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SCREENING OF XYLANASE PRODUCING MICROORGANISMS

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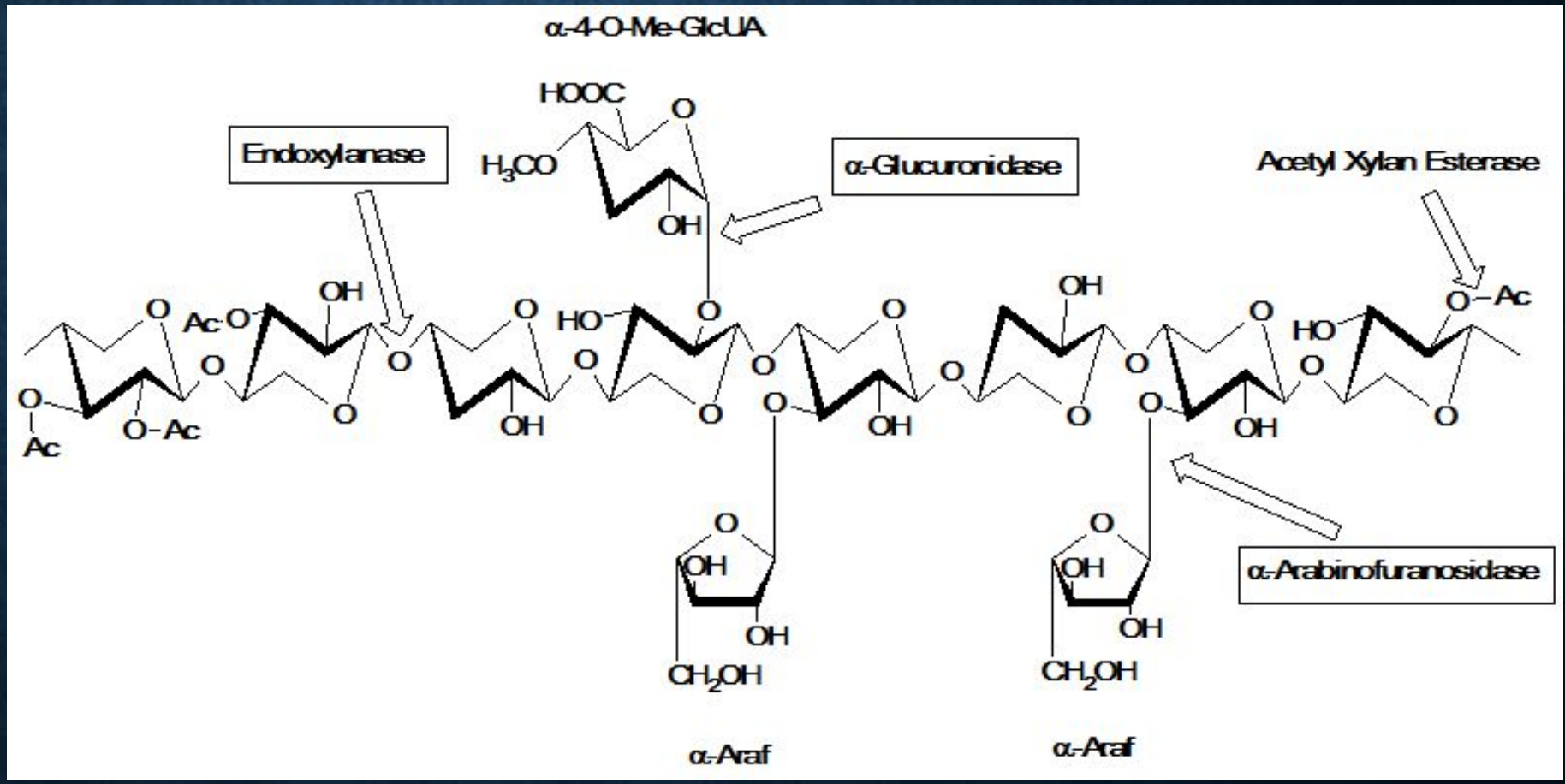
INTRODUCTION

- THEME: Xylanases, a group of enzymes that hydrolyse xylan backbone into small oligomers, are produced by a variety of sources, including bacteria, fungi, yeast, algae, seeds, snails, crustaceans.
- IMPORTANCE: The use of xylanase in different industries (bio-processing of fabrics, biobleaching of pulp, waste paper recycling, bioconversion into higher value products, food and feed) has increased significantly over the years. Thus, the interest in this field has increased, scientists isolating newer microbial strains for xylanase production
- PURPOSE: The study aim was to test different microbial strains regarding their ability to produce xylanases.

