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A comparative study of xylanase producing wild and mutated strains of *Streptomyces variabilis* VITMUVB02 isolated from Kanyakumari salt pan

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The current study aimed to isolate xylanase-producing marine actinobacteria, which is a novel idea; ascribed to the challenges associated with the culturing of marine microorganisms. A total of 5 actinobacterial colonies were isolated from Kanyakumari marine sediments. Among them only one isolate, A1 indicated the elevated xylanase activity manifesting a zone of hydrolysis of around 20 mm during primary screening. On the other hand, secondary screening of A1 *i.e.*, sugar estimation by 3,5-dinitrosalicylic acid method, resulted in A1 exhibiting 400 µg/ml of enzyme activity. The enzyme extracted from the isolate A1 also contained a protein concentration of 600 µg/ml. Physical and chemical mutation studies were carried out to over-express xylanase production. Chemical mutation involving the use of EDTA did not show a significant increase in xylanase production and thus, was not subjected to further analyses.

The isolate, A1 also showed a zone of hydrolysis of 28 mm in the physical mutation study and was named A'1.75. This zone was 8 mm more than the wild-type strain after UV-exposure for 75 min. A'1.75 also exhibited an enzyme activity of 600 µg/ml and protein content of 800 µg/ml. The potential isolate was identified as *Streptomyces variabilis* VITMUVB02 using 16S rRNA molecular sequencing. The molecular weight of the purified xylanase extracted from A1 and A'1.75 was found to be 25 kDa and 20 kDa, respectively. Fourier Transform Infrared spectroscopy (FT-IR) and High-Performance Liquid Chromatography (HPLC) analyses showed the mutagenic effect with the change in the spectral and functional groups.

[**Keywords**: Actinobacteria, Birchwood, Physical mutation, *Streptomyces variabilis*, Xylanase]

Introduction

Enzymes have played a major role in various manufacturing processes, for example, in the production of wine, cheese, bread, starch modification, and in numerous different applications since ancient times. However, the last few decades have seen the application of enzymes extended beyond the food and beverage industry to various other industries like paper, pulp, crafting, bioleaching, and clarification of juices. Production of enzymes in laboratories is generally an expensive process[1]. Hence, scientists are now looking forward to the production and extraction of enzymes from natural "single-cell chemical factories", *i.e.*, microorganisms. In this age and season of quick industrial and innovative development, the world is confronting an enormous issue of disposing of the generated waste. The process of industrial production leads to hazardous waste generation that has serious impact on the environment. Therefore, the search for natural approaches to handle associated waste is of great importance[2]. Microorganisms are ubiquitous in nature and could tolerate adverse climatic conditions. Owing to its nature to adapt to various environmental conditions, they produce numerous enzymes that help them in degrading the substrate[2-4].

Currently, lignocellulosic wastes have become one of the major environmental concerns. Subsequently enzymes obtained from microbial sources shapes an effective method to deal with the problems arising in treating wastes from pulp, paper ventures and oil spills in seas. These comprise enzymes like xylanases which act on hemicellulosic or xylan substrates[1,2,5]. Synthetic hydrolyses of lignocellulosic materials is constantly joined with the aid of the arrangement of poisonous metabolites which harms the surroundings[3,4]. Therefore, it's far vital to analyze the utilization of enzymes that have high particularity and huge substrate selectivity. Versatile enzymes which are harmless and prefer mild reaction conditions are considered more convenient to intercept this issue of waste management[4]. These enzymes are delivered by

a wide range of unicellular organisms. In any case, filamentous fungi and actinobacteria are desired to be a high-quality volume over exceptional microorganisms as they have positive auxiliary enzymes which might be essential for branching and debranching of subbed polysaccharides[1,4]. Xylanase is a xylanolytic enzyme, which as the word describes can be used to degrade xylan; which is the major constituent of hemicellulose. A heteropolymer is made of β-1,4 linked xylopyranose unit branches, xylan contains such as L-arabinofuranosyl and glucopyranosyl residues. Xylanase is a repertoire of hydrolytic enzymes made of β-endoxylanase, β-xylosidases, phenolic acid esterase, α-arabinofuranosidase, α-glucuronidase, and acetyl xylan esterase. Xylan is a heteropolysaccharide comprising of glucopyranosyl, α-arabinofuranosyl, acetyl, feruloyl, and pcumaryl side-chain groups. Xylanase has two distinctive glycosyl families, family 10 and 11. Family 10 has a high molecular weight and low pI. However, family 11 has a low molecular weight but a high pI. Xylanases may be utilized on semi- cellulosic substrates to create outcomes of excessive qualities like ethanol and xylitol[5,6].

Blanching of pulps utilizing xylanase is one of the maximum reasonable programs in the pulp and paper enterprise that can decrease the usage of chlorine or chlorine dioxide synthetic compounds[7-9]. Enzyme manufacturing is co-incited through the occurrence of xylan or natural substrates containing hemicelluloses or even unadulterated cellulose. They belong to E.C. 3.2.1.8 and depolymerized non-crystalline xylan. It is responsible for maintaining the plant cell wall integrity as it has a characteristic adhesion property[10,11]. Endo β-xylanases and β-xylosidases attack xylan and hydrolyze xylooligosaccharides into D-xylose[12-14].

Although almost all micro-organisms and certain plant cells produce xylanases, the enzymes from filamentous fungus are preferred because of the extracellular secretion of enzyme and easy cultivation. However, due to cellulose content and stability problems from fungal xylanases, xylanase from actinobacterial sources are now being studied.

