

**Phylogenetic diversity of culturable marine bacteria from mangrove sediments of goa, india: a potential source of xylanases belonging to glycosyl hydrolase family 10**

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Xylan is one of the most abundant polysaccharides present in softwoods and hardwoods. Microbial xylanases mainly mediate its degradation. Xylanase has biotechnological potential with enormous demand in industries. The present study was initiated to investigate the diversity of culturable bacteria in mangrove sediments of Goa (India) and study the xylanase-coding gene in the isolated bacteria. Phylogenetic diversity was determined, in which proteobacteria was the dominant phylum (57%) with the abundance of *Vibrio* (36%) and *Photobacterium* (9%), while the second most abundant phylum *Firmicutes* (40%) was dominated by *Bacillus* (32%). Among all the culturable isolates screened for xylan degradation, the *Bacillus* genus was dominant (86%) xylan degrader. *Bacillus* isolates were further screened for the xylanase gene. The xylanase gene fragments of representative *Bacillus* were sequenced. All sequences matched to glycoside hydrolase (GH) family 10, thus, showing the dominance of GH10 xylanases in the mangroves regions of Goa (India). GH10 xylanases isolated from *Bacillus* sp. showed a broad pH optima ranged from pH 5.0 to 9.0 and temperature optima ranged from 50 to 60 °C. These properties can be of potential interest for a variety of industrial processes where degradation of lignocelluloses is a crucial process.

**Keywords:** Isolation, screening, 16S rRNA gene, dominance, phylum

Mangroves are coastal wetland, salt-tolerant forest ecosystems that cover approximately 60–75% of the tropical and subtropical coastline globally [1]. Mangroves are known to be biologically diverse and productive ecosystems. Microbes found in mangroves are adapted to various physiological conditions such as periodical changes in temperature, water level, salinity and anoxic conditions [2]. Adaptation to the conditions drives them to follow unique metabolic pathways, which ultimately results in nutrient cycling and mineral transformation by the degradation of organic matter. Microorganisms present in such a dynamic environment can be a potential source of biotechnological resources to be exploited [3].

Xylan is a linear polymer of xylose molecules linked through  $\beta$ -1,4-glycosyl bonds. Xylanase breaks down xylan to give xylose and xylo-oligomers. Xylanase has enormous demand in paper and pulp industry, cattle feed and fodder industry for agro-waste treatment, food industry, bioethanol production, breweries, etc. [4]. Xylanases classified into GH families 5, 7, 8, 10, 11 and 43 based on the sequence similarities of the catalytic domain [5]. Among these, GH10 and GH11 xylanases are the most abundant families found in nature, they have unique three-dimensional structures and mechanisms of action [6], and they also differ in substrate specificity to xylan. Members of GH11 considered as true xylanases due to their stringent substrate specificity on xylans. In the case of GH10 xylanases, previous reports indicate that GH10 xylanases can hydrolyze other polysaccharides along with xylan [7].

The nutritive enrichment of the mangrove swamp soils due to rich leaf litter and high organic materials increases the possibility harvesting bacterial community with GH10 and GH11 xylanases. Also, owing to the adaptation to a harsh environment such as high salinity, mangrove bacteria produce compounds that are different from those produced by their terrestrial counterparts [8]. Thus, this study aimed to reveal the distribution of xylanase-producing bacteria in the mangroves regions of Goa, to investigate their phylogenetic diversity and properties and to look at marine bacteria in decayed mangrove leaves as sources of new and interesting xylanases that could be used in the processing of seafood and saline food.

## **Materials and methods**

**Collection of samples.** Mangrove ecosystem sites from different parts of Goa (India) were selected for sediment and water sample collection, which included 7 sites from North Goa and 6 sites from South Goa (Table 1). From each site, 2 sediment and 2 water samples were collected. The superficial layer of sediment (0 - 5 cm) was collected in an aseptic zip lock bag. Surface water samples from different mangrove regions were collected in sterile plastic tubes. In all sampling locations, the temperature was around 28 - 30°C, whereas surface water temperature

was found to be 25 - 27°C. The samples were immediately transported to the laboratory under cold conditions (4°C) for further processing.

**Isolation and screening of xylanase-producing bacteria.** Serial dilutions of sediment ( $10^0$ - $10^{-5}$ ) and water ( $10^0$ - $10^{-3}$ ) samples were made using sterile 0.8% NaCl solution, and 200  $\mu$ L volume was inoculated on sterile medium. Zobell marine agar (ZMA) (HiMedia, India), MacConkey agar (HiMedia, India), thiosulfate citrate bile salts sucrose (TCBS) agar (HiMedia, India), Eosin methylene blue (EMB) agar (HiMedia, India), cetrinide agar (HiMedia, India), and xylose lysine deoxycholate (XLD) agar (HiMedia, India) were used as cultivation media. The media plates with bacterial inoculum were incubated at room temperature (RT) ( $28 \pm 2^\circ\text{C}$ ) for 24 - 48 h. Based on morphological differences such as colony color, shape, size, and appearance (glossy or matt), the individual colony was isolated on a fresh sterile ZMA plate. The obtained isolates were stored at 4°C for further processing.

Isolated colonies were screened for xylan degrading ability by inoculating them on sterile 1.5% agar plates containing basal salt solution (BSS) supplemented with 0.5% beechwood xylan (Sigma-Aldrich, USA) as sole carbon source, along with 0.2% yeast extract (HiMedia, India) and 0.03% peptone (HiMedia, India). The composition of the BSS medium (w/v) was as follows: NaCl, 30.00 g; KCL, 0.75 g; MgSO<sub>4</sub>, 7.00 g; NH<sub>4</sub>Cl, 1.00 g; K<sub>2</sub>HPO<sub>4</sub> (10%), 7.00 mL; KH<sub>2</sub>PO<sub>4</sub> (10%), 3.00 mL; trace metal solution, 1.00 mL; distilled water, 1000 mL. Trace metal solution has the following composition: H<sub>3</sub>B<sub>3</sub>O<sub>3</sub>, 2.85 g; MnCl<sub>2</sub>·7H<sub>2</sub>O, 1.80 g; FeSO<sub>4</sub>·7H<sub>2</sub>O, 2.49 g; Na-tartrate, 1.77 g; CuCl<sub>2</sub>, 0.03 g; ZnCl<sub>2</sub>, 0.02 g; CoCl<sub>2</sub>, 0.04 g; Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O, 0.02 g; distilled water, 1000 mL [9]. The inoculated plates were incubated at RT for 24 - 48 h. After the incubation period, the plates were flooded with 1% Congo Red. Excess stain was discarded, and plates were washed with 1.5 M NaCl to observe the zone of clearance [10].

