Available online at www.sciencedirect.com
ScienceDirect
Enzyme and Microbial Technology 43 (2008) 137–143
ENZYME AND MICROBIAL TECHNOLOGY
www.elsevier.com/locate/emt

Quantitative linkage mapping of lignin-degrading enzymatic activities in *Pleurotus ostreatus*

Francisco Santoyo [a], Aldo E. González [b], María C. Terrón [b], Lucía Ramírez [a], Antonio G. Pisabarro [a,*]

[a] *Department of Agrarian Production, Public University of Navarre, 31006 Pamplona, Spain*
[b] *Centro de Investigaciones Biológicas CIB, CSIC, 28040 Madrid, Spain*

Received 9 July 2007; received in revised form 29 October 2007; accepted 12 November 2007

Abstract

Pleurotus ostreatus and *Phanerochaete chrysosporium* are two model lignin-degrading basidiomycetes. The genome of *P. chrysosporium* has been released and that of *P. ostreatus* will be available soon. The lignin-degrading strategies of these two fungi are, however, different as *P. ostreatus* lacks lignin peroxidases (LiP) whereas *P. chrysosporium* lacks phenol oxidases (Pox). Both fungi, in addition, contain genes coding for Mn-oxidizing peroxidases [manganese (MnP) and versatile (VP) peroxidases in *P. ostreatus* (*mnp* genes), and manganese peroxidases in *P. chrysosporium*]. We have mapped genetically the genes coding for different ligninolytic enzymes in *P. ostreatus* and we have found them linked to chromosomes IV (*mnp1*, VP activity), V (*mnp3*, MnP activity) and VI (*mnp2,* VP activity; *pox1* and *poxC*, Pox activity). If the enzymatic activities are mapped as quantitative traits instead of mapping the structural genes, the genome regions containing regulators of these activities will be detected in addition to the structural genes. We have used this approach for the Pox and MnP/VP activities and we have identified various genomic regions that control them and that map to linkage groups different from those where the corresponding structural genes had been previously mapped to. These new sites could code for regulatory genes. Using a combination of this information and classic genetic techniques, we have selected new *P. ostreatus* strains enriched in specific activities while maintaining a low biomass production in submerged cultures.

Keywords: *Pleurotus ostreatus*; Manganese peroxidase; Versatile peroxidase; Phenol oxidase; QTL mapping; aQTL; Enzymatic activity

1. Introduction

The production of fungal lignocellulolytic enzymes is of importance for the treatment of paper pulp and in the development of new biotechnologies for the pretreatment lignocellulose material to be used as feedstock for the production of bioalcohol [1]. The lignocellulolytic process includes the degradation of lignin by different types of specialized peroxidases and the degradation of cellulose by carbohydrate active enzymes. In both cases, these activities are controlled by gene families whose members or number varies among different species.

Lignin is mainly degraded by lignin peroxidases (LiP, E.C. 1.11.1.14), versatile peroxidases (VP, EC 1.11.1.16), manganese peroxidases (MnP, E.C. 1.11.1.13), and phenol oxidases (Pox) also known as laccases (E.C. 1.10.3.2) [2,3]. This basic scheme of enzymes is complemented with others such as aryl-alcohol oxidases (AAO, E.C. 1.1.3.7) [4,5] superoxide dismutase (SOD, E.C. 1.15.1.1), glyoxal oxidases (GLO, E.C. 1.2.3.5) and others, that provide some of the substrates used in the corresponding reactions [6]. Cellulose, on the other hand, is degraded by endoglucanases that attack the cellulose at the amorphous regions, and cellobiohydrolases that are exocellulases yielding cellobiose disaccharides from the reducing (type I) or non-reducing (type II) end of the cellulose. The final degradation of cellobiose is carried out by β-glycosidases.

Both in the case of the ligninolytic enzymes and in that of the cellulolytic enzymes, various apparently redundant genes have been found in all the organisms where this has been studied [2]. This redundancy raises the question about their respective functions *in vivo*, and this question is even more pertinent since the cDNAs for some of these genes have not been found or are only detected under special culture conditions.

In microorganisms, the influence of the environment on the phenotype is very high because they are barely isolated by

* Corresponding author. Tel.: +34 948169107; fax: +34 948169732.
E-mail address: gpisabarro@unavarra.es (A.G. Pisabarro).

0141-0229/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.enzmictec.2007.11.007

internal homeostatic systems. Consequently, the response of each cell of a multicellular microorganism, such as a fungus, is essentially independent from that of its fellows. For this reason, the effect of the environment on the final phenotype is higher than in plants or metazoans. Phenotypic traits can be classified into two groups: qualitative traits that are controlled by single or few genes, their inheritance is simple, they follow the Mendelian rules, and the effect of the environment on them is relatively reduced. Hence, the phenotypes produced by these genes can be usually deduced directly from the genotypes. Quantitative traits, on the contrary, are controlled by a high number of genes that are individually inherited following the Mendelian rules [7], and the molecular structure of these complex loci is not fully understood yet [8]. Besides, quantitative traits are highly influenced by the environment probably due to the addition of the environmental influence suffered by each one of the genes involved in this complex type of locus. These complexities make quantitative characters to show a continuous variation in contrast with the discrete variation exhibited by the qualitative traits. Whereas qualitative traits are coded for by genes that can be precisely mapped to specific genome sites, quantitative traits are mapped as genomic regions that influence the quantitative variation of the trait. These regions are known as quantitative trait loci (QTL).

