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Insilico analysis of peptides isolated from *Agaricus bisporus* manifests potential antimicrobial therapeutic activities

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Authors' Contribution | **Fatima, A**. predicted antimicrobial peptides, **F. Haider** evaluated physicochemical properties, **J.M.A. Qaiser** predicted allergenic peptides, **S.U. Jan** predicted haemolytic property of peptides, **S.M. Bakhtiar** conducted functional analysis.

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ABSTRACT

More and more microorganisms are progressively acquiring resistances to conventional antibiotics. Consequently, new antibiotics which are more effective are needed. Antimicrobial peptides (AMPs) are recognized as effective alternative to conventional antibiotics. The AMPs are obtained from different organisms including animals, plants, fungi, algae, and microorganisms. One of such significant sources of AMPs is *Agaricus bisporus* (button mushroom) that is long-familiar for its medicinal values. However, the occurrence and potential of AMPs in *A. bisporus* have not been well characterized till date. This study was aimed to identify AMPs within *A. bisporus* proteome and to further evaluate its antimicrobial potentials through *in silico* analysis. The proteome of *A. bisporus* was explored for antimicrobial peptides and their physicochemical properties were evaluated using bioinformatics tools. The proteome of *A. bisporus* contains 63 AMPs with ample antimicrobial properties such as broad spectrum efficacy, stable, non-allergenic and non-haemolytic attributes. It was further identified that these AMPs putatively target pathogens via membrane disruption and inhibition of ATP-dependent enzymes. This study renders a basis for further evaluation of identified AMPs through *in vitro* experimentations and trials to elucidate their practical use as therapeutic antimicrobial drugs. Resultantly, positive AMPs could be subjected to commercialization as cheaper and effective alternatives to conventional antibiotics.

Keywords: Antimicrobial, antibiotic resistance, button mushrooms, *Agaricus bisporus*, antimicrobial peptides, AMPs, drug resistance, multiple drug resistance, MDR.