**Xylanase assay.** Isolates showing zone of clearance on xylan containing plates as mentioned in the above paragraph were further grown at RT in 5 mL of the same medium for 24-48 h under shaking conditions. Cultures grown in broth were centrifuged at 10625×g, 4°C for 10 min, and supernatant was used for determining xylanase activity. Xylanase assay was performed in triplicate for each bacterial supernatant, and activity was determined by the 3,5-di-nitrosalicylic method described by Khandeparker and Bhosle [9].

**Genomic DNA extraction.** All the bacterial isolates were inoculated in 5 mL ZMB separately and incubated for 24 h at RT. Cell pellet of grown culture was collected by centrifugation at 10625×g. Genomic DNA extraction was carried out using GenElute bacterial genomic DNA kit (Sigma-Aldrich, USA) as per the manufacturer's instructions and stored at -20°C until further use.

**Gene amplification and sequencing.** 16S rRNA gene and genes responsible for the production of GH10 and GH11 xylanases were amplified using genomic DNA as a template and set of gene-specific primers (Table 2) [11]. PCR 96-well thermal cycler (Veriti 9902, Applied Biosystems, USA) was used to amplify genes. PCR reaction program was set, which comprised 35 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 1min [12]. Gene sequencing was carried out by the Taq Dye Deoxy Terminator cycle sequencing kit (PerkinElmer, USA) and analyzed using 373A automated DNA sequencer (3130x/Genetic analyzer, Thermo Fisher Scientific, USA) [10].

**Nucleotide data analysis, identification and accession number.** Obtained nucleotide sequence data were further processed and used for alignment with corresponding 16S rRNA gene as well as GH10 and GH11 xylanase genes using BLAST from the NCBI database. This was carried out to obtain the closest bacterial sequence match present within the respective database. Short sequences (<500bp), sequences with low similarity (<90%), and chimeric sequences were removed. The nucleotide sequences obtained in this study were submitted to the NCBI BankIt database under accession numbers MH767087-MH767389. GH10 gene nucleotide sequences were also submitted to GenBank, and accession numbers assigned were MK911042- MK911051.

**Phylogenetic analysis.** 16S rRNA gene sequences were analyzed using NCBI BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). A single multifasta file was created containing a single representative sequence of each genus along with its reference sequence. All the sequences were aligned, and the phylogenetic tree was constructed using MEGA version 6, with a bootstrap value of 1000. Nucleotide sequences of GH10 and GH11 xylanase gene fragments were translated into amino acids. ExpASy translation tool (<https://web.expasy.org/translate/>) was used for translation. Similarities in protein sequences were assessed using the BLASTp program (<http://www.ncbi.nlm.nih.gov/BLAST/>). Sequences were aligned with known sequences from the GenBank database at the protein level using ClustalW. Phylogenetic trees were constructed with MEGA using the neighbor-joining method. Confidence for tree topologies was estimated by bootstrap values based on 1,000 replicates. A total of 10 representative sequences were selected and used as references for the GH10 gene, and one GH11 sequence was used for phylogenetic tree construction.

**Characterization of GH10 xylanase.** The effect of pH and temperature on GH10 xylanase activity was studied using extracellular xylanase from representative *Bacillus* isolates. Fifty mM (pH 5.0 - citrate, pH 6.0 - 8.0 – Na-phosphate and pH 9.0-10.0 – glycine- NaOH) buffer solutions were used to make crude xylanase aliquots in 1:1 ratio. These aliquots were used for xylanase assay.

Similarly, the effect of temperature on GH10 xylanase activity was also studied by varying incubation temperatures of the enzyme-substrate reaction mixture from 30 to 80°C. GH10 xylanase activity was measured as per the xylanase assay protocol mentioned above.

## Results and discussion

Mangroves are complex, unique, and extremely productive ecosystems found in tropical and subtropical intertidal regions of the world. Microbial populations in mangrove sediments have a significant effect on biogeochemical cycles of the coastal ecosystem [13]. Although the mangrove ecosystem is rich in microbial diversity, less than 5% of the species present have been described; in many cases, neither their ecological role nor their technological potential is known [14].

Lignocellulosic materials are difficult to degrade due to their compact structural features, [15] and microorganisms that specialize in lignocellulose degradation are expected to find in specific environments, such as the mangrove ecosystem, which is rich in such biomass.

In an attempt to explore lignocellulose-degrading bacteria which produce xylanases, 303 bacteria were isolated from mangrove areas along the west coast of India, Goa. Sequence data analysis showed a total of 22 cultivable bacterial genera, of which the top 15 genera are represented in Fig. 1. The evolutionary history of culturable bacterial isolates is represented in the form of a phylogenetic tree (Fig. 2). Mangrove habitat showed the dominance of *Proteobacteria* phylum (57%) comprising of genus *Vibrio* (36%) and *Photobacterium* (9%). Oliveira et al. [16] studied the metagenomic bacterial community of mangrove sediment of Goa and reported that the *Proteobacteria* is dominant in the sediment samples comprising 43-46% of the total population. There are reports showing 88% of bacterial communities belong to *Proteobacteria* in mangrove sediment of southeastern Brazil [17]. Also, a previous study conducted on mangrove sediments of Sanya, Hainan Island, China reported predominant bacterial phylotypes to cluster within *Proteobacteria*, *Bacteroidetes*, *Gemmatimonadetes*, *Actinobacteria* and *Firmicutes* where proteobacteria dominated the community [18]. *Proteobacteria* have been suggested to have an important role in the nitrogen, phosphorous and sulphur cycles in mangrove sediments, such as nitrogen fixation, phosphate solubility and sulfate reduction [19]. *Firmicutes* was the second dominant phylum reported in our study with *Bacillus* (32%) genera being the most abundant. Similar observations were also reported from estuarine and mangrove environments [19]. Phylum *Actinobacteria* (1%) and *Fusobacteria* (2%) were also reported in the current study.

