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RESEARCH PAPER

Isolation, Morphological & Molecular Characterization of *Aspergillus Species* producing α-L-Rhamnosidase from Agro-Industrial Waste

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Abstract

An α-L-rhamnosidase enzyme [E.C.3.2.1.40] has potential application in food and pharmaceutical industry. Naringin is the dominant flavanoid bitter compound found in citrus fruit juices of the objective of present study was to isolate α-L-rhamnosidase producing fungus, study their morphological and molecular characteristics. A total twelve fungal strains were isolated from agro-industrial waste of lemon and sweet lemon peels for α-L-rhamnosidase enzyme production. Of these, only 4 isolates were capable of producing the α-L-rhamnosidase enzyme to hydrolyze naringin for removal of bitter taste from juices. Fungal isolates were selected according to their higher α-L-rhamnosidase enzyme activities (3.481 IU mL^{-1}). For morphological studies, macroscopic features like colony colour, texture, elevation, growth and margins as well as microscopic characteristics such as the size of conidia and conidiophores, and their arrangements were examined for species differentiation. DNA was isolated from the best α-L-rhamnosidase producing fungal strain, and amplification of 18S rRNA gene, using specific primers ITS-1 and ITS-4 was done. This gene was subsequently sequenced and phylogenetic affiliations was accordingly assigned. Fungal isolate was identified as *Aspergillus flavus*.

Keywords: *Aspergillus flavus,* narigin, α-L-rhamnosidase, agro-industrial waste, morphological, molecular characterization

Microorganism are ubiquitous and their presence has not only been observed in a normal environment, but also at an extreme conditions (Bing *et al.* 2013). They are used in various fields especially *Aspergillus* species are used in industries due to their ability to produce various enzymes; among various enzymes α-L-rhamnosidase is of great importance. *Aspergillus* is a large genus composed of more than 180 accepted anamorphic species (Pitt *et al.* 2000). Naringinase is an enzyme complex consisting of α-L-rhamnosidase (EC 3.2.1.40) and β-glucosidase (EC 3.2.1.21). α-L-Rhamnosidase has been reported from plants, yeast, fungi and bacteria (Ribeiro, 2011). Microbial enzymes are gaining special importance in recent days due to cost-effective production due an economically feasible process. Microbial α-L-rhamnosidase has completely replaced the chemical methods of naringin reduction in industries. α-L-rhamnosidase play very important role in biotechnology field and its major application is in debittering of citrus fruit juices, enhancement of wine aroma and also in pharmaceuticals industry (Yadav *et al.* 2017a).

α-L-Rhamnosidase [E.C.3.2.1.40] cleaves terminal α-L-rhamnose specifically from a large number of natural products which include naringin, rutin, quercitrin, hesperidin, terpenyl glycosides and many other natural glycosides containing terminal α-L-rhamnose (Habelt *et al.* 1983; Yoshinobu *et al.* 1995; Caldini *et al.* 1994). Naringin is one of the bitter compounds that is found in citrus fruits. When juice is extracted from citrus fruits, naringin imparts the bitter taste to the juice. Therefore, elimination of naringin or alteration of naringin concentration into bitter free component is important to achieve the consumer acceptance of citrus juice and economically viable if α-L-rhamnosidase production is achieved industrially using microorganism. Apart from above, citrus peel is considered as a waste in the food industry, which has naringin as a principal component. α-L-rhamnosidase was previously produced by using *Aspergillus flavus* in a submerged state fermentation in which culture condition was optimized by applying Taguchi DOE methodology (Yadav *et al.* 2017b).

During this study, 12 fungal cultures were isolated from decaying peels of lemon and sweet lemon peels in different soil and atmospheric condition grown on PDA (Potato Dextrose Agar) plates at 28 °C for 3-4 days . Fungal isolates were selected according to their higher α-L-rhamnosidase enzyme activities (3.481 IU mL^{-1}). For morphological studies, macroscopic features like colony colour, texture, elevation, growth and margins as well as microscopic characteristics such as size of conidia and conidiophores and their arrangements were examined for species differentiation. Molecular characterization of best α-L-rhamnosidase producing fungal isolate was carried out in several steps such as DNA isolation, quantification of genomic DNA, PCR amplification of ITS1 and ITS4 regions, Sanger sequencing, and phylogenetic analysis of the fungi. The ITS region sequence was used to carry out BLAST with the database of NCBI Genbank. Based on maximum identity score, first ten sequences were selected and aligned using multiple alignment software program Clustal W. Distance matrix was generated and the phylogenetic tree was accordingly constructed using MEGA 7.