Marine Actinomycetes occur in a wide range of environments producing a variety of bioactive compounds and enzymes. They are heterotrophic, aerobic, spore-producing, gram-positive, and mesophilic slow growers with a powdery texture and stick firmly to the agar surface[15]. Marine *Streptomyces* species belongs to the actinobacterial subclass, Actinobacteriacae. *Streptomyces* species are known to be the producers of a broad spectrum of secondary metabolites that include antimicrobial, antitumor, antithrombotic, and enzyme inhibitors[16-18]. Xylanases produced from marine actinobacteria can work efficiently under alkaline pH, a temperature range of 40 – 55 °C, and with the help of strain improvement can produce cellulase-free xylanases. In the baking industry, they improve the versatility and quality of the batter accordingly expanding the texture and volume of the bread. In the feed industry, the consolidation of xylanase into the rye-based eating regimen of broiler brings about both weight increase in chicks and their feed change effectiveness. They can likewise be utilized for the change of xylan in wastewater delivered from rural and food ventures into xylose, clarifying juices, liquefaction of products of the soil, degumming of fibers, and deinking of waste papers[19,20]. Xylanase is an inducible enzyme; be that as it may, the chance of it being constitutive has likewise been accounted for. It goes through repression with the presence of glucose and xylose. The gentle release of the inducer particle and the chance of the culture filtrate converting the inducer to its non-metabolizable derivatives are believed to aid the degree of xylanase action[21]. There is a requirement for a mutated strain to lower the expense for enzymatic hydrolysis of biomass that is one of the factors restricting the attainability of the cycle. This study focuses on the production of xylanases from marine actinobacterial sources and increasing its yield by various mutation methods and testing its effectiveness on xylan agar medium.

Materials and Methods

Chemicals and materials

Media and synthetic compounds applied within the experiments have been bought from HiMedia Laboratory Pvt. Ltd., Mumbai.

Sample collection

Salt pan sediments were collected from the seaside locales of Kanyakumari (8°30’26.6” N, 76°58’ 22.8” E). These samples were then transferred to the laboratory in a sterile autoclave bag and refrigerated at 4 °C.

Isolation of microorganisms

Collected sediments were serially diluted and inoculated on Actinomycetes Isolation Agar (AIA) and incubated at 28 °C for 7 days. The isolated action-bacteria were sub-cultured on International Streptomyces Project Medium No. 7 (ISP-7 - Tyrosine Agar).

Primary screening

The xylanase activity was tried utilizing xylan from Birchwood media alongside agar. The cultures on AIA were immunized on xylan agar plates and incubated at room temperature. After 3 – 5 days of incubation, plates were treated with a solution of Congo red and destained with 1 M sodium chloride solution. The observation of a rosy zone of precipitation affirmed the production of xylanase. The colonies of actinobacteria affirmed positive for xylanase production were then sub-cultured on ISP-7 media.

Secondary screening: Xylanase assay

The xylanase producing microorganisms acquired from primary screening were inoculated in Actinomycetes Isolation Broth and incubated in a rotational shaker at hundred and twenty rpm for 7 days at 28 °C. On the 7 day, the flask with the broth was exposed to filtration on Whattmann no. 1 channel paper. The acquired supernatant was then utilized for xylanase assay. Xylan from birchwood was used as an enzyme-substrate for xylanase activity. The reaction blend was established by adding 2 ml of 95 % ethanol and 22.5 ml of distilled water in 0.25 g of xylan. This mixture was then transferred into a glass beaker covered with aluminum foil and was warmed on a hot plate at 100 °C for 10 min. Using distilled water, the volume of the mixture was then made up to 25 ml. Sodium acetic buffer (1 ml) along with 0.1 ml of the culture was added to 0.5 ml of the solution and incubated in a water bath for 15 min at 40 °C. The solution mixture was shaken at regular intervals for proper dissolution of the contents. The assessment of the reducing sugar released was performed utilizing 3,5-dinitrosalicylic acid (DNSA) and glucose as the standard followed by heating of the solution for 5 min in a water bath. The optical density was checked using a UV-V is spectrophotometer at 540 nm. Under these particular examination conditions, the measure of catalyst needed to liberate 1 µ mol of xylose per minute defined 1 unit of enzyme activity[1,21].

Estimation of protein by Lowry's method

The amount of protein was assessed by the Lowry technique with Bovine Serum Albumin (BSA) as the standard[22]. The optical density of the culture was recorded at 670 nm using a UV-Vis spectrophotometer.

Strain improvement of xylanase producing Actinobacteria

The two isolates exhibiting potent xylanase activity were further selected to study the effect of the mutation on xylanase production. Strain improvement can help to increase enzyme production using techniques of classical mutagenesis such as subjecting the microbe to a physical mutagen (UV-rays) and chemical mutagen (EDTA).

Strain improvement using the physical method

A total of 18 sterile xylan agar plates were prepared and the selected isolate was streaked on 6 of these plates. The isolate was plated in triplicates. They were then subjected to UV exposure of 6 W, 280 nm short wavelengths with a constant distance of 10 cm for a treatment interval of 15 min. The UV treated plates were taken out at regular intervals of 15, 30, 45, 60, 75, and 90 min and subsequently incubated at room temperature for 48 h, in complete darkness by covering the plates using aluminum foil to prevent photo repair mechanism. The microbial colonies which survived the UV treatment were screened for xylanase production using congo red stain and NaCl destaining procedure. The isolates which showed maximum zones of precipitation were further sub-cultured on ISP-7 media. These isolates were further subjected to xylanase assay to confirm the presence of xylanase producing actinobacteria and calculate the enzyme activity utilizing the DNSA method (sugar estimation) and the Lowry's method (protein estimation).