INTRODUCTION: The first microorganism found resistant to antibiotics was a strain of *Escherichia coli* that was capable of producing penicillinase against penicillin (Abraham and Chain, 1940). Since then, the number of microorganisms resistant to a drug or multiple drugs either through natural or acquired resistance mechanisms has been promptly increasing (Reygaert, 2018). These resistances are globally comprehended as alarming concern in public health – a reason why the global scientific community has devoted their efforts through intensive and extensive researches in finding effective and efficient alternatives of conventional antibiotics (Ventola, 2015). Most widely accepted efficient as well as effective alternatives to antibiotics discovered till date include the bacteriophages, antibodies, probiotics, and antimicrobial peptides (Ghosh *et al.*, 2019). Among these, the antimicrobial peptides (AMPs) hold reasonably unique blend of attributes to conventional antibiotics due to their lesser toxicity and broader range of activities. Moreover, the microbial cells rarely develop resistance against AMPs (Hancock and Patrzykat, 2002). The discovery of lysozyme in 1922 by Alexander Fleming set a benchmark in the production of antibiotics and later on AMPs (Huan *et al.*, 2020). Gramicidin was the first AMP discovered by René Dubos who isolated it from a soil bacterium *Bacillus brevis* (Dubos, 1939). The gramicidin exhibited both *in vivo* and *in vitro* bacteriostatic or bactericidal activity against broad range of gram positive bacteria including *Pneumococci* (Dubos, 1939). Previous findings have shown successful disinfection of bacteria through application of gramicidin on infected skin wounds of guinea pig, therefore, was allowed for clinical use due to their effective therapeutic potential (Gause and Brazhnikova, 1944). Subsequent detailed and structural analysis revealed that, being a member of N-formylated peptides, gramicidin comprises of homogeneous six AMPs with alternating D- and L-amino acids (Sarges and Witkop, 1965). This structural and functional characterization of gramicidin thus rendered a boost in the strive of finding more AMPs to effectively encounter bacterial resistance against drugs including multiple drugs resistance (MDR). Likewise, another AMP named as purothionin was isolated from wheat (*Tritucum aestivum*) in 1941 (Balls *et al.*, 1942) which also showed efficacy against pathogenic bacteria and fungi (Ohtani *et al.*, 1977). AMPs are a diverse group of small peptide molecules with varying lengths of amino acids ranging from 10 to 100 amino acids (Mabrouk, 2022). Till date, more than 5000 AMPs have been identified (Zhao *et al.*, 2013) from wide range of species including plants (Moyer *et al.*, 2019), mammals (Wang *et al.*, 2020), microorganisms (Yazici *et al.*, 2018), reptiles (Van Hoek *et al.*, 2019), amphibians (Li *et al.*, 2019), fish (Kim *et al.*, 2018), and invertebrates (Lee *et al.*, 2019). Generally, the AMPs are positively charged (cationic) and amphiphilic that possess both the hydrophilic and hydrophobic ends (Lei *et al.*, 2019) which facilitates the antimicrobial activity of AMPs in diverse range of environments. In animals, the AMPs usually reside in organs with higher exposure to airborne pathogenic entities and play their part as troops of innate immunity (Zasloff, 2002; Schauber and Gallo, 2008) defending the body against bacterial, viral or fungal infections (Radek and Gallo, 2007). AMPs are successful against resistance of several drugs but the most significant attribute of AMPs lies in their activity against MDRs (Brogden, 2005; Roversi *et al.*, 2014). Although the AMPs are primarily involved in preventing an infection from occurring, yet, the AMPs are highly diverse on the basis of their mode of action in various organisms (Hancock and Sahl, 2006). Furthermore, AMPs obtained from different organisms also perverts the nature as well as activities of AMPs. *Agaricus bisporus*, commonly known as button mushroom, is a most popular specie of edible fungi consumed worldwide (Mehta *et al.*, 2011). Previous studies have reported that the extracts of button mushrooms prepared with methyl alcohol possess antimicrobial activities against bacteria, yeasts, and dermatophytes (Abah and Abah, 2010; Akyuz *et al.*, 2010). Microbial inhibition through extracts from *A. bisporus* (Ndungutse *et al.*, 2015) suggests potential use of the stipes of button mushrooms as natural antimicrobial agents. The net aqueous protein extracts of the cultivated button mushrooms possess significant antibacterial activity against *Staphylococcus aureus*, particularly against the methicillin-resistant *Staphylococcus aureus* (MRSA) (Tehrani *et al.*, 2012; Atila *et al.*, 2019). These studies suggest the presence of putative AMPs within *A. bisporus* proteome which require confirmation and functional characterization through advanced wet lab procedures. Generally, the wet lab based detailed characterization of such putative AMPs is preceded by identification of AMPs through *in silico* tools. However, there is a gap of concise studies that could report the number and nature of AMPs contained in *A. bisporus* as well as their potential as antimicrobial agents.

OBJECTIVES: : The current study was designed with aims (1) to identify antimicrobial peptides from *A. bisporus* proteome through *in silico* tools and further (2) evaluate the physicochemical properties of the predicted peptides. This would allow evaluation of therapeutic potential of the identified AMPs. In addition, (3) the assessment of physicochemical properties will highlight the compatibility of peptides with human body.

MATERIALS AND METHODS: The methodological pipeline adopted in identification and study of AMPs within *A. bisporus* proteome is shown in figure 1. The button mushroom's reference proteome (ASM827154v1) was retrieved from NCBI. Among all the retrieved peptide sequences, the partial sequences as well as the hypothetical or low annotated peptides were removed. The remaining peptide sequences were evaluated by an online platform for scanning antimicrobial sequences known as AMPA tool (http://tcoffee.crg.cat/apps/ampa/do). The putative AMPs were predicted along with their percentage efficacy. The AMPs with efficacy threshold greater than 10% were selected for further analysis.