Mangrove sediments studied here showed a high abundance of *Vibrio*, and *Bacillus*, while *Vibrio* dominated. High numbers of pathogenic bacteria such as *Escherichia coli*, *Listeria*,

*Salmonella*, and *Vibrio* sp. are previously reported from Goan mangroves by Poharkar et al. [20]. Family *Vibrionaceae* is one of the most fundamental bacterial groups in marine environments. Members of this family often predominate in the bacterial flora of seawater, plankton, and fish. In a survey carried out in the West Pacific Ocean, *Vibrios* accounted for nearly 80% of the bacterial population in surface seawater. The genus *Bacillus* contains phylogenetically and phenotypically diverse species, which are ubiquitous in all the habitats, including terrestrial, freshwater also widely distributed in seawater.

Preliminary screening of xylan-degrading bacteria based on Congo Red assay revealed 94 isolates (32%) representing 6 genera (*Bacillus*, *Vibrio*, *Fusobacterium*, *Catenococcus*, *Staphylococcus*, and *Klebsiella*) with xylan-degrading capacity (Fig. 3). A total of 17 representative isolates from 6 genera were further studied for the quantitative estimation of xylanase activity (Table 3). It was seen that *Bacillus* strains produced a high amount of xylanase compared to *Vibrio* and other genera (Fig. 4). *Bacillus* alone represented 86% of xylan degraders in the study area. *Bacillus* genus has been reported as a potential source of xylanases production, and a large number of bacilli, such as *B. circulans*, *B. stearothermophilus*, *B. amyloliquefaciens*, *B. subtilis*, *B. pumilus*, *B. halodurans* and *B. tequilensis*; [10, 21] have been reported to have significant xylanase activity. In this study, 10 strains of *Bacillus* were found to be involved in hemicellulose degradation. *B. megaterium*, *B. cereus*, *B. subtilis*, *B. proteolyticus*, *B. tropicus*, *B. tenquilitis*, *B. amyloliquefaciens*, *B. safensis*, *B. vietnamensis* and *B. plakortidis* are among them. Some of the *Bacillus* species reported in this study are already reported for hemicellulose degradation [10].

Most of the earlier investigations are focused on microbial ecology-based taxonomic studies, which help in understanding microbial populations in a particular environmental niche [22], although few efforts have been made to study the microbial diversity by exploring the functional potential of microbes [23]. Our understanding of the functional diversity of xylanases of microorganisms from different mangrove regions can possibly help to elucidate the mechanisms of the transformation of organic substances in marine ecosystems and to fulfil the requirements of modern biotechnology. To study the functional diversity of *Bacillus* screened in study area, xylan-degrading *Bacillus*, comprising 10 representative strains, were further screened for the presence of GH10 and GH11 xylanase gene fragments. DNA of each bacterial sample was PCR amplified using GH10 and GH11 gene primers (Table 2). Amplification with GH10 primers indicated the presence of the GH10 xylanase gene in all ten representative *Bacillus* strains isolated from mangrove sediments of Goa. The PCR product was sequenced. BLASTx analysis inferred all sequences showing high similarities to GH10 xylanase gene fragments. Partial sequences of GH10 xylanases obtained in this study and

GH11 xylanase sequence from *Bacillus* sp. BT21 reported earlier [10] were used to construct a phylogenetic tree (Fig. 5). All the sequences reported in this study shared high identity with GH10 xylanases from *Bacillus* sp. where GH11 xylanase was seen as out-group (Fig 7). According to the Pfam database (<http://pfam.sanger.ac.uk/>), GH11 xylanase is mainly distributed in fungi, while GH10 sequences are mainly from bacteria. This suggests that bacteria and fungi are the main producers of GH10 and GH11 xylanases, respectively [11]. Although GH11 xylanases are most frequently chosen for the industrial processes, GH10 enzymes display certain enzymological characteristics that can make them better candidates for enzymatic degradation of lignocelluloses biomass [24]. According to Hu and Saddler [7], GH10 xylanases have higher accessibility towards the xylan backbone within pre-treated biomass, thus, enhancing biomass degradation. This characteristic makes GH10 xylanases a potential enzyme for biomass utilization.

As per the earlier report, bacterial isolates from mangrove regions have potential to produce a broad spectrum of xylanases. According to Guo et al. [25], xylanases from marine bacterium *Glaciecola mesophila* are stable at a lower temperature (25°C). On the contrary, Khandeparker and Bhosle [9, 26] reported xylanases from *Enterobacter* sp. and *Arthrobacter* sp. isolated from an estuarine environment had temperature optima of 100°C. Alkaline pH tolerance of xylanases of bacterial isolates from mangrove reported by Khandeparker and Bhosle [9, 26] and Annamalai et al. [27] was found to be 9.0, whereas, Khandeparker et al. [10] revealed *Bacillus* sp. xylanase having pH optimum of 6.0 which was also isolated from bacteria of mangrove sediment. Palavesam and Somanath [28] reported xylanase, which had organic solvent tolerance ability up to 25%. Xylanases with organic solvent tolerance can be of great potential in industries involved in the saccharification of sugars to produce bioethanol. Salt tolerance of xylanase obtained from bacterial isolates from mangroves have also been widely studied and reported.

Extracellular GH10 xylanases from marine *Bacillus* sp. reported in the current study showed optima pH from 5.0 to 9.0 and temperature optima in the range of 50° to 60°C (Table 3, Fig. 7). *Bacillus* sp. possessing accession number MH767108 and MH767158 produced xylanase with optimum pH of 9.0 and 8.0, respectively, and temperature optimum at 60°C. Xylanases active at high temperatures and alkaline pH are applied in biobleaching of pulp [29]. The effectiveness of bacterial xylanase in pulp bleaching has been studied previously for *Bacillus* sp. [30, 31], where *Bacillus* sp. NCIM 59 xylanase had a temperature and pH optima of 60°C and 6.0, respectively, while *Bacillus* sp. Sam-3 xylanase showed optimum pH 8.0 and temperature optimum of 60°C. *Bacillus circulans* is reported for its xylanase application in biobleaching of eucalyptus pulp [32], the optimum pH for the enzyme was 6.0–7.0, while temperature optimum was 80°C.

