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Extremophilic enzymes from enriched cultures with wheat straw

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The demand for functional ingredients (such as prebiotics) has risen as an effect of increasing health consciousness. Amongst them, xylooligosaccharides (XOS) shows prebiotic effect at lower doses, in contrast with fructooligosaccharides (FOS), isooligosaccharides (IOS) or galactooligosaccharides (GOS).

We have developed a process for obtaining XOS from wheat straw applying an alkali pre-treatment to the raw material followed by enzymatic hydrolysis with xylanases. Using thermophilic xylanases, the arabinoxylan extraction from the solid wheat straw was increased significantly compared to mesophilic counterparts. However, a neutralization (and washing) step of the pre-treated wheat straw was still needed before the enzymatic treatment. Therefore, finding thermophilic and alkaliphilic endo-xylanases would offer a substantial advantage for the overall optimization of XOS production.

In order to increase the possibilities of finding endo-xylanases and beta-xylosidases in thermophilic and alkaliphilic environments able to act on wheat straw xylan, we carried out an enriched culture using wheat straw as inoculum and the sole C source and sterile Tinteiro thermal spring waters (Ourense) as solvent and source of mineral micronutrients. Tinteiro thermal waters are characterized by a high pH (8.6), moderate temperature (50 ºC) and a high content in sulfur and carbonate compounds.

Samples were taken periodically from the culture medium and were grown in xylan-agar plates. Positives strains were visualized with Congo-red stain and identified by 16S gene sequencing. Their full-length 16S genes were amplified and sequenced. We have obtained two promising candidates strains. Their 16S rRNA gene sequences exhibited similarity to genus *Paenibacillus* and *Neobacillus*. From the *Paenibacillus* strain, identified as *P. barengoltzii*, produces different lignocellulose degrading enzymes have been described. The *Neobacillus* strain was related to *N. thermocopriae* and *N. sedimentimangrovi* and presents highly promising enzymes. Endo-xylanase highlights for its ability to work at pH 7-10, with maximal activity at 10. Also, beta-xylosidase was detected which presumably shares alkaliphilic characteristics. Higher characterization of the enzymes should be performed prior to industrial XOS production.

In conclusion, a microorganism characterized as *Neobacillus sp*. that produces endo-xylanases with extremophile characteristics was isolated from a non-extremophile environment incubating the inoculum at the desired conditions of the final application.

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