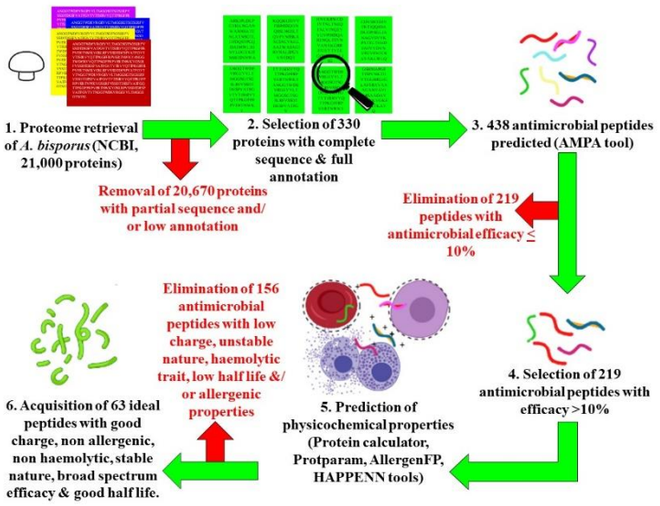

Figure 1: The pipeline adopted for the prediction and evaluation of antimicrobial peptides from proteome of Agaricus bisporus.

Predicted AMPs were evaluated through online ProtParam Expasy tool (https://web.expasy.org/protparam/), to determine their lengths, molecular weights, stability, half-life and GRAVY. All the AMPs with low half-life (<1 hour) and unstable nature were eliminated. The calculated physicochemical properties were further evaluated for their potential candidacy to be used as therapeutic peptide. The AMPs of efficacy greater than 10% were submitted to Protein Calculator *(v3.4)* (http://protcalc.sourceforge.net/). All the AMPs with charge between +2 and +9 were selected. The online tool Allergen FP *(v1.0)* (http://ddg-pharmfac.net/AllergenFP/) was used to identify if the predicted AMPs had the probability to cause allergy. All the allergy causing AMPs were eliminated. The AMPs with ideal physicochemical properties were submitted to HAPPENN online tool (https://research.timmons.eu/happenn_show_prediction?request=20210223064203377020). The peptides with haemolytic activity were excluded. The remaining peptides were regarded as finally selected putative AMPs within *A. bisporus* proteome which were further assessed through functional analysis. Finally selected putative AMPs were evaluated to identify one with the best qualities among all the predicted peptides. The evaluation was based on stability, allergenicity, non-haemolytic nature, charge, percentage efficacy, molecular weight, half-life and GRAVY. The AMP with best properties was procured from catalase protein (XP_006456321). The sequence of protein catalase was aligned using BLAST-P and family of protein was also observed. The aligned sequences were uploaded to MEGA *(v5.05)* to study their evolutionary relationship. The tree was constructed using neighbour joining method. Furthermore, to identify motif of this AMP, the sequence was submitted to the web-based MOTIF search tool (https://www.genome.jp/tools/motif/). The number and location of motifs along with corresponding sequence was noted.

RESULTS AND DISCUSSION: The proteome of button mushroom contained approximately 21,000 proteins. A total of 330 protein sequences remained after manual exclusion of redundant, partial, unannotated, and hypothetical sequences. The AMPA tool predicted existence of 438 peptide sequences as putative AMPs (Supplementary Information: Table S1). Since, the therapeutic drug was being formulated with focus on MDR and XDR pathogens, strict parameters were applied in final selection of putative AMPs. For instance, all the AMPs with ≤10% efficacy were removed, while those with greater than 10% efficiency were selected. Likewise, the charge, percentage antimicrobial efficacy, and length were assessed for the identified potential AMPs. Charge entails the mode of action of AMPs because AMPs develop electrostatic forces of attraction with target pathogenic membranes to neutralize them. In addition, the AMPs with their charge ranging from +2 to +9 show broad spectrum efficacy (Hancock, 2001; Bahar and Ren, 2013). Using protein calculator *(v3.4)*, charge of AMPs was calculated. All the AMPs were assessed where the AMPs with their charge ranging between+2 to +9 were selected while the AMPs with charge outside the selected range (+2 to +9) were eliminated (Supplementary Information: Table S2). The AMPs with charge in selected range will show broad spectrum efficacy. Among the selected AMPs, the highest charge was observed as 5.4 while the lowest was 2.1. The length essentially elucidates the mode of action of AMPs. All the AMPs with amino acids less than 100 disrupt cell membranes and interact with ATP directly to inhibit ATP dependent enzymes. On the other hand, AMPs with amino acids greater than 100 usually are involved in cell lysis. They sequester/ quench the proteins and macromolecules leading to cell death (Brogden, 2005; Akhtar *et al.*, 2012; Moghaddam *et al.*, 2014). The length of AMPs was calculated from ProtParam. The length of predicted AMPs ranged from 12-19 amino acids (Supplementary Information: Table S3). This means that these AMPs will kill the target pathogens by disrupting cell membranes and interacting with ATPs (to inhibit ATP dependent enzymes). Another major concern with AMPs is that the AMPs usually are unstable with low half-life (Lei *et al.*, 2019). Using ProtParam stability and half-life of all the AMPs with ideal charge (+2 to +9) were calculated (Supplementary Information: Table S3). The half-life of 4 AMPs was less than 1 hour, these were discarded due to such short half-life. The final AMPs had half-life ranging from 1 to 100 hours in mammalian cells.