In this study, acidic xylanase isolated from *Bacillus sp.* MH767110 and having optima at pH 5.0 and 50°C can also be a potential candidate for the food industry as the main desirable properties for xylanases for this use are high stability and optimum activity at acid pH. As per our knowledge, there are fewer reports on bacterial xylanases in this area; mostly fungal xylanases are reported in the food industry [33]. Singh and Singh [34] have reported *B. subtilis* for improving the nutritional value of poultry feed with optimum pH of 4.0 and temperature optimum of 35 °C. Looking at the properties of the *Bacillus sp.* isolated from mangroves, the potential application of these bacterial cultures in various industries which require acidic/alkaline conditions and elevated temperatures during processing can be targeted which includes fruit juice, bakery, seafood processing industries, and paper and pulp industry [35].

Mangroves are natural terrains which contain diverse microbial communities with immense potential. Mangrove bacteria play a very important role in these dynamic conditions as they are adapted to extreme conditions, like the higher salinity and the low availability of oxygen, which make them a rich source for novel metabolite and enzyme discovery. The current study was carried out to investigate the bacterial community in mangrove sediments of Goa (India) and to explore the potential GH10 xylanase producers from this community. Results obtained clearly indicate that mangrove regions could be potential hotspots for xylan-hydrolyzing bacteria, mainly *Bacillus* strains producing GH10 xylanase. Halophilic, alkalophilic, thermophilic and acidophilic xylanases reported from mangroves areas can be further studied for their novel applications.

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### **COMPLIANCE WITH ETHICAL STANDARDS**

The authors declare that they have no conflict of interest.



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## Legends to figures to the paper of Parab et al.

**Fig. 1.** Total bacterial diversity in mangroves of Goa region.

**Fig. 2.** The evolutionary history of culturable *Proteobacteria* (a) and *Firmicutes* and other bacterial (b) isolates from mangroves of Goa. \*Highlighted strains possess xylan-degrading capacity.

**Fig. 3.** Diversity of xylanase-producing bacteria in mangroves of Goa region.

**Fig. 4.** Xylanase production by xylanase-producing bacterial representatives. 1 - *Bacillus* MH767089; 2 - *Fusobacterium* MH767093; 3 - *Bacillus* MH767098; 4 - *Vibrio* MH767099; 5 - *Bacillus* MH767100; 6 - *Fusobacterium* MH767101; 7 - *Staphylococcus* MH767105; 8 - *Bacillus* MH767158; 9 - *Bacillus* MH767108; 10 - *Bacillus* MH767109; 11 - *Bacillus* MH767110; 12 - *Bacillus* MH767164; 13 - *Vibrio* MH767266; 14 - *Catenococcusthiocycli* MH767274; 15 - *Klebsiella* MH767341; 16 - *Bacillus* MH767342; 17 - *Bacillus* MH767343.

**Fig. 5.** Phylogenetic tree constructed by MEGA using the neighbor-joining method. The lengths of the branches indicate the relative divergence among the amino acid sequences.

**Fig. 6.** Phylogenetic relationships of a GH10 xylanase of *Bacillus* species from mangroves with GH11 xylanase of previously reported *Bacillus* species constructed using NJ-tree method with bootstrap values (1000 replicates) as a percentage at the nodes.

**Fig. 7.** Temperature and pH optima of a GH10 xylanase from *Bacillus* species. 1 – *Bacillus* MH767089; 2 - *Bacillus* MH767098; 3 - *Bacillus* MH767100; 4 - *Bacillus* MH765158; 5 - *Bacillus* MH767108; 6 - *Bacillus* MH767109; 7 - *Bacillus* MH767110; 8 - *Bacillus* MH767164; 9 - *Bacillus* MH767342; 10 - *Bacillus* MH767343.

**Table 1.** Name and location details of bacterial sampling sites.

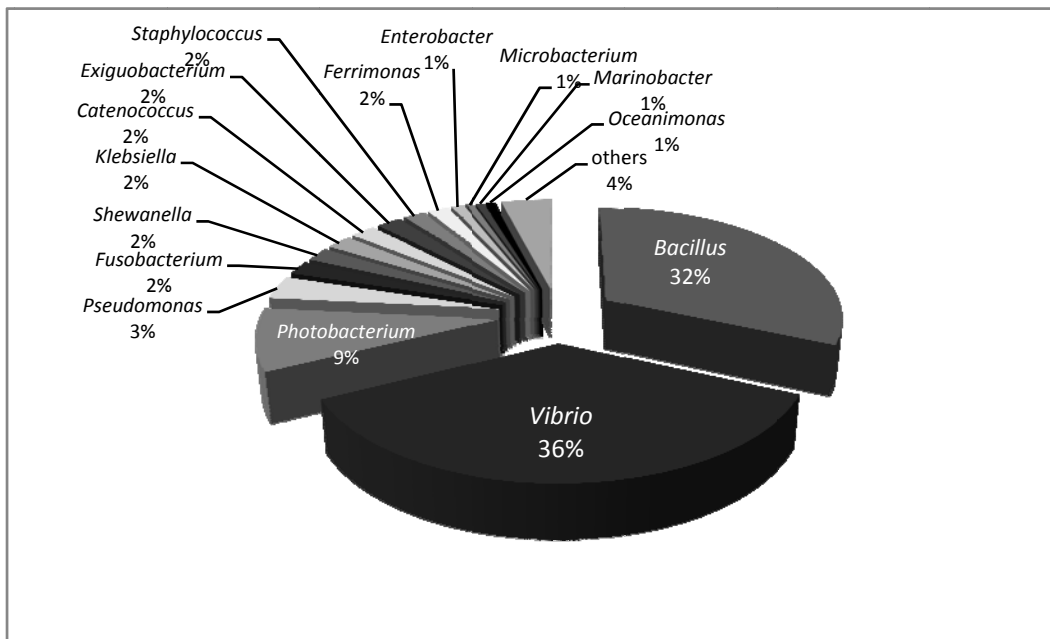
<b>North-Goa, India</b>		<b>South-Goa, India</b>	
<b>Name of the sampling site</b>	<b>Location on map</b>	<b>Name of the sampling site</b>	<b>Location on map</b>
Vagalim	15°38'06.1"N 73°46'13.0"E	Cortalim	15°24'17.7"N 73°53'49.9"E
Shiollim	15°36'55.1"N 73°45'16.6"E	Chandor	15°19'08.7"N 74°00'23.5"E
Sarmanas(Piligao)	15°32'39.8"N 73°57'37.7"E	Navelim	15°14'31.8"N 73°58'29.1"E
Marcel	15°32'42.0"N 73°56'39.4"E	Assolna	15°10'00.7"N 73°57'06.7"E
Diwar	15°30'18.6"N 73°53'39.8"E	Talpona	14°59'06.9"N 74°02'35.7"E
Chorao	15°30'42.4"N 73°51'29.6"E	Galgibag	14°57'38.1"N 74°03'03.7"E
4-Pillar (Panjim)	15°29'13.1"N 73°50'02.9"E		