XYLAN AND XYLANASES

- Xylan
- Xylanases:
  - β-1,4-endoxylanase (E.C.3.2.1.8)
  - β-xylosidase (E.C.3.2.1.37)
  - α-glucuronidase (E.C.3.2.1.1)
  - α-L-arabinofuranosidase (E.C.3.2.1.55)
  - acetyl xylan esterase (E.C. 3.1.1.6)
  - feruloyl esterase (E.C. 3.1.1.73)



Nair and Shashidhar , 2008

SOURCES OF XYLANASE

- Main sources for these enzymes are fungi and bacteria. According to the source, xylanases have different characteristics which makes them useful for an application or another.
- Xylanases produced by aerobic bacteria (*Bacillus spp.*, *Pseudomonas spp.*, *Streptomyces spp*. etc) are efficient in a broader pH range of 5 to 9 and temperature of 35-60ºC. They are useful in different industries due to their alkali tolerance and thermostability, like the pulp and paper industry.
- Fungal xylanases (*Aspergillus spp.*, *Fusarium spp.*, *Penicillium spp.*, *Trichoderma spp.* etc) are effective at a pH range of 4 to 6 and temperature below 50ºC, thus being used in limited industrial applications.They are important producers due to their higher xylanase activity compared with bacteria or yeast, their high yields and extracellular release of the enzymes.

OBJECTIVES

- Objective 1: Qualitative screeening of microbial strains for xylan degradation
- Objective 2: Preliminary data concerning the quantitative screening of the isolated strains
- Objective 3: The influence of the culture media in the screening process

OBJECTIVE 1: QUALITATIVE SCREEENING OF MICROBIAL STRAINS

Microorganisms used in experiments

BACTERIA

- *Bacillus amyloliquefaciens B4*
- *B.amyloliquefaciens BN7*
- *B.licheniformis B40*
- *B.subtilis ICPC*
- *B.subtilis 832 S*
- *B.subtilis USA 2*
- *B.subtilis ATCC 11774*
- *Bacillus spp. B5*
- *Bacillus spp. B6*
- *B.amyloliquefaciens BIR*
- *Streptomyces spp. S6*
- *Streptomyces spp Str S1*
- *Streptomyces spp Str. S9*

FUNGI

- *Trametes versicolor*
- *Alternaria sp.*
- *Rhizoctonia solani*
- *Aspergillus flavus T11*
- *A.flavus AFR*
- *A.niger prot.*
- *A.niger An4*
- *A.brasiliensis ATCC 16404*
- *Trichoderma atroviride TK20*
- *T.viride UV*
- *T. viride Tv 2*
- *T.harzianum TK25*
- *T. harzianum P8*
- *Fusarium graminearum G82*
- *F. oxysporum*
- *F.culmorum FC28*
- *Penicillium digitatum*
- *P. verruculosum KUCC 47345*