The AMPs with molecular mass >500Da have low oral bioavailability and hence, must be administered via injections (Craik *et al.*, 2013). The molecular weight was calculated using ProtParam of all the AMPs with ideal stability and half-life. The molecular weight of AMPs ranged from 1100–2300 Da (Supplementary Information: Table S3). Since all AMPs have molecular mass >500Da, these AMPs oral bioavailability is low and they should be administered via injections. The solubility (GRAVY) of AMPs was also noted using ProtParam. The AMPs with negative gravy are hydrophilic in nature and those with positive gravy values are hydrophobic in nature (Supplementary Information: Table S3). The final selected AMPs had 47 hydrophilic while 16 hydrophobic AMPs. Many proteins and AMPs have the potential to cause allergy (Huby *et al.*, 2000). The AMPs were evaluated to see if they had allergenic nature or not, using AllergenFP *(v1.0)*. The non-allergenic AMPs (64) and allergenic AMPs (18) can be observed in Supplementary Information: Table S4. The 18 allergenic AMPs were eliminated. The non-allergenic AMPs have good candidacy to be developed as a drug, which does not cause any allergic reactions. One of the major problems with AMPs is their haemolytic activity. Since they have a charge based mode of action, they will interact with negatively charged surfaces of RBCs and cause their lysis (Melo *et al.*, 2009). In order to address this problem, haemolytic activity of AMPs was evaluated. Only one AMP had haemolytic activity and it was removed (Supplementary Information: Table S5). The 63 AMPs were non haemolytic (table 1). For conduction of functional analysis, the ideal AMPs were scrutinized to select one AMP with the best traits among all the 63 AMPs predicted. The AMP procured from catalase protein (XP_006456321) had the best ideal qualities among 63 final AMPs. The AMP had 19% antimicrobial activity, 3.1 charge, length of 13 amino acids, molecular weight of 1443.67Da, half-life 100h, stable nature, hydrophilic, non-allergenic and non-haemolytic trait. The catalase protein was hence, selected for functional analysis.

The catalase protein was used in BLAST-P for alignment. The protein belonged to catalase like superfamily. This enzyme is present in both prokaryotes and eukaryotes. It provides protection against the peroxides' toxic effects. The enzyme is involved in production of water and molecular oxygen by catalysing the conversion of hydrogen peroxide. It can also use H_2O_2 for oxidation of substrates like alcohols and phenols. The fungal catalase (NCBI: cd08157) is relatively small in size and binds to heme b (protoheme IX). The enzyme forms tetramers and can usually be found in peroxisomes. The phylogenetic tree was constructed using MEGA *(v5.05)*. The tree was constructed using neighbour joining method. The tree (figure 2) showed that *A. bisporus* was closely related to an edible fungus called *Laccaria bicolor* (bicoloured deceiver). The motifs of catalase protein were located using MOTIF Search tool. The tool showed two motifs along with corresponding sequence (figure 3). The motif 1 was called catalase with pfam ID: PF00199. It was located from 10-387 amino acids. The second motif was called Catalase-related immune-responsive with pfam ID: PF06628 and located at 431-490 amino acids. The AMP predicted in this research lied in the second motif (figure 3). The second motif has function in immune responsiveness which corresponds with findings of this research, as the predicted AMP also has antimicrobial function.

