	0.0075	0.0075	0.0112	0.0000	0.0000		0.0000	0.0070
KP012989.1	10	0.0000	0.0037	0.0037	0.0075	0.0075	0.0112	0.0000	0.0000	0.0000		0.0070
AB733344.1	11	0.0265	0.0226	0.0226	0.0265	0.0226	0.0226	0.0265	0.0265	0.0265	0.0265	

Table 4. **Mycochemical composition of aqueous extract of fruiting body of** *Trametes sanguine*

Mycochemicals	Present/Absent in extract
Carbohydrate	+
Glycosides	+
Protein	+
Alkaloid	+
Steroid	+
Triterpene	+
Flavonoid	+
Tannin	+
Lipid	+
Saponin	+
Phenol	+

Note: "+" — present; "–" — absent.

Morphological characterization also needs accuracy of basic knowledge on the phenotypic characters of mushrooms [17].

Due to certain variable sections, the nuclear D2 region of the 28S RNA gene is commonly utilised in phylogenetic research, and the 28S RNA gene also contains core portions that are well conserved and valuable for taxonomic purposes. Divergent D-domains or expansion segments are terms used to characterise the highly preserved core segment [6, 18]. The coexistence of variability and conserved regions along with the 28S gene makes the D-region pertinent to estimate relationships among species on the basis of phylogeny. Since variation in sequences provide information about phylogeny, whereas the conserved organization easily identify the homology [6, 18.

The D-region fragment ranges between 500 to 700 base pair have the high probability of identification for the wide range of eukaryotes [19]. It has been reported that only a small segments of the whole DNA material are sequenced to get the consensus sequence [20]. The internal transcribed spacer (ITS) sections of rDNA have been described as the most widely employed genomic for fungal molecular characterization. It was previously reported that the ITS segments of rDNA was utilized to identify macrofungal species of the genus *Tramates* with 96% to 99% alikeness to genes of Tramates species in NCBI GeneBank [21]. In another study nucleic acid sequence of D1and D2 region of the 28s rDNA and the ITS region of large subunit were used for the identification of filamentous fungi and reported the DNA section of D1 and D2 region and ITS region score 99% to 100% similarity with the existing gene sequence in the database [8].

Mushrooms of the genus Trametes are polypore and have therapeutic properties for the liver, kidneys, and heart, as well as immune system support [22]. Polypores are global mushrooms that have been extensively studied for their potential to combat multidrug-resistant infections [23].

Secondary metabolites such as sesquiterpenes, other terpenes, steroids, anthraquinones, polysachharides, and quinolines, oxalic acid, and certain peptides obtain from higher fungi have been shown antibacterial action against pathogenic

Table 5. **Zone of inhibition (ZOI in mm) and minimum inhibitory concentration (MIC in µg) of *Trametes sanguine* extract against the tested bacteria**

Concentration of extract and Bacteria	25 µg	50 µg	100 µg	200 µg	400 µg	800 µg	MIC µg
S. typhi	0	0	0	5	12	15	200
S. aureus	0	0	3	5	9	13	100

Table 6. **Zone of inhibition (ZOI in mm) and minimum inhibitory concentration (MIC in µg) of Gentamycin against the tested bacteria**

Concentration of Gentamycine Bacteria	25 µg	50 µg	100 µg	200 µg	400 µg	800 µg	MIC µg
S. typhi	2	13	16	21	25	27	25
S. aureus	13	18	21	25	27	34	25