Strain improvement using chemical method

To mutate the microorganism using chemical agents, EDTA was the chemical chelating agent of choice and was added in the range of 10 – 100 ppm concentration in ISP-7 media. The selected isolate was inoculated by the pour plate method and incubated at room temperature for 48 h. The colonies were isolated on the 3 day and plated on xylan agar plates to screen for its xylanolytic activity. The colonies were then screened using congo red staining and NaCl destaining method. The selected isolates were then sub-cultured on ISP-7 media. Enzyme activity of these mutated isolate was calculated by performing the xylanase assay.

Preparation of crude enzyme extract

To prepare the crude enzyme extract obtained from the wild type and the mutant isolate, a production broth containing soluble starch was prepared. Conical flasks containing 300 ml of production broth were prepared - one for wild type and one for the physically mutated isolate. After inoculation, the flask with isolates had been incubated in a rotary shaker at hundred and twenty rpm for 7 days at 28 °C. Post

incubation, the contents of the flasks was centrifuged individually at 10000 rpm for 20 min at 4 °C. The ensuing supernatant thus formed was gathered and refrigerated for further investigations.

SDS-PAGE

The molecular weight of the crude enzyme extract was determined using Sodium Dodecyl-Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE). The crude enzyme extracts obtained from both the wild type as well as the UV treated mutants was analyzed. The polyacrylamide gel prepared consisted of 10 % separating gel and 5 % stacking gel. 20 µl of the crude enzyme samples were loaded onto the polyacrylamide gel and run for 4 h at 110 volts. Coomassie brilliant blue R-250 was used to stain the gel. Destaining was performed using a solution of 10 % acetic acid and 40 % methanol. The standard protein molecular weight marker (10 – 250 kDa) was obtained from Sigma Aldrich.

High-Performance Liquid Chromatography (HPLC)

HPLC was performed to examine xylanase qualitatively. The crude extract (100 ml) was mixed with ethyl acetate (100 ml) and stored in a solvent extraction funnel for 24 h. This enabled the solvent layers to get separated. The procedure was repeated for the supernatants obtained from both the wild type as well as the UV treated isolates. Post 24 h, the supernatants were extracted from the funnels into separate beakers. Half of the wild type and UV treated supernatants were subjected to HPLC analysis. C18 reversed-phase column was used for HPLC. A mixture of acetonitrile (75 %, w/v) and distilled water (25 %, w/v) constituted the mobile phase. The components of the HPLC system included an automatic injector, a pump, and a refractive index detector. The flow rate was 0.5 ml/min, and the injection volume for the sample was 10 µl. Absorbance was measured at 280 nm with a run time of 20 min.

Fourier Transform-Infrared spectroscopy (FT-IR)

FT-IR is used to analyze the absorption and emission of infrared spectra of the samples. The FT-IR spectrometer is capable of collecting high spectral resolution data over a wide range simultaneously. The crude enzyme extract-supernatants (wild-type and UV treated) solubilized in ethyl acetate were subjected to FT-IR. The pH of the supernatants was checked before analysis. The FT-IR spectrometer (8400 Shimadzu, Japan, with Hyper IR-1.7 software for Windows) was attached with a helium-neon laser lamp as the source of IR radiation. At a frequency range of 400 – 500 cm^{-1} and room temperature, the IR spectra of the supernatants were recorded. To prepare the analysis supernatants, they were mixed with potassium bromide in a ratio of 1:100 in a mortar to form pressed pellets.

Identification of marine Actinobacteria

The potential strains were identified using the Nonomura key by observing the aerial mass color, melanoid pigment, reverse side pigment, and soluble pigment on ISP media[23]. Morphological characterization was performed using microscopy, spore chain, and SEM. The mutated isolate was characterized molecularly by 16S rRNA sequencing using the primers 1492R (5'-GGTTACCTTGTTACG ACTT-3') and 27F (5'-AGAGTTTGATCCTGGC TCAG-3'). The 16S rRNA sequence and the DNA extracts from cells had been decided with the aid of the fluorescent dye terminator method using ABI Prism Big dye terminator cycle sequencing ready reaction kit v.3.1. Products were run on an ABI13730XL capillary DNA sequencer (ABI Prism 310 genetic analyzer, Tokyo, Japan). The aligned sequences were computed using ClustalW software and the sequence homologies were determined using BLASTn to create an evolutionary distance matrix[23].

Results and Discussion

Isolation of microorganisms

A total of five colonies named A1, A2, A3, A4 and A5 were isolated on AIA media. All the isolates exhibited an abundant growth pattern and a powdery, greyish aerial mycelium. These were then sub-cultured on ISP-7. Out of these 5 colonies, only A1, A2 were further proceeded for screening. The A1 isolate displayed a smooth texture with an off-white mycelium whereas the A2 isolate was powdery and greyish in color. Similar research work on marine *Streptomyces* sp. isolated from Andaman and Nicobar Island resulted in the isolation of light brown to pink to light brown to red mycelium on ISP-7 and AIA, respectively[24].