There are different strategies for identifying the genomic regions involved in the control of a quantitative trait (QTL mapping) [7]. Essentially, QTL mapping is aimed at finding discrete genome segments that are statistically correlated to differences in the value of the quantitative trait when a segregating population of individuals is studied. For such a study, two sets of paired data are used as input: the quantitative value of the trait in a given individual and the genetic constitution of that individual. This genetic constitution is deduced from the particular set of alleles that the individual bears in each one of the genetic loci of the genetic linkage map of the corresponding species. The QTL mapping output consists in a graph that represents a measurement of the likelihood (LOD value) of a positive correlation between given genomic segment a significant variation in the quantitative trait (*Y* axis), against the chromosome linkage length (*X* axis).

Considering the high number of genes involved in the degradation of lignocellulose, and the high impact of the environment on microorganisms (even in multicellular filamentous fungi), we hypothesize that the lignocellulolytic activities must behave, as a whole, as quantitative traits amenable to the analytic procedures developed for this type of genes. Within this hypothesis, we predict that the QTL mapping of these enzymatic activities should uncover the genome positions for the genes coding for the enzyme structural genes plus genes coding for regulators of these activities. This prediction can be expanded to other enzymatic activities that can be studied in microorganisms. In fact, previous works [9,10] have shown differences in the expression level of fungal genes involved in lignocellulose degradation depending upon differences in culture conditions. In more complex natural growing conditions, the expression of these genes and the corresponding enzymatic activities could be expected to be affected by multiple factors.

Pleurotus ostreatus (Jacq.: Fr.) Kumm. (Pleurotaceae, *Agaricales*) [11,12] is a white rot basidiomycete that produces phenol oxidase (laccase), and Mn-oxidizing peroxidases (manganese-peroxidase and versatile peroxidases) as the main ligninolytic activities. Lignin peroxidase activity, on the other hand, has not been detected in this fungus. This enzymatic portfolio contrasts with that of the model white rot fungus *Phanerochaete chrysosporium* that produces LiP and MnP but in which *pox* genes have not been found. According to Hattaka [3], *P. ostreatus* could be included in the group of fungi which are efficient lignin degraders in nature and especially suitable for selec-

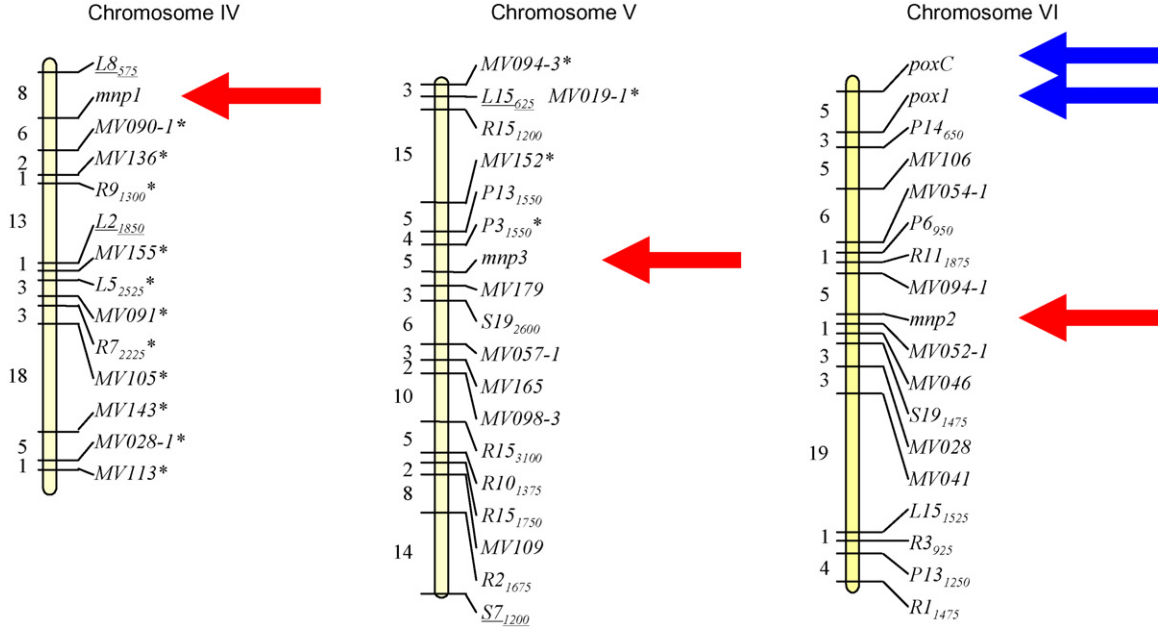

Fig. 1. Genetic linkage map of the chromosomes carrying the genes coding for Mn-oxidizing peroxidases (*mnp*) (*mnp*, red arrows in the web version) and phenol oxidases (*pox*) (*pox*, blue arrows in the web version) in *P. ostreatus*.