**Table 2.** List of primers used for gene amplification.

Gene fragment to be amplified	Set of primer used	Nucleotide sequence of primer
16S rRNA gene	27F	5'-AGAGTTTGATCCTGGCTCAG-3'
	1492R	5'-GGTTACCTTGTTACGACTT-3'
Glycosyl hydrolase 10 gene (GH10)	X10-F	5'-CTACGACTGGGAYGTNIBSAAYGA-3'
	X10-R	5'-GTGACTCTGGAWRCCIABNCCRT-3'
Glycosyl hydrolase 11 gene (GH11)	X11-F	5'-CCGCACGGACCAGTAYTGNKIRAANGT-3'
	X11-R	5-'AACTGCTACCTGKCNITNTAYGGNTTGG-3'

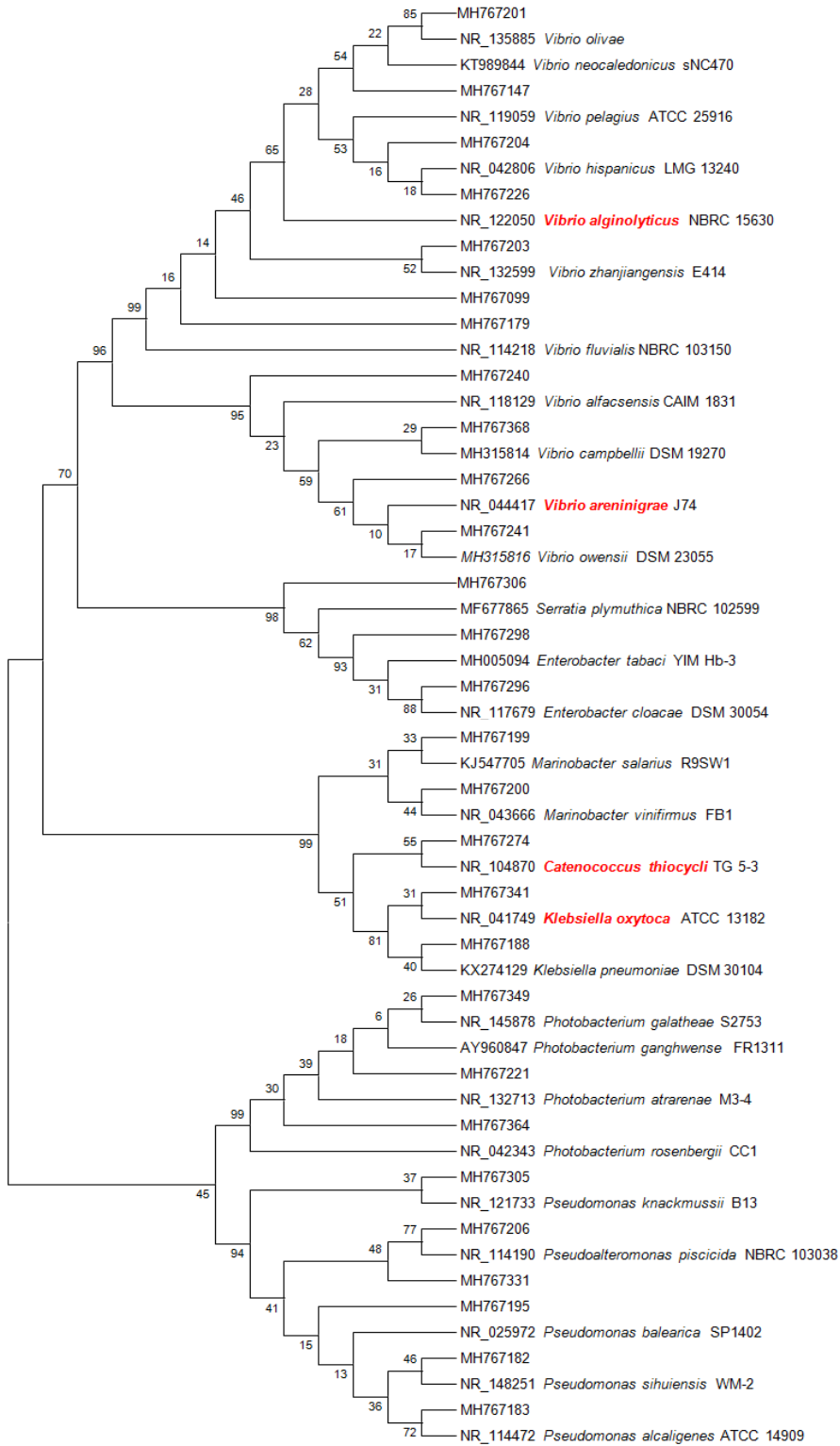
**Table 3.** Xylanase activity and pH and temperature optima of representative bacterial isolates.