Xylanase assay

In primary screening, only A1 isolate showed a visible zone of precipitation measuring 20 mm (Fig. 1a); whereas, there was no zone of hydrolysis formed by the A2 isolate (Fig. 1b). This further proves that the environment of the microbe actually shows its metabolic capability. The isolate A1 was found in an environment where a high amount of xylose substrate

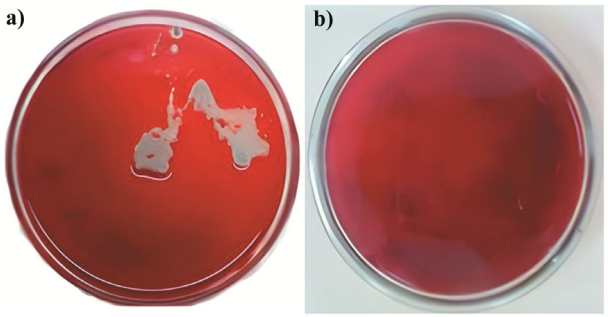

Fig. 1 — Xylanase activity (zone of hydrolysis) of active isolated strains: a) A1; and b) A2

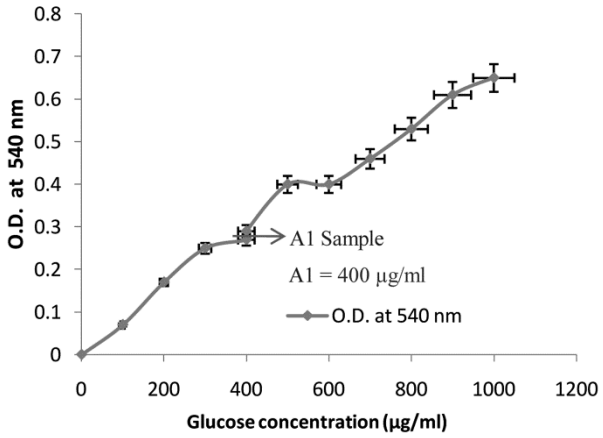

Fig. 2 — Graph showing the estimation of glucose in A1 isolate by DNSA method

is present, thus being an inducing factor, helping the isolate to produce more amount of xylanase. On solid agar medium, hallow zone around the isolates were seen which is because of the presence of enzyme emitted close to the growing colony on the agar plate[26]. In secondary screening (xylanase assay) sugar estimation by the DNSA method resulted in the isolate, A1 exhibiting 400 µg/ml of enzyme activity (Fig. 2).

Estimation of protein by Lowry's method

The complete estimation of protein was determined by performing a biochemical assay called the Lowry's method, which is displayed by the color change of the sample solution concerning the protein concentration. In this assay, a wide range of amino acids react with the Folin-Ciocalteu reagent, resulting in the reduction of the same and oxidation of aromatic residues[27]. From this assay, the quantity of protein present in the isolate A1 was found to be 600 µg/ml (Fig. 3).

Strain improvement using the physical method

The isolate, A1 was exposed to UV-irradiation for a time-interval of 15, 30, 45, 60, 75, and 90 min and

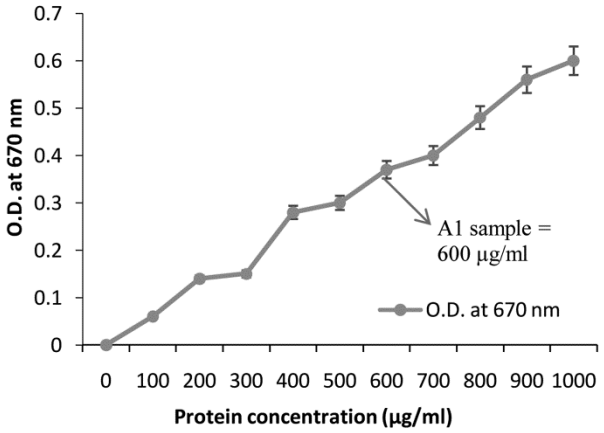

Fig. 3 — Graph showing the estimation of protein content in A1 isolate by Lowry method

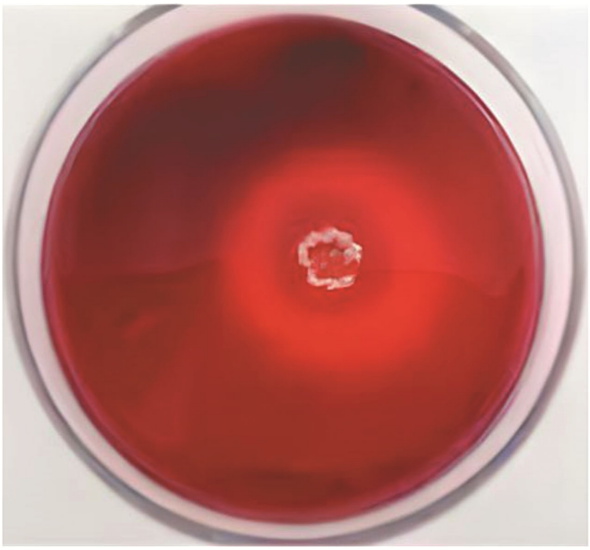

Fig. 4 — UV treated isolate, A'1.75 showing a zone of inhibition of 28 mm

were named A'1.15, A'1.30, A'1.45, A'1.60, A'1.75 and A'1.90, respectively. The replicates of each time exposed isolate were named as R1, R2, and R3. The isolate, A1 exposed to UV at various time intervals showed variations in the zones of hydrolysis formed on xylan agar. Compared to the Wild-Type (WT) A1 isolate, the UV-treated replicates showed a marked increase in xylan degradation for the time periods of 15, 30, 60, and 75 min. Decreased xylanase activity was observed in the time periods of 45 and 90 min of UV exposure. The isolates were considered dead after exposing them to UV for 90 min. The mutated isolate A'1.75 showed the maximum increase in the zone of inhibition *i.e.* of 8.0 mm (Fig. 4) and was used for further studies. According to the research performed