OBJECTIVE 1: QUALITATIVE SCREEENING OF MICROBIAL STRAINS

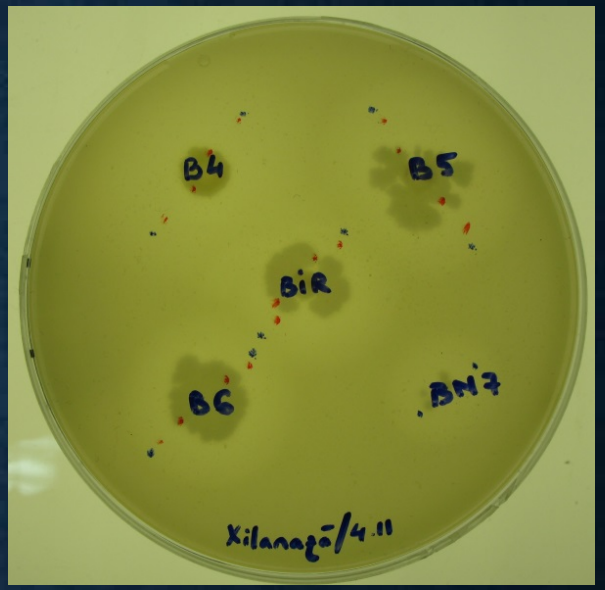
- **METHOD**: Plate screening method

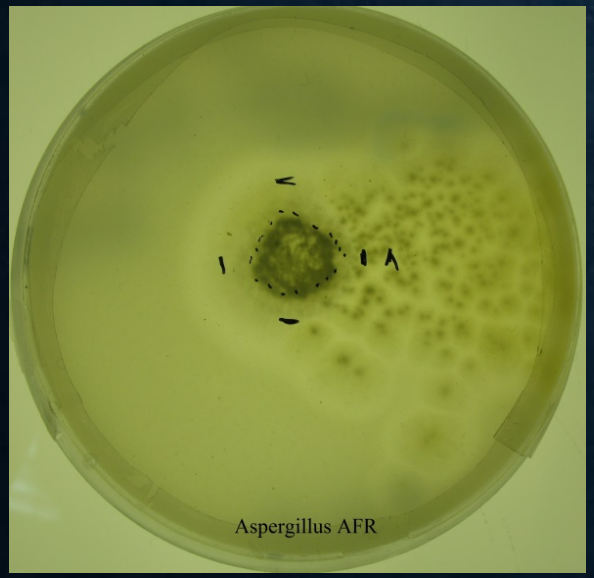
- **CULTURE MEDIA**:
  - Minimal agar medium with 0.5% oat spelt xylan as the only carbon source
  - Different medium constituents for the bacterial and the fungal strains

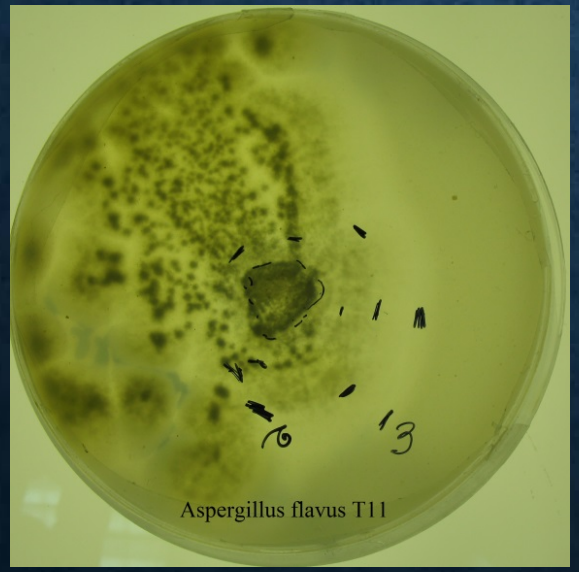
- **DETECTION OF XYLANOLYTIC ACTIVITY**: Based on the clear zones of hydrolysis of xylan around the microbial colonies.