Accession number obtained	Nearest neighbor in NCBI database	Similarity %	Xylanase activity, IU/mL	Standard error	Optimum pH	Optimum temperature, °C
MH767089	<i>Bacillus altitudinis</i> 41KF2b	99.69	0.722	0.015	6.0	50
MH767093	<i>Fusobacterium varium</i>	81.78	0.046	0.017	6.0	60
MH767098	<i>Bacillus subtilis</i> subsp. spizizenii strain NBRC 101239	97.40	0.473	0.020	7.0	50
MH767099	<i>Vibrio alginolyticus</i> strain NBRC 15630	99.21	0.239	0.013		
MH767100	<i>Bacillus proteolyticus</i> strain MCCC 1A00365	99.86	0.732	0.016	6.0	55-60
MH767101	<i>Fusobacterium necrogenes</i>	83.19	0.047	0.022	6.0-7.0	40
MH767105	<i>Staphylococcus edaphicus</i> strain CCM 8730	99.89	0.214	0.110		
MH767158	<i>Bacillus safensis</i> strain FO-36b	99.48	0.841	0.041	8.0	60
MH767108	<i>Bacillus aerius</i> strain 24K	99.62	0.946	0.010	9.0	50
MH767109	<i>Bacillus subtilis</i> strain JCM 1465	95.80	0.353	0.009	6.0	60
MH767110	<i>Bacillus cereus</i> strain IAM 12605	99.85	0.134	0.031	5.0-6.0	50
MH767164	<i>Bacillus amyloliquefaciens</i> strain MPA 1034	99.04	0.617	0.023	6.0	55-60
MH767266	<i>Vibrio areninigræ</i> strain J74	96.54	0.306	0.012		
MH767274	<i>Catenococcus thiocycli</i> strain TG 5-3	97.67	0.072	0.030	7.0	60
MH767341	<i>Klebsiella oxytoca</i> strain ATCC 13182	97.65	0.281	0.017		
MH767342	<i>Bacillus plakortidis</i> strain P203	96.29	0.513	0.012	7.0-8.0	55-60
MH767343	<i>Bacillus vietnamensis</i> strain NBRC 101237	97.82	0.189	0.010	7.0	50



**Fig. 1.**

(a)





(b)

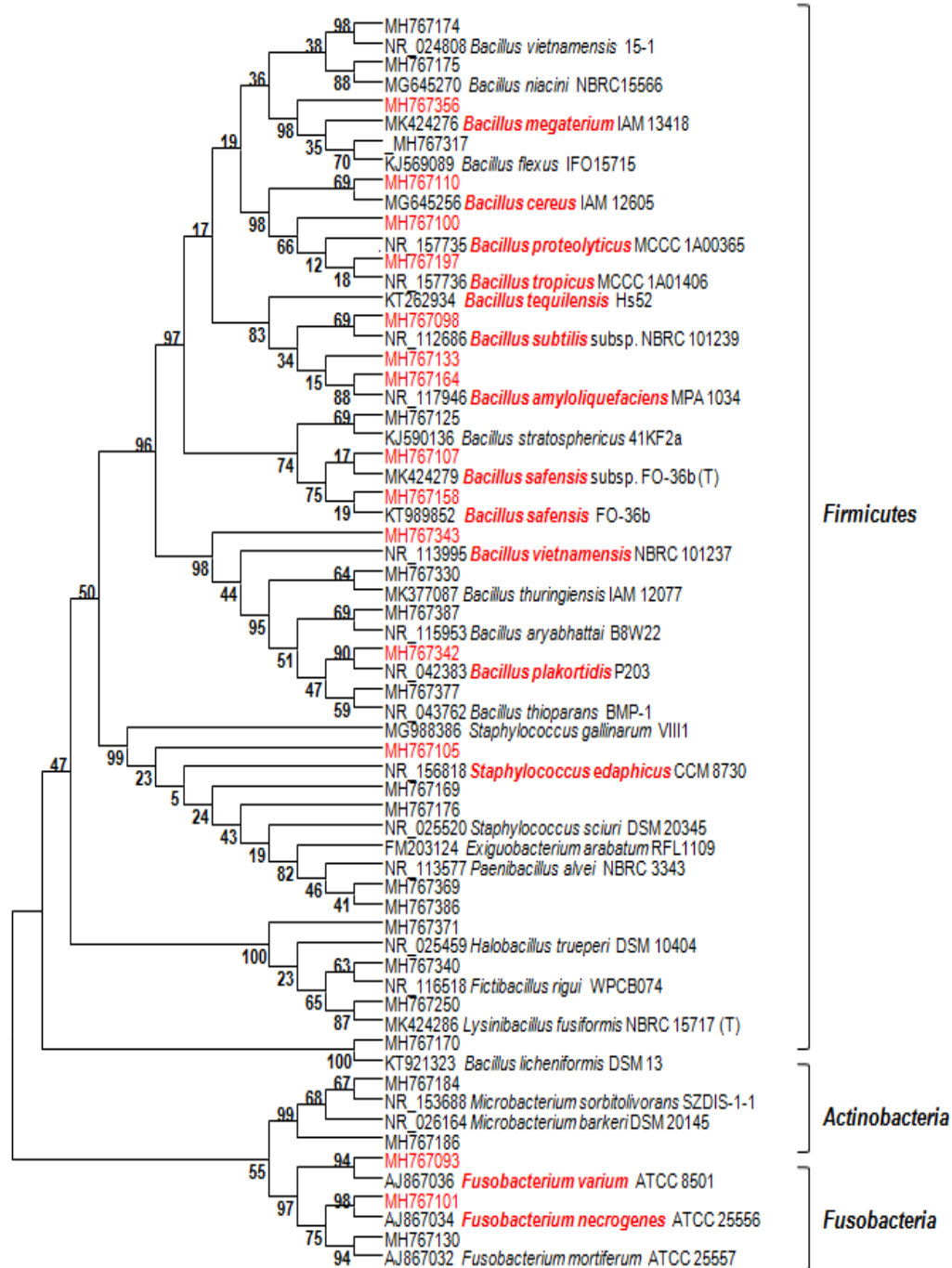
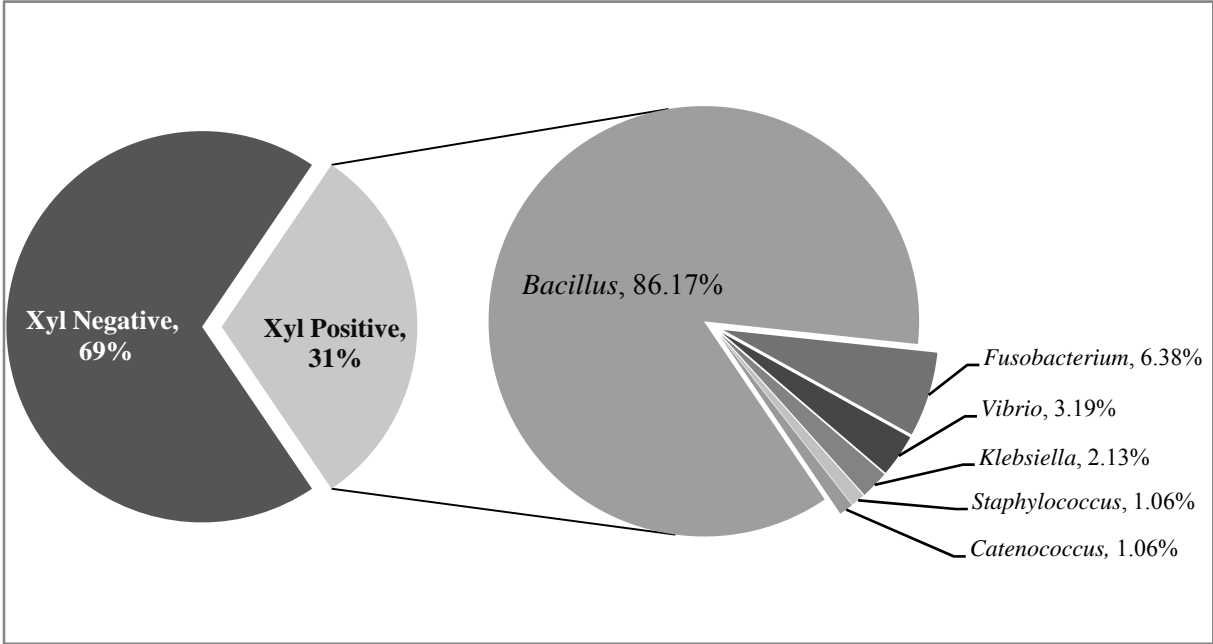
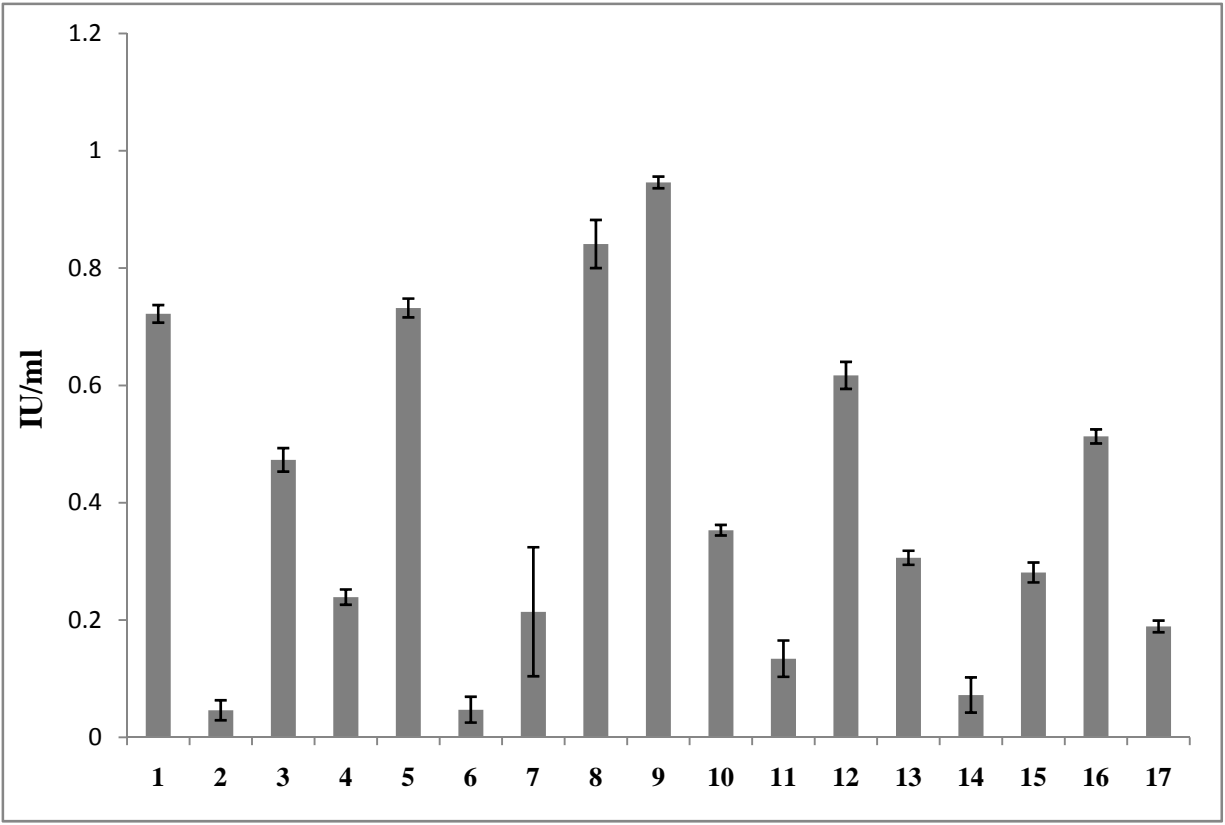