MATERIALS AND METHODS

Chemicals

Naringin and *p*-nitrophenyl- α-L-rhamnopyranoside were obtained from sigma, India. The other culture media (Potato dextrose Agar, Czapek Dox Agar and Broth) and different carbon and nitrogen sources were obtained from Hi-Media. All other reagents used were of analytical grade.

Sample collection

For the isolation of fungi, agro-waste of lemon and sweet lemon peels were procured from different region of Varanasi, which was allowed to spoil in air and soil. The spoiled citrus peels were collected in sterilized Hi-Media bag. Fungi were also isolated from lemon and sweet lemon peels powder in which peels were washed in running tap water, dry in tray dryer at 50 °C for 72 hours and grounded into fine particles.

Isolation of Strain

For the isolation of naringin hydrolyzing fungus, decaying peels of citrus lesions were streaked aseptically on fresh PDA plates. After 3 days, fungi were distinguished according to the macroscopic characteristics features. Spores of different morphological fungi were streaked on separate PDA plate under aseptic conditions. This process was continued until a pure culture was obtained.

Preparation of inoculums for enzyme production

Production of enzyme

The production of α-L-rhamnosidase by the isolated fungal strain was done in the liquid culture medium having composition: water (distilled) 1000 mL, KCl 0.5g, $MgSO_4.7H_2O$ 0.5g, KH_2PO_4 1g, $NaNO_3$ 3g, Sucrose 30g, Naringin 0.2%, mineral solution-10mL/100mL medium (mineral solution- $ZnSO_4.7H_2O$-0.07%, $CuSO_4$. $5H_2O$-0.07%, $FeSO_4.7H_2O$-0.07%) and the initial pH was adjusted to 6.0 and was sterilized at 121

°C, 15 psi for 15 min. The production medium was inoculated with 10 mm bead size cut with the help of cark borer from 4 days old spore on PDA medium plate aseptically in to liquid culture medium (20mL) kept in 100 mL culture flask that also contain 0.2% naringin as inducer (Vinita *et al*. 2010). The culture flasks were incubated in a B.O.D. incubator at 25 °C under stationary condition. Aliquots of 1mL of the culture filtrate were taken out at an interval of 24h in eppendorf tube and were further evaluated for the presence of α-L-rhamnosidase activity (Davis, 1947).

Assay of α-L-rhamnosidase activity

The activity of α-L-rhamnosidase was assayed by Davis method (1947) in which naringin was used as a substrate. The reaction solution constituted of 2.5 mL of 0.86 mM naringin dissolved in 0.2 M sodium acetate buffer, pH 4.5, maintained at 60 °C. 0.25 mL of enzyme extract was added to the above solution, 0.1 mL aliquot was withdrawn immediately and 5 mL of 90% diethylene glycol was added. After that 0.1 mL 4M NaOH was added to the mixture. The sample were kept at ambient temperature for 10 min. The intensity of resultant yellow color was determined at 420 nm. One unit of activity (IU) was defined as the amount of enzyme required to hydrolyse 1μmol of naringin under the above assay conditions.

Morphological characterization of α-L-rhamnosidase producing *Aspergillus species*

Identification of fungal species was done on the basis of cultural and morphological characteristics. The morphology of fungi and their appearance on potato dextrose agar was examined, after incubating at 28±2 °C for 3-4 days. For microscopic variability, fungal slides were prepared with the help of lactophenol cotton blue strain. Macroscopic features like colony colour, texture, elevation, growth and margins as well as microscopic characteristics such as size of conidia and conidiophores and their arrangements were examined for species differentiation as per the standard method (Afzal *et al.* 2013).

