BIOSCREENING OF FUNGI FOR EXTRACELLULAR CHITIN DEACETYLASE

Ursula Stoll[a] / Christoph Kulmann[a] / Jürgen Warrelmann[a] / Wolfgang Heyser[a]*

[a]UFT, University of Bremen, Leobener Str., 28359 Bremen

Abstract

In the last years there has been a growing interest in chitosan, since there is a wide range of applications (medical and pharmaceutical applications, water treatment, personal-care products etc.) (Kumar, 2000). Presently, chitosan is produced from chitin, which is got out of crab shells using a thermo-chemical procedure. This process exhibits great disadvantages such as environmental problems and a broad and heterogeneous range of products with different molecular masses and deacetylation degrees. A biotechnological approach availing the use of chitin deacetylase (CDA) for preparation of chitosan polymers and oligomers offers the possibility of the development of an enzymatic process that could potentially overcome most of these drawbacks (Tsigos et al., 2000).

The main focus of this project is not to substitute the thermo-chemical process, but rather to develop a biotechnological subsequent procedure, in which the heterogeneous chitin/chitosan from the chemical procedure is used to obtain a well defined product (chain-length and deacetylation degree).

Introduction

One objective of the project is the establishment of a convenient analytical method for determination of chain length and degree of deacetylation. Despite the strongly increasing interest in chitin/chitosan in the last decades, there is no standardised method for characterization so far. Usually dyes or colorimetric reactions are used, for example Calcofluor White (Trudel & Asselin, 1990) or Ninhydrin (Curotto & Aros, 1993). But these methods are not able to give really detailed information. In order to determine the activity of CDAs, it is very important to find a sensitive method to detect even small changes of relevant parameters.

A further objective is to find fungi with extracellular CDA of high enzyme activity. The function of CDA in fungi is versatile: fungi of the class zygomycetes for example need CDA for cell wall biogenesis, because their cell wall consists not only of chitin but also of chitosan (Davis & Bartnicki-Garcia, 1984). In contrast, plant pathogenic fungi use CDA during penetration to convert the chitin of their cell wall into chitosan in order to resist the plant´s chitinolytic enzymes, which are part of the defence mechanisms of the host plants (Mendgen et al., 1996; Siegrist & Kauss, 1990). Consequently, distinct fungi show high CDA activities under different conditions and in different growth phases, which have to be determined.

Another objective is the extraction of chitin from fungi, which shows some advantages against chitin from crabs. Chitin from crabs is often polluted with marine-borne heavy metals, furthermore this chitin contains proteins (Groeger et al., 2006), which may cause allergenic reactions.

Materials and Methods

Fungi: Currently, *Colletotrichum lindenmuthianum* (Fig.1) and *Metarhizium anisopliae* (Fig.2) are investigated as model organisms with respect to their enzymatic potential and the growth conditions under which extracellular CDA is produced.

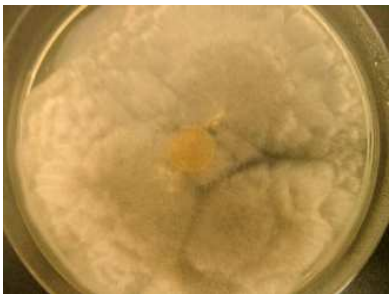

Figure 1: Colletotrichum lindemuthianum, 1 week old culture on MMN (Modified Melin Norkrans) agar.

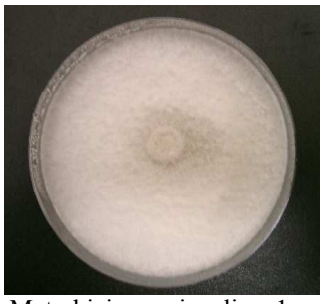

Figure 2: Metarhizium anisopliae, 1 week old old culture on MEX (Malt Extract) agar.

Concentration of proteins in media supernatants: Concentration on Vivaspin centrifugal concentrators, addition of tetraborate buffer (pH 8.5) and glycerol for stabilization. These extracts are used directly for electrophoresis.

Modified Sodium Dodecylsulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE): We use standard Lämmli gels (Lämmli, 1970) where glycol- chitin is included in the separating gel as the substrate. Proteins are applied to the gel without mercaptoethanol and without boiling. After electrophoretic separation, proteins are renatured in HEPES buffer containing 1% Triton X-100 and incubated in reaction buffer. Chitosan, as a result of chitin deacetylase action, can then be visualized after staining with Calcofluor White under UV illumination. The result of electrophoresis of *Colletotrichum lindemuthianum* is shown in figure 3.

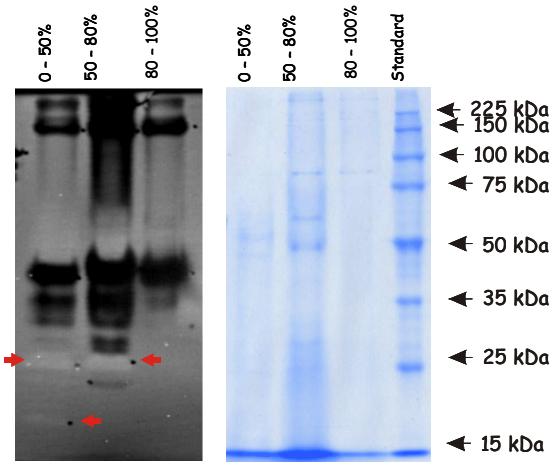

Figure 3: Ammoniumsulfate-fraction from culture super-natant of *Colletotrichum lindemuthianum* (V8-medium) after electrophoretic separation in 10% SDS-PAGE including 0,01% glycolchitin.

Left side: Gel after renaturation with Triton X-100, incubation in Tris-HCL (pH 8.5) and staining with Calcofluor white. Light bands (red arrows) indicate chitosan as a product of activity of CDA. Dark bands indicate degradation of glycolchitin due to chitinase activity.

Right side: Identical electrophorese, but staining with protein specific dye EZBlue. The molecular mass standard is shown to the right. CDAs shown here have a molecular mass from 26 to 20 kDa.

The next steps of the project are as follows

- Searching for more fungi with extracellular chitin deacetylase.
- Evaluation and further development of mass spectroscopy for chitin/chitosan analysis.
- Searching for other analytical methods.

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