by Shokoofeh *et al.*[28], the UV lamp used was 30 W leading to the exposure time of the isolates being 3 – 4 min. Since the UV lamp used by our research team was only of 6 W, the exposure time had to be prolonged[28]. The enzyme (xylanase) activity of the mutated strain, A'1.75 calculated using the DNSA method (sugar estimation) was 600 µg/ml (Fig. 5a), whereas that calculated using the Lowry's method (protein estimation) was 800 µg/ml (Fig. 5b).

Strain improvement using chemical method

The isolate, A1 was inoculated in ISP-7 supplemented with a chemical chelating agent, EDTA at different concentrations ranging from 10 – 100 ppm with an interval of 10 ppm per plate. These isolates were named A*1.10, A*1.20, A*1.30, A*1.40, A*1.50, A*1.60, A*1.70, A*1.80, A*1.90, and A*1.100, respectively. The replicates of each isolate subjected to different concentrations of EDTA were named R1, R2, and R3. Compared to the Wild-Type (WT) A1 isolate, the replicates showed marked variations in the zones of hydrolysis formed on xylan agar. Even though the isolate subject to an EDTA concentration of 10 and 20 ppm showed a slight increase in the zone of precipitation formed, a subsequent increase in the amount of EDTA resulted in lesser degradation by xylanase. The idea behind using EDTA was to crosscheck the negative effect of mutation caused by chelation. Since the exposure of the isolate to EDTA resulted in decreased zones of hydrolysis, the study was inconclusive and thus, the isolates were not subjected to further analyses.

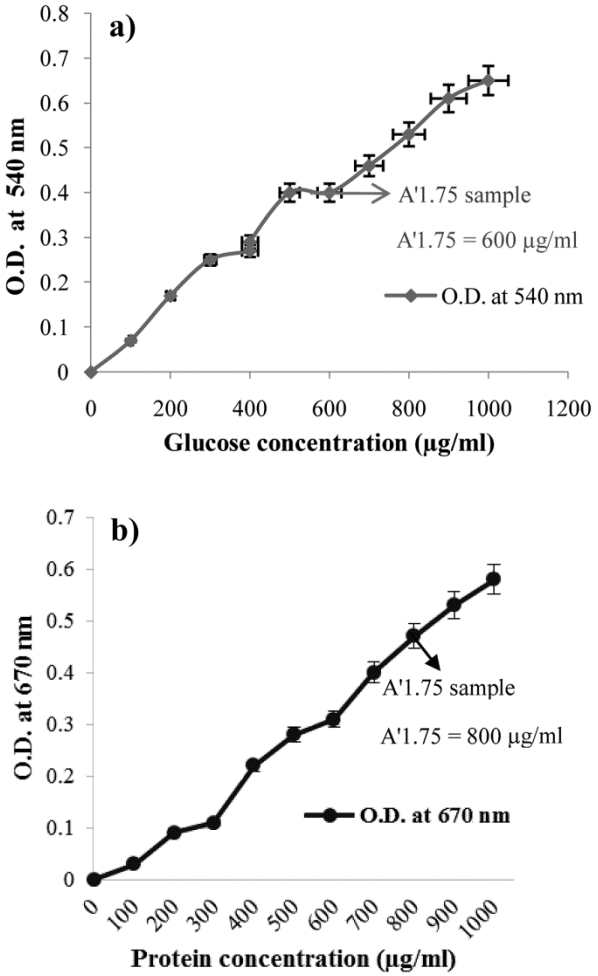

Fig. 5 — Graph representing: (a) estimation of sugar content in A'1.75 isolate by DNSA method; and (b) The estimation of protein content in A'1.75 isolate by Lowry method

Molecular weight determination using SDS-PAGE

The molecular weight of the enzyme isolated from VITMUVB02 analyzed using SDS-PAGE was found to be 20 kDa when compared with standard xylanase which is 25 kDa. The image of the gel is given in Figure 6. Various research works on the determination of the molecular weight of xylanase isolated from different bacterial sources had ranged from 20 to 145 kDa[29] even as high as 340 kDa in some cases[30,31].

HPLC

The HPLC chromatogram obtained for the WT and VITMUVB02 samples were analyzed to elucidate the compounds formed as a result of xylanase degradation. The WT sample showed 3 individual and distinct peaks suggesting the breakdown of xylan in