Molecular characterization of best α-L-rhamnosidase producing *Aspergillus species*

Molecular characterization of best α-L-rhamnosidase producing fungal isolate was done in several steps such as DNA isolation, quantification of genomic DNA, PCR amplification of ITS1 and ITS4 regions, Sanger sequencing, and phylogenetic analysis of the fungi. Forward and reverse DNA sequencing reaction of PCR amplicon was carried out with ITS1 and ITS4 primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. Consensus sequence of the PCR amplicon was generated from forward and reverse sequence data using aligner software. The ITS region sequence was used to carry out BLAST with the database of NCBI Genbank. Based on the maximum identity score first ten sequences were selected and aligned using multiple alignment software program Clustal W. Distance matrix was generated and the phylogenetic tree was constructed using MEGA 7.

Fungal DNA isolation

Total DNA from the fungi was isolated by using method of Mishra *et al.* (2014). Isolation of fungal genomic DNA was done by growing the fungi in Potato Dextrose Broth (PDB) for 3-4 days. Mycelium was harvested and filtered through a double layered sterilized filter paper, dried in between two layers of filter paper under aseptic conditions and stored at −80°C. Deep freeze fungal mycelium was grounded to fine powder using liquid nitrogen in pre-sterilized and cool mortar and pestle. DNA quality was evaluated on 1.0% Agarose gel.

DNA amplification, Sanger sequencing of amplified product

Fragment of ITS region was amplified by PCR. The PCR amplicon was purified to remove contaminants. Forward and reverse DNA sequencing reaction of PCR amplicon was carried out with ITS1 and ITS4 primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer.

Phylogenetic analysis

Sequence alignments were conducted with the help of Clutal W program (Thompson *et al.* 1994). The ITS (internal transcribed spacer) region sequence was used to carry out BLAST with the database of NCBI Genbank. Based on maximum identity score first ten sequences were selected and aligned using multiple alignment software program Clustal W. Distance matrix was generated and the phylogenetic tree was constructed using MEGA 7 (Kumar *et al.* 2015).

RESULTS AND DISCUSSION

Isolation of fungal strain

A total twelve fungal strain were isolated from agro-industrial waste of lemon and sweet lemon peels for α-L-rhamnosidase enzyme production. Out of these, only four isolates were capable of producing the α-L-rhamnosidase enzyme labelled as L31, M41. LW12 and MPP12 (Table 1).

Table 1: α-L-Rhamnosidase activity of different fungal isolates in submerged state fermentation after 7 days

Sl. No.	Culture Code	Enzyme Activity (U mL^{-1}
1	L31	1.246 ± 0.07
2	M41	1.058 ± 0.02
3	LW12	3.481 ±0.03
4	MPP12	1.342 ±0.04

Morphological characterization of α-L-rhamnosidase producing fungi

α-L-rhamnosidase producing fungal isolates (L31, M41, LW12 and MPP12) were grown in Potato dextrose agar medium and colonies on plate were observed macroscopically for colony color, texture, reverse side of the plate, Sclerotia shape and diameter etc. and microscopically observed features such as Conidiophore length by width, Conidiophore color, Conidial diameter and conidia surface as shown in Table 2 and Fig. 1.

On the basis of morphological characters and growth pattern on Potato Dextrose Agar medium, isolate LW12, the best α-L-rhamnosidase producer could be identified as may be *Aspergillus flavus* and similar finding was reported by Srikantha *et al.* (2016).

Molecular Characterization of best α-L-rhamnosidase producing fungi

Genomic DNA was isolated from best α-L-rhamnosidase producing LW12 strain of *Aspergillus species*. DNA purity and quality was tested by using 1% Agarose gel followed by staining with ethidium bromide. DNA obtained was an intact band of good quality as shown in Fig. 2.