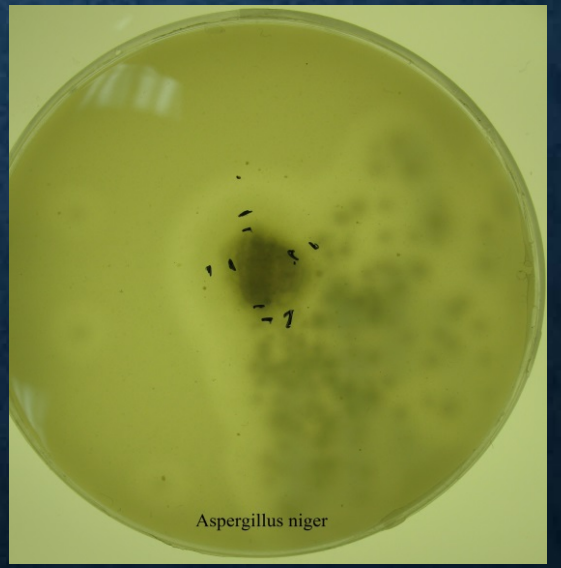
OBJECTIVE 1: QUALITATIVE SCREEENING OF MICROBIAL STRAINS

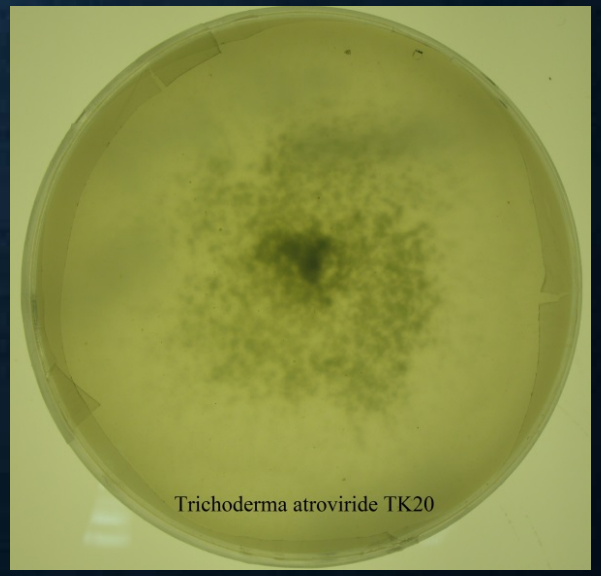
- The plates were incubated at 28±2ºC for 3 to 10 days, depending on the strain and analyzed at every 24 hours for the occurrence and evaluation of the halo diameter.