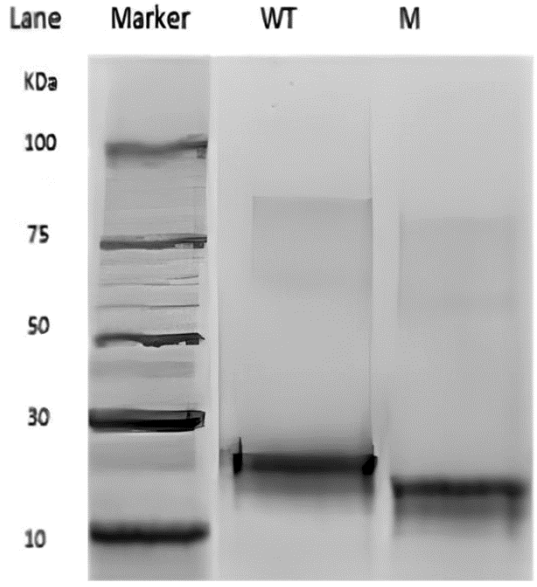

Fig. 6 — Molecular weight determination of Wild-type *S. variabilis* and UV-treated mutant *S. variabilis* VITMUVB02

the agar into three compounds with a peak retention time of 3.083, 4.083, and 7.795 min (Fig. 7a). On the other hand, the chromatogram of VITMUVB02 exhibited 5 new, individual, and distinct peaks at 2.317, 3.087, 3.985, 5.438, and 7.391 min which suggest the breakdown of xylan into five new compounds indicating that the UV-treated sample is more capable of degrading xylan than its WT counterpart. It also had a lesser peak retention time of 7.391 min highlighting its efficacy (Fig. 7b).

FT-IR

FT-IR was executed to analyze the functional groups and the vibration design present in the A1 and A'1.75 isolates (Fig. 8a & b) to understand the extent of structural changes that might have taken place as a result of exposing the WT isolate to UV irradiation. In the FT-IR spectra of the WT isolate (A1), the vibration band at 3277.06 cm^{-1} and 2929.87 cm^{-1} reveals the existence of an alkyne functional group with strong sharp C-H stretch alkanes and alkyls with an asymmetric and symmetric C-H stretch, respectively. Similarly, the bands obtained at 1728.22 cm^{-1}, 1631.78 cm^{-1} and 1230.58 cm^{-1} shows the existence of aldehydes with a strong C=O stretch, amides (strong C=O stretch), and alcohols (Ar-O-H) with medium to strong C-O stretch, respectively. The vibration bands at 1074.35 cm^{-1}, 1020.34 cm^{-1}, and 524.64 cm^{-1} represent functional groups like alcohols ($R\text{-}CH_2\text{-}OH$ or C=C-CH(R)-OH) with medium to strong C-O stretch, alkyl halides (R-F) with a very strong C-F stretch and alkyl halides (R-Br) with a strong C-Br stretch, respectively. But, compared to the WT isolate, major rearrangement of peaks have been observed in the case of the UV treated mutant isolate (A'1.75). The vibration band at 3257.77 cm^{-1}

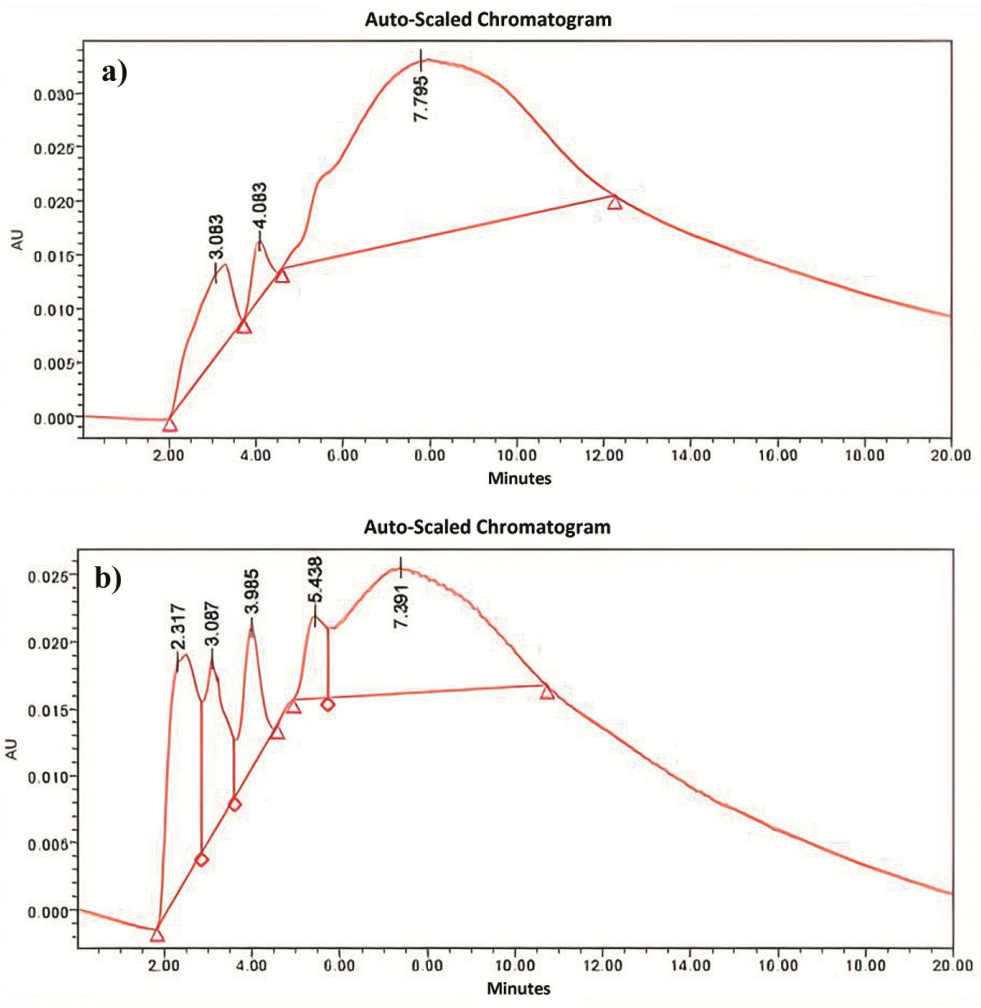

Fig. 7 — HPLC chromatogram of a) wild-type A1 isolate, *Streptomyces variabilis*; and b) UV treated mutant, A'1.75 isolate, *Streptomyces variabilis* VITMUVB02