The PCR amplification of 18S rRNA gene was done by using gene specific primer ITS-1 and ITS-4. The nuclear small submit of ribosomal DNA (18S rDNA) was selected for characterization and identification of

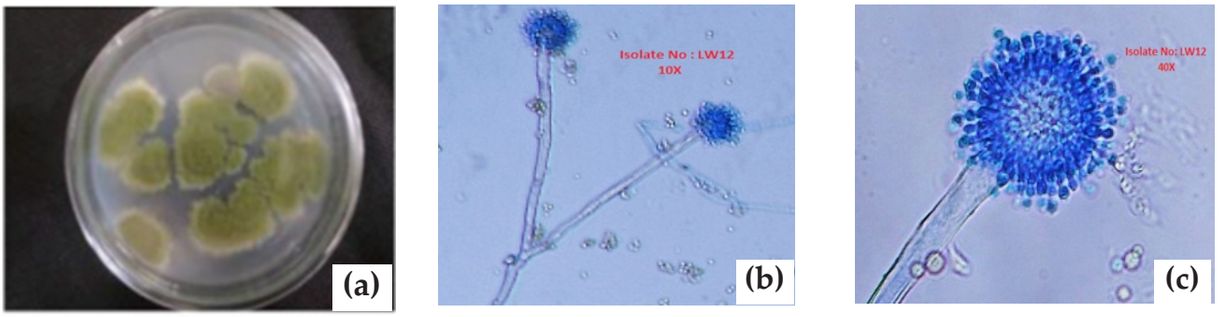

Fig. 1: (a) LW12 strain on PDA plate after 4 days of incubation at 28 °C. **(b) & (c)** Light microscope picture of strain LW12 at 10X and 40X respectively

Table 2: Morphological characterization of fungal isolates obtained from agro-industrial waste

Macroscopic feature of *Aspergillus species*	**Culture code**			
Characteristics	**L31**	**M41**	**LW12**	**MPP12**
Colony colour	Brown	Slightly black	Green	Dark grey
Texture				
Reverse side	Light Yellow	Hyaline	Hyaline	Hyaline
Sclerotia shape	—	—	Globose	—
Sclerotia diameter	—	—	400-700	—
Microscopic feature of *Aspergillus species*				
Conidiophore length by width	80-110 by 50-90	80-150 by 40-60	250-350 by 200-300	150-250 by 45-55
Conidiophore colour	Hyaline	Hyaline	Hyaline	Hyaline
Conidial diameter	2-3	3.5-4-5	3.0-4.5	1.5-2.5
Conidia Surface	Smooth slightly rough	Very rough irregular	Smooth finely roughened	Smooth

LW12 strain firstly, because available universal fungal primers based on conserved regions of 18S rDNA. Secondly, the large numbers of 18S rDNA sequence are easily available in NCBI GenBank which help to make similarity searches convenient.

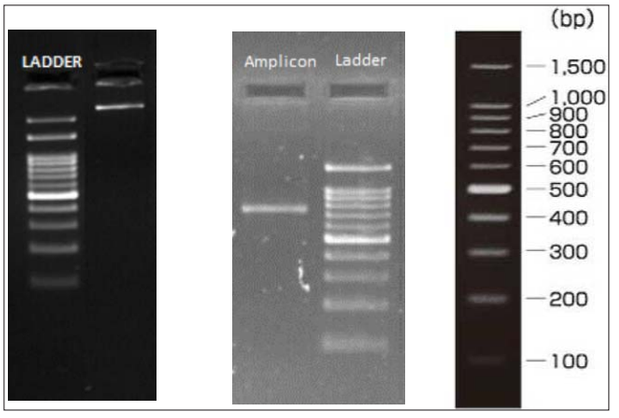

Fig. 2: (a) Agarose gel electrophoresis of DNA isolated from best α-L-rhamnosidase producing enzyme LW12 *Aspergillus species;* (**b)** PCR amplification of 18S rRNA gene of LW12 *Aspergillus species*

Another researcher have also reported characterization of fungi based on 18S rRNA gene sequence analysis (Smit *et al.,* 1999: Majid *et al.,* 2015). The forward sequence data (ITS-1), reverse sequence data (ITS-4), and LW12 consensus sequence data are given in figures 3(a) 3(b) and 3(c) respectively.

Forward and reverse DNA sequencing reaction of PCR amplicon was carried out with ITS-1 and ITS-4 primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. Consensus sequence of the PCR amplicon was generated from forward and reverse sequence data using aligner software.

Further, the ITS region sequence was used to carry out BLAST with the database of NCBI Genbank (Fig. 4). Based on maximum identity score first ten sequences (Fig. 5) were selected and aligned using multiple alignment software program Clustal W. Distance matrix was generated and the phylogenetic tree was constructed using MEGA 7.