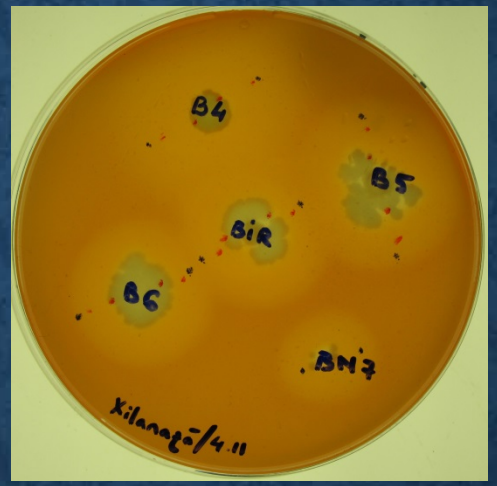


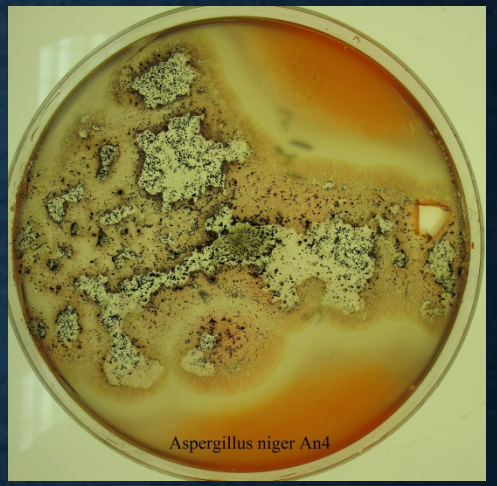


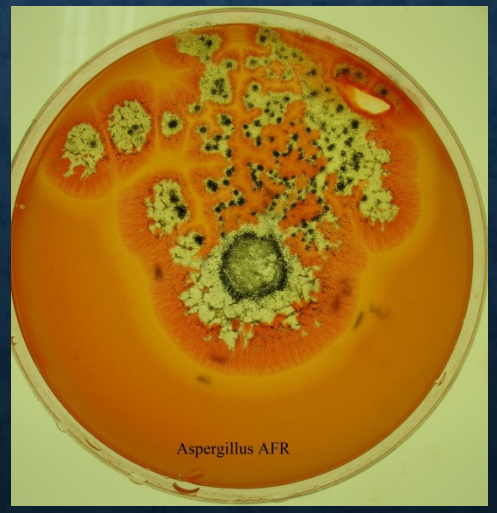


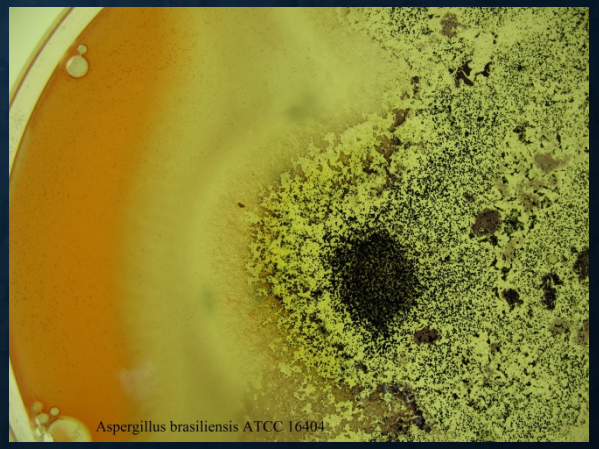
OBJECTIVE 1: QUALITATIVE SCREEENING OF MICROBIAL STRAINS

- The use of Congo red dye improved the halo evaluation
- After the evaluation, the microbial strains that showed xylanase activity were: *Bacillus amyloliquefaciens*, *Aspergillus flavus*, *A.niger*, *A.brasiliensis*, *Trichoderma atroviride*, *T.harzianum*, *T. viride*, *Rhizoctonia solani*, *Penicillium digitatum*, *Fusarium graminearum*, *F. oxysporum*, *P. verruculosum*









OBJECTIVE 2: PRELIMINARY DATA CONCERNING THE QUANTITATIVE SCREENING OF THE ISOLATED STRAINS

Xylanase activity – fungal strains

- 17 microbial strains were selected for further analysis
- Cultivation – liquid medium with 0.5% oat spelt xylan as the carbon source
- Incubation: 28±2ºC in an incubator with shaker at 120 rpm for 5-9 days
- Xylanase activity was determined according to the DNS assay for reducing sugars
- Protein assay by Lowry method was carried out in order to calculate the specific enzymatic activity.

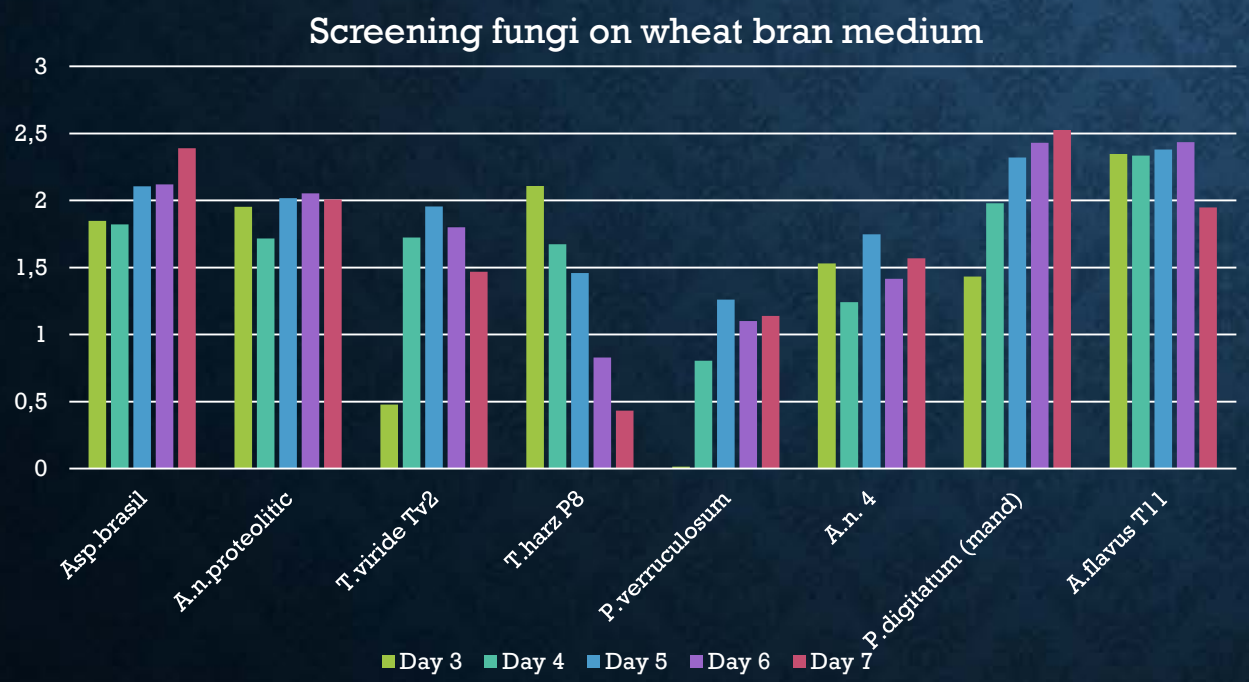
OBJECTIVE 2: PRELIMINARY DATA CONCERNING THE QUANTITATIVE SCREENING OF THE ISOLATED STRAINS