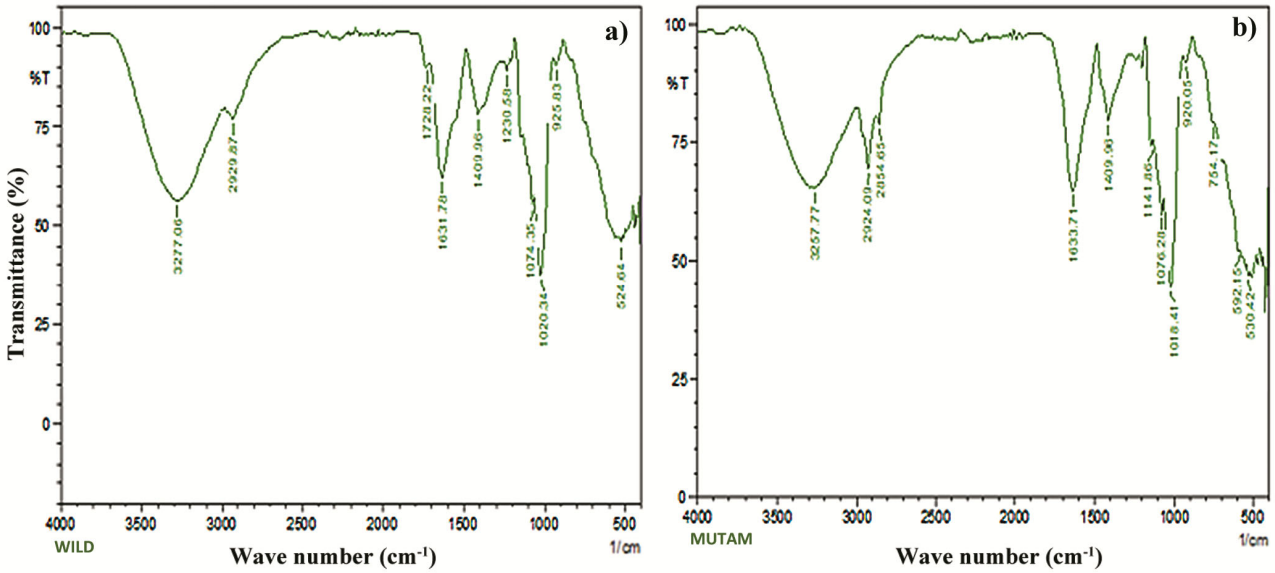

Fig. 8 — FT-IR spectral analysis of a) wild-type isolate A1, *Streptomyces variabilis*; and b) UV treated mutant, A'1.75 isolate, *Streptomyces variabilis* VITMUVB02

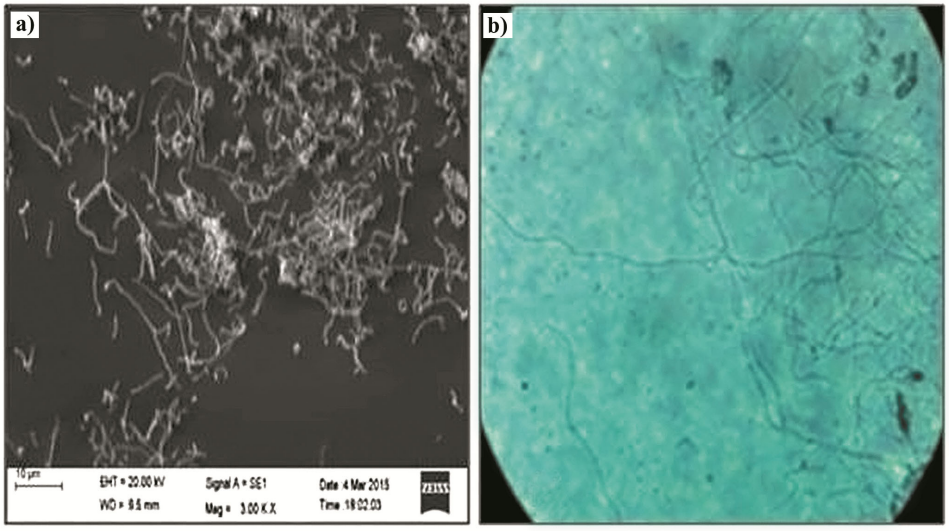

Fig. 9 — (a) Scanning electron Microscopy image; and (b) Spore chain morphology of UV treated mutant, *Streptomyces variabilis* VITMUVB02

shows the presence of alkynes with a strong sharp C-H stretch. The FT-IR spectra of the A'1.75 isolate shows absorption at two nearby peaks, 2924.09 cm^{-1} and 2854.85 cm^{-1}, both representing alkanes with a strong C-H stretch. The bands obtained at 1633.71 cm^{-1}, 1141.86 cm^{-1}, and 1076.28 cm^{-1} reveal the existence of amides with a medium to strong C=O stretch and alcohols (for the last two peaks) with a medium to strong C-O stretch, respectively. Absorption bands at 1018.41 cm^{-1}, 592.15 cm^{-1} as well as a band at 530.42 cm^{-1} depicts the presence of alkyl halides like R-F with a very strong C-F stretch and R-Br with a strong C-Br stretch, respectively. Hence the analysis revealed various structural changes of xylanase enzymes produced in between the wild type and the mutant isolates.