The result of the present study also revealed that the sequence information of ITS region could differentiate the *Aspergillus* strain and ITS region can be used for authentic identification for molecular characterization of fungal isolates.

The strain LW12 showed high similarity with *Aspergillus flavus* based on nucleotide homology and phylogenetic analysis.

>Forward Seq data

TCGTAGTGACCTGCGGAGGATCATTACCGAGTGTAGGGTTCCTAGCGAGCCCAA
CCTCCCACCCGTGTTTACTGTACCTTAGTTGCTTCGGCGGGCCCGCCATTCATGGC
CGCCGGGGGCTCTCAGCCCCGGGCCCGCGCCCGCCGGAGACACCACGAACTCTG
TCTGATCTAGTGAAGTCTGAGTTGATTGTATCGCAATCAGTTAAAACTTTCAACA
ATGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAG
TGTGAATTGCAGAATTCCGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCC
TGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCATCAAGCACGGC
TTGTGTGTTGGGTCGTCGTCCCCTCTCCGGGGGGGACGGGCCCCAAAGGCAGCG
GCGGCACCGCGTCCGATCCTCGAGCGTATGGGGCTTTGTCACCCGCTCTGTAGGC
CCGGCCGGCGCTTGCCGAACGCAAATCAATCTTTTTCCAGGTTGACCTCGGATCA
GGTAGGGATACCCGCTGAACTTAAGCATATCAA

Figure 3(a) forward sequence data (ITS-1)

>Reverse Seq Data

ATCCGAGGTCACCTGGAAAAGATTGATTTGCGTTCGGCAAGCGCCGGCCGGGCC
TACAGAGCGGGTGACAAAGCCCCATACGCTCGAGGATCGGACGCGGTGCCGCCG
CTGCCTTTGGGGCCCGTCCCCCCCGGAGAGGGGACGACGACCCAACACACAAGC
CGTGCTTGATGGGCAGCAATGACGCTCGGACAGGCATGCCCCCCGGAATACCAG
GGGGCGCAATGTGCGTTCAAAGACTCGATGATTCACGGAATTCTGCAATTCACAC
TAGTTATCGCATTTCGCTGCGTTCTTCATCGATGCCGGAACCAAGAGATCCATTG
TTGAAAGTTTTAACTGATTGCGATACAATCAACTCAGACTTCACTAGATCAGACA
GAGTTCGTGGTGTCTCCGGCGGGCGCGGGCCCGGGGCTGAGAGCCCCCGGCGGC
CATGAATGGCGGGCCCGCCGAAGCAACTAAGGTACAGTAAACACGGGTGGGAG
GTTGGGCTCGCTAGGAACCCTACACTCGGTAATGATCCTTCCGCAGGTTCACCTA
CGGAAACCTTGTTACGACTTTTACTTCCTCT

Figure 3(b) Reverse sequence data (ITS-4)

>LW12 Consensus data

AGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCA
TTACCGAGTGTAGGGTTCCTAGCGAGCCCAACCTCCCACCCGTGTTTACTGTACC
TTAGTTGCTTCGGCGGGCCCGCCATTCATGGCCGCCGGGGGCTCTCAGCCCCGGG
CCCGCGCCCGCCGGAGACACCACGAACTCTGTCTGATCTAGTGAAGTCTGAGTTG
ATTGTATCGCAATCAGTTAAAACTTTCAACAATGGATCTCTTGGTTCCGGCATCG
ATGAAGAACGCAGCGAAATGCGATAACTAGTGTGAATTGCAGAATTCCGTGAAT
CATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGT
CCGAGCGTCATTGCTGCCCATCAAGCACGGCTTGTGTGTTGGGTCGTCGTCCCCT
CTCCGGGGGGGGACGGGCCCCAAAGGCAGCGGCGGCACCGCGTCCGATCCTCGAG
CGTATGGGGCTTTGTCACCCGCTCTGTAGGCCCGGCCGGCGCTTGCCGAACGCAA
ATCAATCTTTTTCCAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAA
GCATATCAA