CONCLUSION: Antimicrobial peptides are a centre of attention to combat the hydra headed monster of antibiotic resistance. The AMPs are being evaluated as the alternative to conventional

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Sr. No.	PPAN	PAP	AE (%)	C	L	MW (Da)	HL (h)	S	II	G	A	HA	HP
1.	XP_006453831	RTKQTARKSTGGK	16	4.9	13	1418.62	1	✓	23.88	-2.008	✗	✗	0.005
2.	XP_006453905	KYGYNHPKAIRL	18	3.1	12	1459.71	1.3	✓	-7.38	-1.125	✗	✗	0.003
3.	XP_006454128	RFRLRVNMYTIK	15	3.9	12	1596.96	1	✓	30	-0.475	✗	✗	0.059
4.	XP_006454409	PSKILYRAIRGMVP	12	2.9	14	1600.99	20	✓	26.35	0.15	✗	✗	0.003
5.	XP_006454411	KAPRSTWAKHFNN	20	3.1	13	1556.75	1.3	✓	20.95	-1.546	✗	✗	0.268
6.	XP_006454562	HWKQLFKSLKLP	16	3.1	12	1524.87	3.5	✓	15.88	-0.625	✗	✗	0.042
7.		AKILQLYQIQKIQHGL	13	2.1	16	1894.29	4.4	✓	24.69	0	✗	✗	0.032
8.	XP_006454582	RKVLNLLFTRKALGIY	14	3.9	16	1905.36	1	✓	-1.24	0.362	✗	✗	0.047
9.	XP_006454865	AKAHPRAKLVIRI	11	4.1	13	1472.84	4.4	✓	-11.85	0.062	✗	✗	0.002
10.	XP_006454991	GIRTLITKRYSKD	13	2.9	13	1550.82	30	✓	21.98	-0.877	✗	✗	0.002
11.	XP_006455144	RALAKLIGKRAAR	16	4.9	13	1423.77	1	✓	21.25	-0.185	✗	✗	0.005
12.	XP_006455223	RNYLFNGYRRIS	15	2.9	12	1558.76	1	✓	33.49	-1.1	✗	✗	0.002
13.	XP_006455253	NAKGRNFAVKYTIT	15	2.9	14	1582.82	1.4	✓	-6.82	-0.521	✗	✗	0.012
14.	XP_006455356	RLKSTRGKQQRP	12	4.9	12	1454.7	1	✓	39.78	-2.333	✗	✗	0.003
15.	XP_006455519	VVKFLSVMQRHGY	13	2.1	13	1563.88	100	✓	1.52	0.269	✗	✗	0.023
16.	XP_006455553	NKKFTLGFIHRMFG	12	3.1	14	1696.05	1.4	✓	35.01	-0.136	✗	✗	0.007
17.	XP_006455709	YIRHSLRLKFEKNRYVTD	12	3.2	18	2338.7	2.8	✓	17.22	-1.111	✗	✗	0.004
18.	XP_006455838	RKFPVLGKIATWTLRT	17	3.9	16	1887.3	1	✓	16.09	-0.056	✗	✗	0.012
19.	XP_006455909	RYTANIVKNQKYN	12	2.9	13	1611.82	1	✓	16.15	-1.469	✗	✗	0.002
20.	XP_006455943	NRKALRKTTSSFW	13	3.9	13	1594.84	1.4	✓	38.86	-1.215	✗	✗	0.001
21.	XP_006456126	WYRQARGFNKTA	21	2.9	12	1497.68	2.8	✓	23.78	-1.4	✗	✗	0.009
22.	XP_006456321	VKNVSGHFKNVKS	19	3.1	13	1443.67	100	✓	-14.74	-0.654	✗	✗	0.001
23.	XP_006456355	RCRYLRDWRKQHE	14	3.1	13	1846.1	1	✓	36.46	-2.423	✗	✗	0
24.	XP_006456415	SLRQTVFGILKTLVVR	13	2.9	16	1830.25	1.9	✓	34.88	0.769	✗	✗	0.011
25.	XP_006456433	YLRTNARVNRVIF	14	2.9	13	1621.91	2.8	✓	12.48	-0.092	✗	✗	0.041
26.	XP_006456448	VWKERTGRQKVEKI	14	2.9	14	1757.07	100	✓	4.85	-1.45	✗	✗	0
27.		VKGGLWVRFHKAT	16	3.1	13	1498.79	100	✓	39.88	-0.085	✗	✗	0.009
28.	XP_006457225	SKIGVARHRGID	19	2.2	12	1308.51	1.9	✓	3.99	-0.517	✗	✗	0.001
29.	XP_006457341	RVFKIERKLHGRE	26	3.2	13	1667.98	1	✓	10.43	-1.277	✗	✗	0.005