Identification of marine Actinobacteria

The morphological characteristics of the isolate, A1 are given in a tabular form below in Table 1. The microscopic view depicting the spore chain morphology of the mutated isolates is given in Figure 9. 16s rRNA sequencing analysis of the mutated (UV-treated) isolate A'1.75 confirmed it to

Table 1 — Colony characteristics of wild-type (A1) and UV treated (A'1.75) *S. variabilis*

Characteristics	A'1.75 (VITMUVB02)
Size	0.1 mm
Color	Sandy-brown (upper side), greyish brown (back side of agar)
Shape	Circular
Margin	Regular
Elevation	Flat
Opacity	Opaque
Consistency	Much finer (powdery) compared to the Wild- type
Gram character	Positive

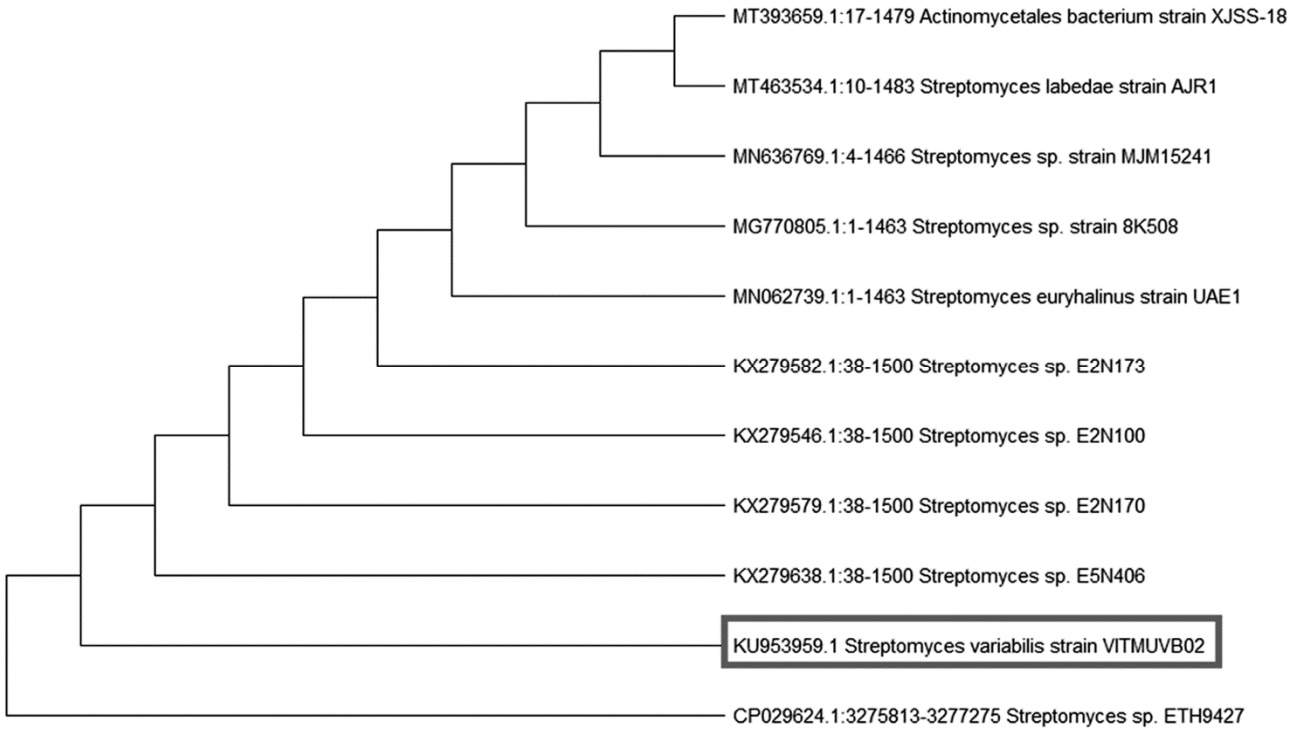

Fig. 10 — Phylogenetic tree analysis of the isolate by neighbor joining method

belong to the genus *Streptomyces*[25]. The mutant was identified to be *Streptomyces variabilis* VITMUVB02 and deposited in the NCBI GenBank (Accession No: KU953959). Phylogenetic tree was constructed as shown in Figure 10. *Streptomyces* sp. is considered to be prolific producers of stable, cellulase free xylanase.

Conclusion

Thus, to conclude, screening of naturally occurring microorganisms might be the most perfect approach to get enzymes for commercial applications. More current techniques are being contrived every day to decrease the utilization of toxic synthetic substances for the treatment of different commercial waste. Introducing advancements in enhancing the introduction of compounds with the aid of novel microorganisms may be a good thought to spare the surrounding from demolition. Subsequently, as have to be apparent by using genetic modifications, as an example, strain improvement strategies or by enhancing the fermentation parameters of those novel enzymes may be multiplied and made beneficial for remedy of environmental wastes.

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Conflict of Interest

The authors do not express any conflict of interest either in the work done and manuscript writing.

Author Contributions

UB & SM: Executed the work and wrote the manuscript, VS: Proposed and developed the idea and performed parts of the study, and KVB: Supervised, executed and monitored and corrected the manuscript.

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