Figure 3(c) LW12 consensus sequence data

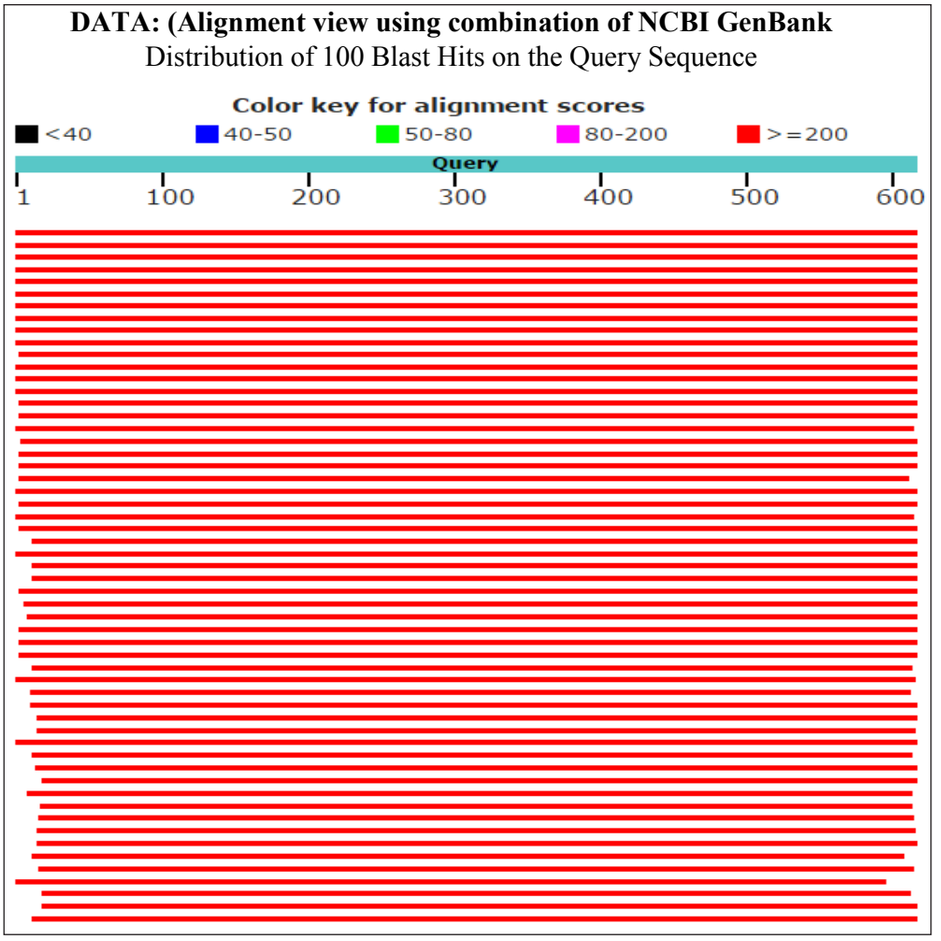

Fig. 4: Alignment view of ITS region by using BLAST (Basic local alignment Search Tool) in combination with NCBI GenBank

For phylogenetic analysis, LW12 strain isolated from agro-industrial waste of lemon and sweet lemon peel, sequence was aligned with other sequence of *Aspergillus flavus* available in NCBI database using molecular evolutionary genetic analysis (MEGA) software version 7. An optimal tree was constructed (Fig. 6) and different taxa were clustered together in a bootstrap test with 1000 replicated (Fig. 5).

The evolutionary history was concluded by using the Maximum Likelihood method based on the Kimura 2-parameter model (Kimura M. 1980). The bootstrap consensus tree deduce from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed (Felsenstein J. 1985). Branches corresponding to partitions found that in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the linked taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein J. 1985). Initial trees were constructed and obtained by applying Neighbor-Joining algorithms and Maximum Composite Likelihood (MCL) approach. The analysis involved 11 nucleotide sequences. Codon positions included were 1^{st} + 2^{nd} + 3^{rd} + Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 607 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar S. *et al.* 1985).