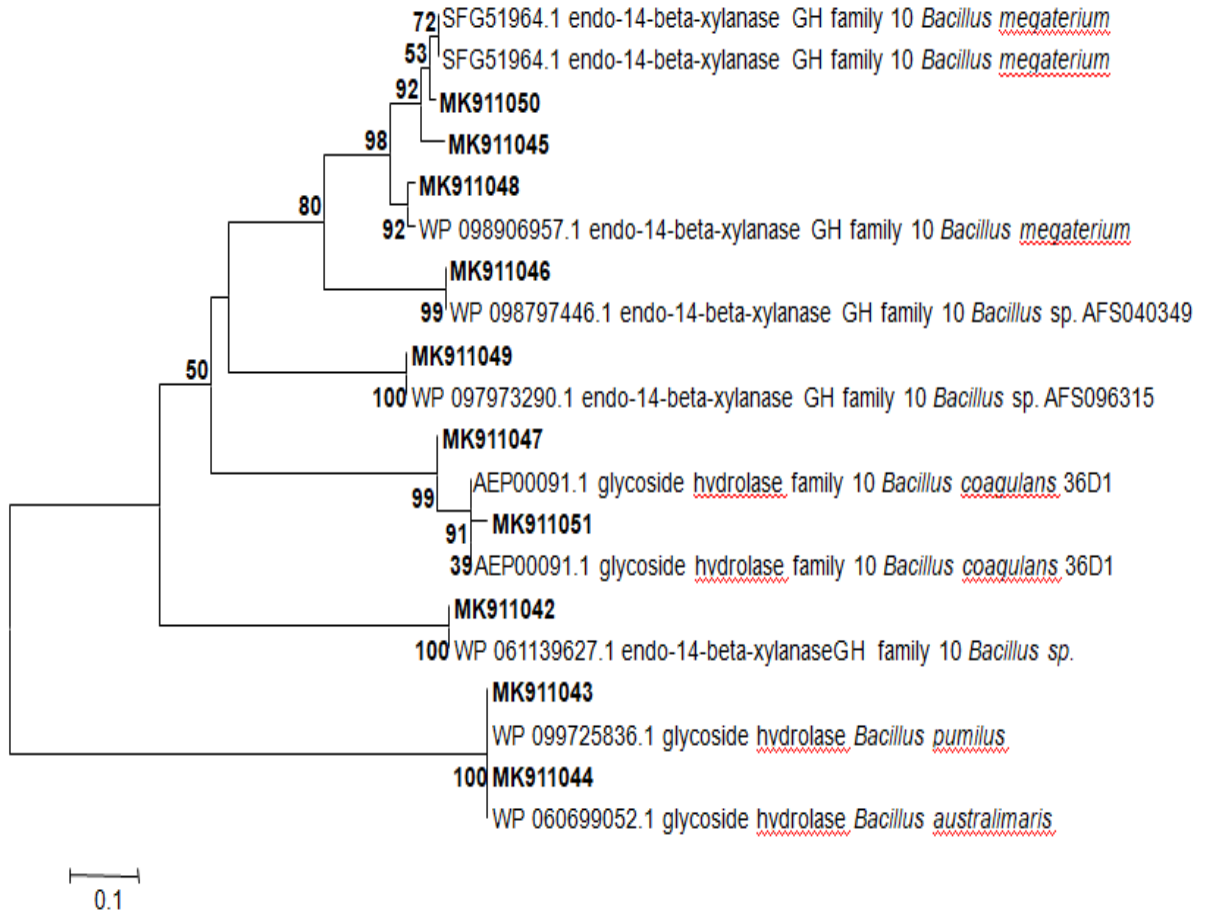
Fig. 2.



