# Coning of a novel xylanase gene from the mixed genomic DNA of rumen fungi and its expression in *Lactobacillus reuteri*

**Je-Ruei Liu(劉嘉睿)[1,*], Bi Yu(余碧)[2], Shiou-Hua Lin(林淑華)[3], Kuo-Joan Cheng(鄭國展)[4], and Yo-Chia Chen(陳又嘉)[3]**

**[1] Department of Biotechnology, National Formosa University; [2] Department of Animal Science, National Chung-Hsing University; [3] Institute of Biotechnology, National Pingtung University of Science and Technology; [4] Institute of BioAgricultural Sciences, Academia Sinica**

* Assistant Professor, 64 Wenhua Road, Huwei, Yunlin, Taiwan.

Tel: (05)6315506, Fax: (05)6315502, E-mail: jrliu@sunws.nfu.edu.tw

## Abstract

Rumen fungi are able to degrade the most-resistant plant cell-wall polymers, thus, the rumen fungal population represents a rich and underutilized source of novel enzymes with tremendous potential for industrial and agricultural applications. Those cellulases and xylanase produced by these fungi are among the most-active fibrolytic enzymes described to date. To the best of our knowledge, however, it is estimated that more than 90% of the total microbial population can not be isolated by currently known methods, and the isolation of such microbes will become one of the limitations to future successes at attempting to isolate novel genes and to comprehend the fibrolytic systems from rumen ecosystem. In order to overcome such a problem and to avoid complicated microbe-isolation protocols, it would appear feasible to directly obtain mixed genomic DNA from unpurified ruminal microbes as a gene source. In this study, we directly obtained a novel xylanase gene, *xynR8*, from the mixed DNA samples prepared from unpurified rumen fungal cultures by means of a polymerase chain reaction (PCR), and cloned this xylanase gene into *Lactobacillus reuteri* Pg4, a strain of probiotic bacteria isolated from the gastrointestinal tract of healthy broiler chickens. The DNA sequence of *xynR8* revealed that the gene was 884 bp in size and encoded amino acid sequences with a molecular weight of 27.9 KDa. XynR8 belonged to glycosyl hydrolase family 11, and the catalytic site residues were also found in its amino acid sequence. The main hydrolysis products of XynR8 were xylobiose, xylotriose and xylotetrose, which indicated that it belonged to the endoxylanase. The *xynR8* gene was constructed so as to express and secrete under the control of the *Lactococcus lactis lac* A promoter and its secretion signal, and transformed into *L. reuteri* Pg4. The *L. reuteri* transformants harboring *xynR8* not only acquired the capacity to break down xylan but also maintained their high adhesion efficiency to mucin and mucus, and their resistance to bile salts and acid.

**Keywords:** Lactobacillus reuteri, Orpinomyces, Rumen fungi, Xylanase gene.