Sequences producing significant alignments:

Description	Max score	Total score	Query cover	E value	Ident	Accession
Aspergillus flavus isolate F4 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1129	1129	100%	0	100%	JF951750.1
Aspergillus flavus culture-collection MUM:10.206 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1129	1129	100%	0	100%	HQ340106.1
Aspergillus flavus culture-collection MUM:10.204 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1129	1129	100%	0	100%	HQ340104.1
Aspergillus oryzae isolate 7 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1129	1129	100%	0	100%	EU301638.1
Aspergillus flavus strain 176 1 B2 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1123	1123	100%	0	99%	KP784374.1
Aspergillus flavus strain UOA/HCPF 10337-18 isolate ISHAM-ITS_ID MITS127 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1123	1123	99%	0	100%	FJ878654.1
Aspergillus flavus culture-collection MUM:10.220 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1122	1122	100%	0	99%	HQ340108.1
Aspergillus flavus culture-collection MUM:10.202 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1122	1122	100%	0	99%	HQ340103.1
Aspergillus flavus culture-collection MUM:10.200 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1122	1122	100%	0	99%	HQ340101.1
Aspergillus oryzae isolate A-4 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1118	1118	99%	0	99%	GU120193.1

Fig. 5: Nucleotide BLAST analysis of LW12 strain (*Aspergillus flavus*) with other sequences available at NCBI GenBank

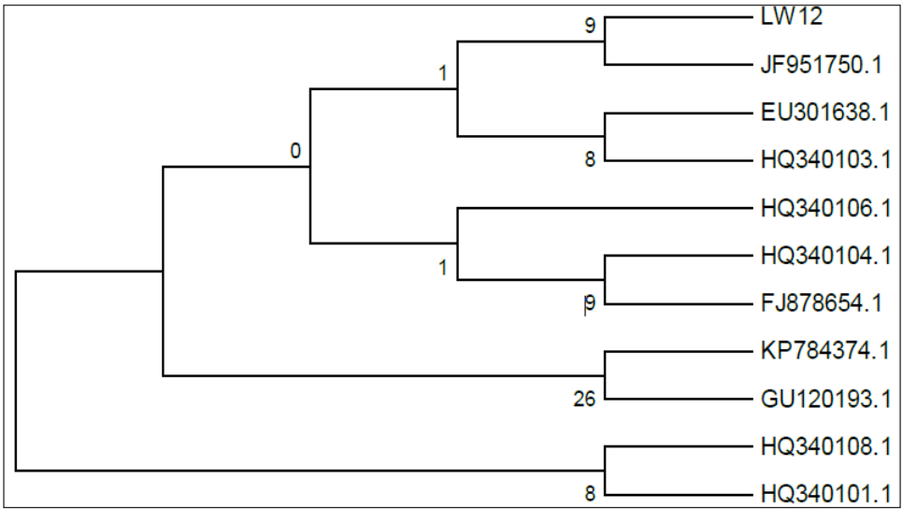

Fig. 6: Molecular Phylogenetic analysis by Maximum Likelihood method

CONCLUSION

The present investigation suggest that α-L-rhamnosidase production by *Aspergillus flavus* strain LW12 have significant values which can be exploited for industrial production of α-L-rhamnosidase. Moreover, this enzyme have an important biotechnological application including removal of bitterness from citrus fruit juices, enhancement of grape wine aroma, removal of hesperidin crystals from orange juices, tomato pulp digestion and also help in conversion of clinical important steroids. α-L-rhamnosidase enzyme producing *Aspergillus flavus* species isolated from agro-industrial waste of lemon and sweet lemon peels were comprehensively characterized relying on various morphological and molecular approaches. Depending on macro and microscopic morphological features, the isolates was identified as *Aspergillus flavus*. *A. flavus* 18S rRNA gene flanking ITS1, ITS4 regions, was amplified, sequenced and aligned with the closely related fungal isolates data available at NCBI GenBank. From phylogenetic analysis, the isolate was determined as *A. flavus*, according to the genetic loci of *Aspergillus* species isolates deposited on database. Among the isolated strains of fungal from agro-industrial waste of lemon and sweet lemon peels, *Aspergillus flavus* (LW12 strain) is the best α-L-rhamnosidase enzyme producer under submerged state fermentation system.

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CONFLICT OF INTEREST

No conflict of interest declared.

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