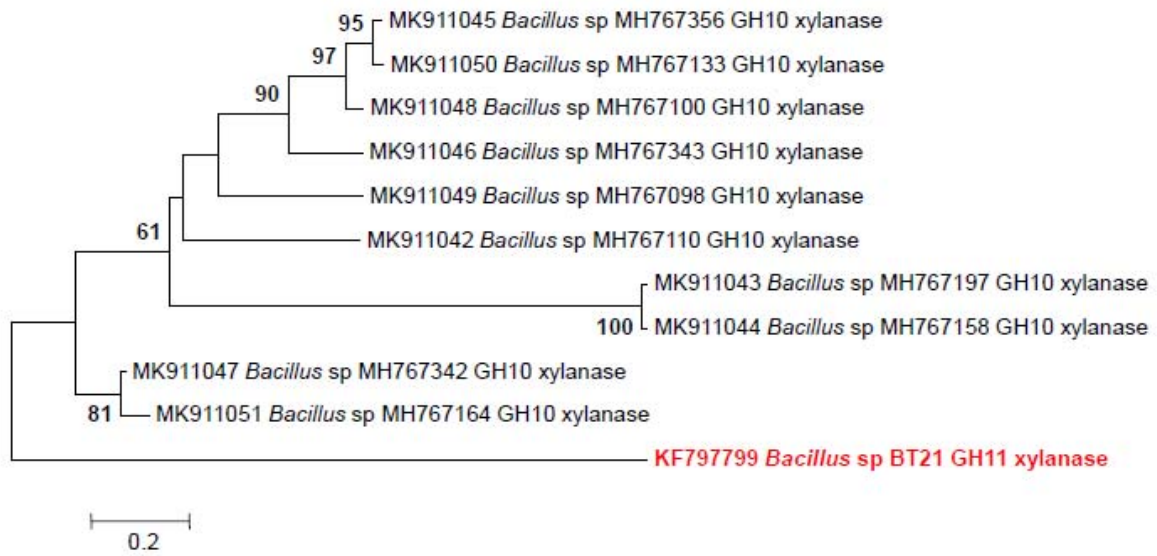
**Fig. 3.**



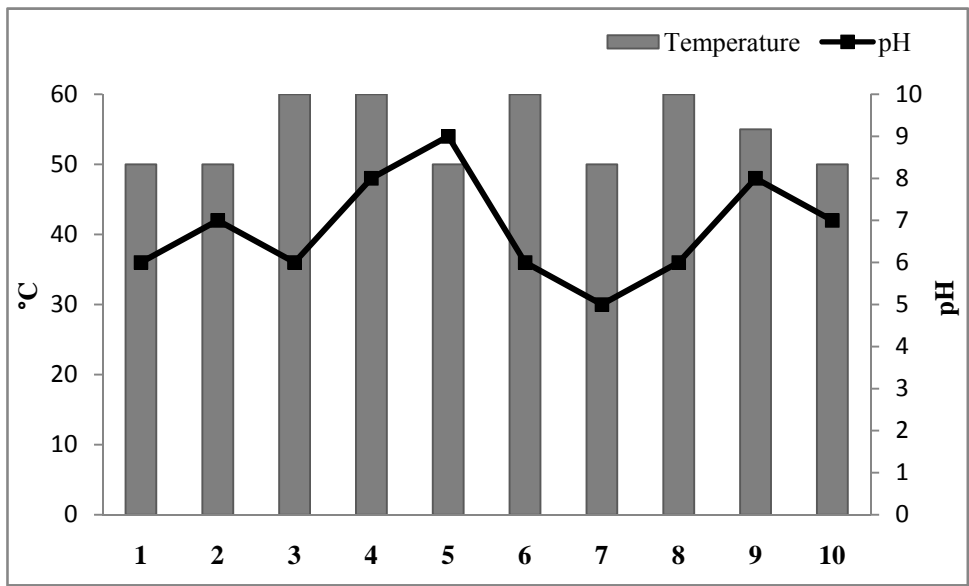
**Fig. 4.**



**Fig. 5.**



**Fig. 6.**



**Fig. 7.**