30.	XP_006457793	PRKNHQQHKKPQVLGK	11	5.4	16	1923.25	20	✓	28.81	-2.256	✗	✗	0.001
31.	XP_006457952	NVKGLFKKIAMS	17	2.9	12	1335.67	1.4	✓	16.71	0.217	✗	✗	0.023
32.	XP_006458195	VKNAINVVQLWKASKI	13	2.9	16	1811.2	100	✓	17.07	0.319	✗	✗	0.054
33.	XP_006458398	VRIIIKGGVWKNT	12	2.9	13	1483.82	100	✓	-0.87	0.285	✗	✗	0.006
34.	XP_006458632	LKNQGHKVVLVSS	18	2.1	13	1408.66	5.5	✓	23.4	0.015	✗	✗	0.013
35.	XP_006458905	KLNTVCNRQGWKLTK	12	3.9	15	1789.13	1.3	✓	13.42	-1.007	✗	✗	0.003
36.	XP_006459026	AQKRLARGVKLNRT	11	4.9	14	1610.93	4.4	✓	18.39	-1	✗	✗	0
37.	XP_006459296	RNLLTKWLSNKGY	18	2.9	13	1592.86	1	✓	33.37	-0.923	✗	✗	0.05
38.	XP_006459344	FRKQKFGRVINTAS	11	3.9	14	1651.93	1.1	✓	20.14	-0.686	✗	✗	0.004
39.	XP_006459626	SVKNPILRYHFHP	15	2.4	13	1607.88	1.9	✓	27.93	-0.638	✗	✗	0.003
40.	XP_006459715	LSIRALYRTRAQ	13	2.9	12	1447.7	5.5	✓	-4.92	-0.342	✗	✗	0.007
41.	XP_006459917	PRKHLTIIHKSNVLSV	12	3.4	16	1842.22	20	✓	27.18	-0.069	✗	✗	0.001
42.	XP_006460038	AFWIGVRVQQFRGKIALH	11	3.1	18	2126.54	4.4	✓	1.81	0.311	✗	✗	0.715
43.	XP_006460096	NKKGPKYKHPGYVV	16	4.1	14	1614.91	1.4	✓	12.01	-1.464	✗	✗	0.007
44.	XP_006460374	TAKTLGRQAKKAQ	18	3.9	13	1400.64	7.2	✓	24.05	-1.215	✗	✗	0.001
45.	XP_006460791	VKNLNRLQIGKH	15	3.1	12	1419.69	100	✓	26.86	-0.842	✗	✗	0.012
46.		FKHYKGQHMICLN	11	2.4	13	1618.93	1.1	✓	39.75	-0.569	✗	✗	0.004
47.		KPKVPGIQRKSAS	17	3.9	13	1395.67	1.3	✓	5.18	-1.108	✗	✗	0.001
48.	XP_006460815	KSKAVLVTCRGVGN	13	2.9	14	1431.72	1.5	✓	-14.97	0.186	✗	✗	0.001
49.		VKKQLKQLKLSG	21	3.9	12	1369.71	100	✓	25.01	-0.683	✗	✗	0.015
50.	XP_006460859	TKRLSAIKNQRN	17	3.9	12	1428.66	7.2	✓	33.26	-1.558	✗	✗	0.004
51.	XP_006461504	GNRNRIYPRGTR	18	3.9	12	1459.63	30	✓	2.22	-2.075	✗	✗	0.003
52.		GTYRGRHVYTNHA	13	2.4	13	1531.65	30	✓	-25.92	-1.362	✗	✗	0.002
53.	XP_006462763	RQRFEKVYHRIA	21	3.1	12	1602.86	1	✓	22.14	-1.3	✗	✗	0.003
54.	XP_006463027	SRIGIRFLIGQH	14	2.1	12	1396.66	1.9	✓	18.14	0.233	✗	✗	0.074
55.	XP_006463506	KYKAIVTRITAI	23	2.9	12	1376.71	1.3	✓	-4.98	0.525	✗	✗	0.001
56.		GSRIRVGSVKGNVGHL	17	3.1	16	1635.89	30	✓	-1.61	-0.119	✗	✗	0.002
57.		ALRGSGRHTVKI	15	3.1	12	1294.52	4.4	✓	18.43	-0.342	✗	✗	0.004
58.	XP_006463614	RHVFSTGIRNFKIP	17	3.1	14	1671.97	1	✓	-9.86	-0.307	✗	✗	0.003
59.		LSRKLKPYIRISHT	11	4.1	14	1712.07	5.5	✓	0.14	-0.614	✗	✗	0.002
60.		HPRGALRIITVS	26	2.1	12	1319.57	3.5	✓	2.38	0.258	✗	✗	0.001
61.	XP_006464052	KIKSLSLKGVIS	21	2.9	12	1272.59	1.3	✓	-19.13	0.525	✗	✗	0.007
62.	XP_006464125	YTRKNPKRAYYLSL	12	3.9	14	1773.07	2.8	✓	33.26	-1.279	✗	✗	0.003
63.		KSIRTTAKMGKFS	15	3.9	13	1454.75	1.3	✓	27.78	-0.662	✗	✗	0.004