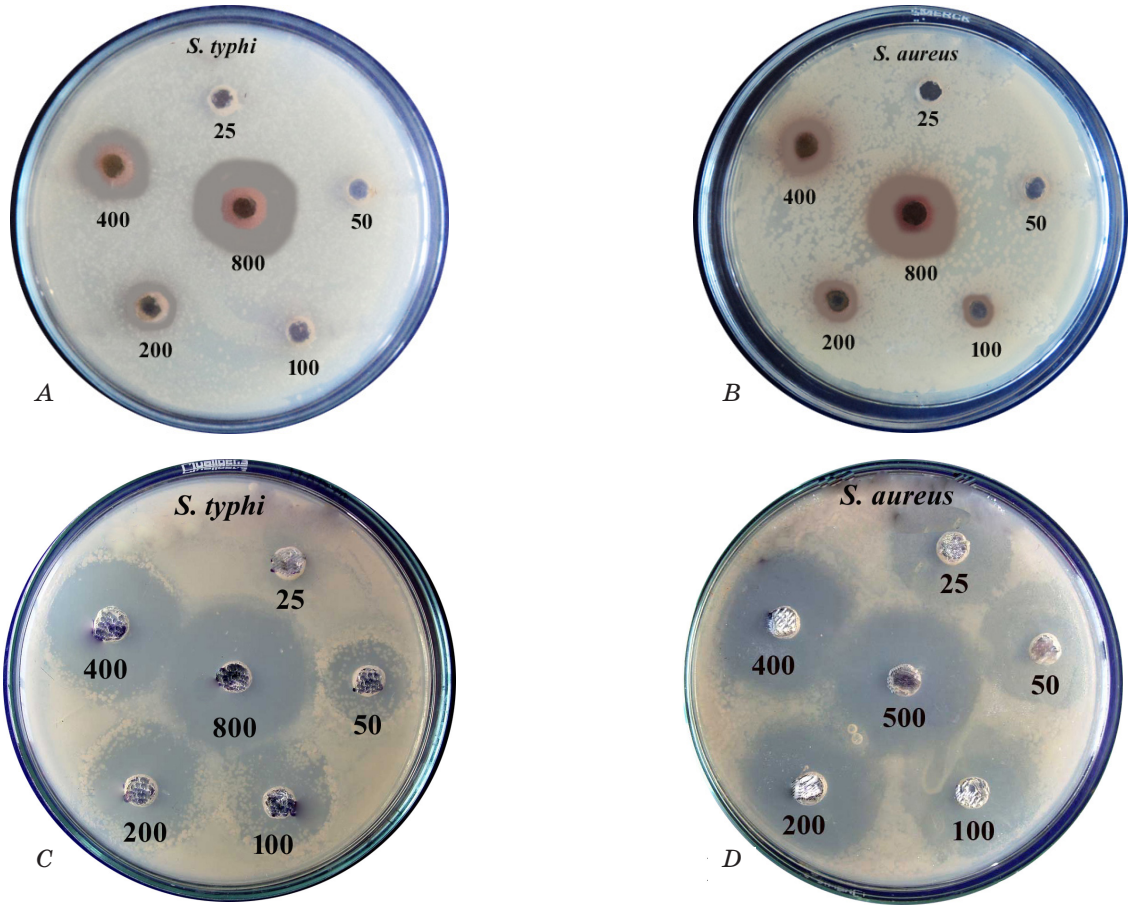

Fig. 6. **Zone of inhibitions of *Trametes sanguine* extrac against *S. typhi* (*A*) and *S. aureus* (*B*); Zone of inhibitions of Gentamycin against *S. typhi* (*C*) and *S. aureus* (*D*)**

microorganisms [24]. Landingin *et al* [25] reported secondary metabolites from higher fungi *Cyclocybe cylindracea* and *Pleurotus cornucopiae* possess antibacterial properties as well as other medicinal properties such as antioxidant, anticarcinogenic, antiviral antiparasitic activities. Compounds isolated from polypore fungus, such as 8-epidioxy-5, 22-dien-3-ol, 5-ergosta-7, 8-ergosta-6, and others, have been found to have antibacterial action against both gram-positive and gram-negative pathogenic bacteria [26]. In the present study bactericidal activity of polypore macrofungus *Trametes sanguine* extract might be due to present of such antibacterial compounds.

Conclusion

The collected macrofungus having morphological identity similar to *Pycnoporus sanguineus* now can be said *Trametes sanguine* on the basis of molecular identification of 28S large subunit rDNA of D2 region. The macrofungus *Trametes sanguine* also possess high antibacterial activity against pathogenic bacteria *S. typhi* and *S. aureus*.

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МОЛЕКУЛЯРНА ІДЕНТИФІКАЦІЯ ТА АНТИБАКТЕРІАЛЬНА АКТИВНІСТЬ МАКРОГРИБА ДО *Trametes sanguineus* (L.)

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Мета. Молекулярна ідентифікація *Pycnoporus sanguineus*, раніше морфологічно визнаного гриба, була проведена для визначення антибактеріальної активності щодо патогенних бактерій *Staphylococcus aureus* і *Salmonella typhi*.

Методи. Фрагмент ділянки D2 28S рДНК ампліфікували за допомогою ПЛР, секвенували та проводили BLAST з використанням консенсусної послідовності. Для побудови філогенетичного дерева використовували максимальний бал ідентичності. Для вивчення антибактеріальної активності застосовували дифузію в агарі.

Результати. Було проведено секвенування ПЛР-амплікону на 700 парах основ і згенеровано 616 пар основ ділянки D2 гена великої субодиниці. 100 бласт-попадань на ділянку D2 гена LSU показали схожість із *Trametes sanguineus* voucher PRSC95 (номер доступу GenBank: JN164795.1) на основі гомології нуклеотидів і філогенетичного аналізу. Антибактеріальний скринінг показав, що сирий екстракт має вищу активність щодо *Staphylococcus aureus* із зоною інгібування від 3 мм до 13 мм і мінімальною інгібіторною концентрацією 100 мкг порівняно з *Salmonella typhi*. *Salmonella typhi* мала зону інгібування від 5 мм до 15 мм і мінімальну інгібіторну концентрацію 200 мкг.

Висновок. Згідно з отриманим результатом морфологічно ідентифікований гриб *Pycnoporus sanguies* можна віднести до *Trametes sanguine*, і його можна використовувати для виготовлення антибактеріальних засобів.

Ключові слова: макрогриби; *Trametes*; ПЛР; філогенетичний аналіз; антимікробна активність.