Microorganism	Xylanase activity (µmol/mL/min)
B.amyloliquefaciens B4	1.71
B.amyloliquefaciens BN7	0.22
A.flavus AFR	2.71
A. flavus T11	2.42
A.niger An4	2.03
A.brasiliensis	3.05
T.atroviride TK20	1.89
T.harzianum TK25	2.01
T. viride Tv2	2.03
T.viride UV	0.81
F.culmorum FC 28	0.72
Rhizoctonia solani	0.09
P.digitatum	2.42
F.graminearum G82	0.32
F. oxysporum	0.41
T. harzianum P8.	2.9
A.niger prot.	2.03

Microorganism	Specific enzymatic activity (µmol/mg protein)
B.amyloliquefaciens B4	1.35
B.amyloliquefaciens BN7	0.11
A.flavus AFR	0.48
A. flavus T11	0.57
A.niger An4	1.87
A.brasiliensis	1.14
T. viride Tv2	1.06
P.digitatum	0.49
T. harzianum P8.	0.74
A.niger prot.	1.03

OBJECTIVE 3: THE INFLUENCE OF THE CULTURE MEDIA IN THE SCREENING PROCESS

Cultivation - liquid medium with 0.5% wheat bran as the only carbon source



Microorganism	Xylanase activity (µmol/mL/min)	
	Xylan medium	Wheat bran medium
B.amyloliquefaciens B4	1.71	1.65
A. flavus AFR	2.71	1.2
A. flavus T11	2.42	2.43
A.niger An4	2.03	1.74
A.brasiliensis	3.05	2.39
T. viride Tv2	2.03	1.95
P. digitatum	2.42	2.52
T. harzianum P8	2.9	2.1
A.niger prot.	2.63	2.05

CONCLUSIONS

- In this work, 31 microbial strains were subjected to a screening for their ability of xylan degradation. Among them, 17 were examined for xylanase activity from a qualitative point of view.
- The highest xylanase activity was obtained with *Bacillus amyloliquefaciens B4* and *Aspergillus brasiliensis ATCC 16404*.
- The best specific xylanase activities were detected in *B.amyloliquefaciens* B4 and in *Aspergillus niger An4*.
- A less studied microorganism for xylan degradation, *Penicillium digitatum*, showed a high xylanase activity in both xylan medium and wheat bran medium.

CONCLUSIONS

- The cultivation of selected microorganisms in xylan medium and in a medium where the carbon source was represented by wheat bran allows the observation that no significant differences in enzymatic activity are related to the medium composition in our experimental conditions.
- It was determined that wheat bran could be useful as a cheap alternative substrate for cultivation of xylanase producing microorganisms.
- These results are significant for further studies regarding lignocellulosic biomass biodegradation by a microbial enzymatic complex.

BIBLIOGRAPHY

- Ja'afaru M.I., 2013. Screening of fungi isolated from environmental samples for xylanase and cellulase production. ISRN microbiology 2013: 1-7
- Mandal A., 2015. Review on microbial xylanases and their applications. International Journal of Life Sciences, 4(3):178-187
- Miller G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry 31(3): 426-428
- Nair S.G., Shashidhar S., 2008. Fungal xylanase production under solid state and submerged fermentation conditions. African Journal of Microbiology Research, 2(4):82-86
- Polizeli M.L.T.M., Rizzatti A.C.S., Monti R., Terezni H.F., Jorge J.A.,Amorim D.S., 2005. Xylanases from fungi: properties and industrial applications. Applied Microbiology and Biotechnology, 67:577-591
- Techapun C., Poosaran N., Watanabe M., Sasaki K., 2003. Thermostable and alkaline-tolerant microbial cellulase-free xylanases produced from agricultural wastes and the properties required for use in pulp bleaching bioprocess: a review. Process Biochemistry, 38:1327-1340
- Ten L.N., Im W.T., Kim M.K., Kang M. S., Lee S.T., 2004. Development of a plate technique for screening of polisaccharide-degrading microorganisms by using a mixture of insoluble chromogenic substrates. Journal of Microbiological Methods 56(3): 375-382
- Uday U.S.P., Choudhury P., Bandopadhyay T.K., Bhunia B., 2016. Classification, mode of action and production strategy of xylanase and its application for biofuel production from water hyacinth. International Journal of Biological Macromolecules, 82:1041-1054

Thank you !