Table 1: The physical, chemical, and molecular attributes of finally selected 63 AMPs identified in A. bisporus proteome.
(PPAN = Parent Protein Accession Number: the proteins from which AMPs were predicted. PAP = Predicted Antimicrobial Peptide. AE = Antimicrobial Efficacy expressed in percentage. Only AMPs with AE > 10% were selected. C = Charge of AMPs: AMPs with their charge ranging between +2 and +9 were selected as they demonstrate broad spectrum efficacy. L = Length of AMPs i.e. the number of amino acids in each AMP. MW = Molecular Weight of AMPs expressed in daltons (Da). HL = Half-life of AMPs in mammalian cells expressed in hours (h): The range of half-life was 1-100 hours where, all the AMPs with half-life lower than 1 hour were removed. S = Stability: the symbol "✓" implicates AMP is stable. II= Instability Index. The score of II < 40 suggests that AMP is stable while the score II ≥ 40 entails the higher probability of AMP being unstable. G = GRAVY: The solubility of AMPs where the negative GRAVY value indicates the hydrophilic AMPs while positive GRAVY values indicate hydrophobic AMPs. A = Allergenic nature of peptides where, the symbol "✗" represents non-allergic AMPs suggesting that the AMP has no potential to cause allergy. HA = Haemolytic Activity: As the aim was to have a drug with least side effects all the peptides with no haemolytic activity were selected. HP = Haemolytic Probability: This score gives probability that a peptide is haemolytic or not. The HP score ranges between 0 and 1.

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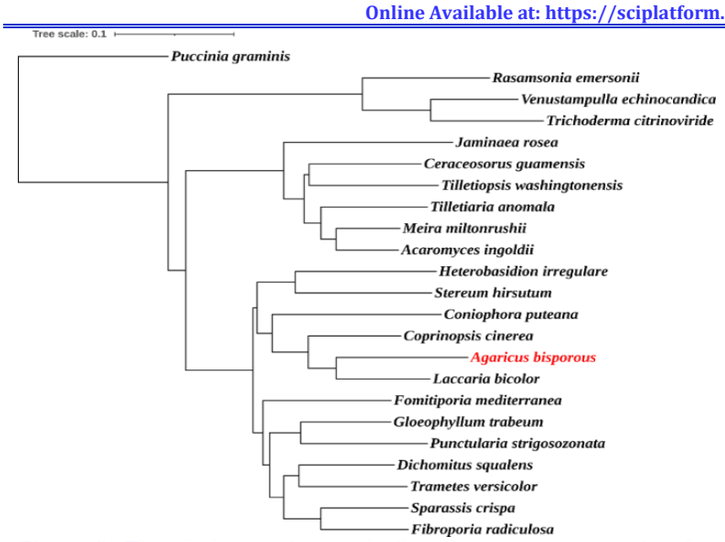

Figure 2: The phylogenetic tree built using catalase protein of *A. bisporus* with neighbour joining method. The closest relative of *A. bisporus* is *Laccaria bicolor*, followed by *Coprinosis cinera, Coniphora puteana, Stereum hirsutum and Heterobasidion irregulare*. The tree has 23 leafs and an outgroup *Puccinia graminis*.

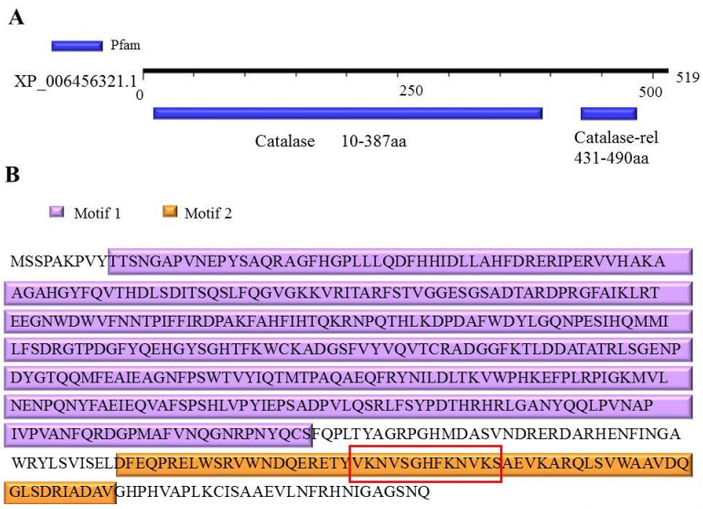

Figure 3: The two motifs of catalase protein. A) The location of motifs can be noted. B) The sequence corresponding to motif 1 and motif 2 is shown. The red highlighted region in motif 2 is the therapeutic peptide predicted as antimicrobial peptide in this research.

antibiotics which are rendered useless against many MDR and XDR pathogens. The *A. bisporus*' proteome was explored for AMPs. The AMPs were evaluated for their potential candidacy to be used as drug on base of physicochemical properties. The research allowed identification 63 AMPs with stable nature, good half-life and non-allergenic traits. These AMPs have broad spectrum efficacy as their charge lies from +2 to +9. Furthermore, the length of AMPs ranges from 12 to 19 amino acids. This means these AMPs will inhibit ATP dependent enzymes of the target pathogens by interacting with ATP directly. These AMPs will also neutralize the pathogens by disruption of pathogenic cell membranes. The AMPs identified were non haemolytic and hence, will not cause lysis of erythrocytes. It was further noted that the AMP with best features was from catalase protein of *A. bisporus*. The *A. bisporus* closest relative is *L. bicolor* based on tree constructed using catalase protein. The other close relatives include *C. cinera, C. puteana, S. hirsutum and H. irregular*. The antimicrobial nature of predicted AMP was confirmed by identifying motifs of the catalase protein. The protein motif has a function in immunity thereby confirming the findings of this research that the AMP predicted was indeed antimicrobial in nature. The AMP's efficacy and side effects can be evaluated in animal trials. This AMP can be produced in vitro by proteolytic digestion of catalase protein. It can also be mass produced by inserting the transgene in bacterium via genetic engineering. This will allow easy production & isolation, high yield and commercialization of the therapeutic AMP..

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