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NEW HYBRID MATRIX FOR IMMOBILIZATION OF *TRICHODERMA VIRIDE* SL-45

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Abstract

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In this work *Trichoderma viride* SL-45, was investigated in new a hybrid matrix by immobilizing method. Vegetative culture of this strain was immobilized in four variations of sol-gel hybrid silica materials, composed of tetraethylorthosilicate as an inorganic precursor, 10 % polyethylene oxide and 5, or 10% carboxymethylcellulose as organic compounds. It was found that the new hybrid matrix of immobilization had positive effect on cellulase activity of strain. Biosynthetic activity of immobilized cultures was investigated by batch cultivation up to 840th h. The production of cellulase from immobilized culture in hybrid matrix composed of tetraethylorthosilicate (as precursor) and 5% carboxymethylcellulose (as organic compound and substrate) was found to be about twice higher than the activity of free *Trichoderma viride* SL-45 culture.

Key words: immobilization; hybrid matrix, *Trichoderma*; cellulase activity

Introduction

Cellulolytic enzymes are involved in enzymatic hydrolysis of cellulose, one of the most widely used organic material that can be converted to products with significant commercial interest. Bioconversion of cellulose to monomeric sugars has been intensively studied for decades to produce bioethanol and bio-based products, food and animal feeds and many valuable chemicals (Haapala et al., 1995; Adsul et al., 2007; Jäger et al., 2010; Adsul et al., 2011). A good strategy to improve the low stability of free enzymatic preparations with cellulase activity could be the immobilization in/on inorganic porous supports (Paljevac et al., 2007; Takimoto et al., 2008; Dragomirescu et al., 2010). Silica sol-gels can be prepared from alkylsiloxane precursor tetraethylorthosilicate (TEOS) that undergo hydrolysis and polymerization (Peralta-Perez et al., 2001; Shchipunov et al., 2004; Samuneva et al., 2008; Takimoto et al., 2008; Meunier et al., 2010). Retention of the biologically active species is achieved by entrapping them in the porous matrix that is created during sol–gel formation. Under the right conditions, this can prolong cell viability, protect cells from contamination and increase the production of primary metabolites such as hydrolytic enzymes (Haapala et al., 1995; Shchipunov et al., 2004; Wang et al, 2005, Ruanglek et al., 2007).

The aim of this study was to investigate the relationship between strain immobilization, total cellulase activity and different variations of matrix compositions. The effects of the immobilization method and the influence of environmental parameters on the cellulase activity were determined. We report that this method of immobilization had positive effect on cellulase activity of strain.

Materials and Methods

Microorganism and fermentation conditions

The fungal strain *Trichoderma viride* SL-45 was obtained from strain collection of Department of Biotechnology, Faculty of Biology, Sofia University St. Kliment Ohridski (Bulgaria) and used in the present study. The cultures were maintained on potato dextrose agar at 28°C for four days. Inoculums were obtained in 500 cm^3 flasks which contained

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100 ml Mandels mineral salt medium (Mandels et al., 1974) with added 2% glucose and 1% maize extract at 28°C with constant shaking (250 rpm) for 24 hours. Fermentation mixture (50 ml) was composed of Mandels mineral salt medium, 1% microcrystaline cellulose Micricel® and 1% wheat bran. For immobilized cells cultures of *Trichoderma viride* SL-45, we used the same fermentation media, consisting only of 1% microcrystaline cellulose Micricel® as a substrate. Fermentation process for free and immobilized cells cultures was held at 28°C with constant shaking 250 rpm and endo-1,4-β-glucanase (Cx) activity was measured at every 24 hours.

Immobilization technique

Sol-gel transparent silica hybrid matrices were synthesized at room temperature. The hybrid materials were prepared by substituting part of the inorganic precursor tetraethylorthosilicate with 5 and 10% carboxymethylcellulose and two variants of 10% PEO (polyethylene oxide) (Chernev et al., 2006). The cell immobilization was carried out using 10 ml mycelium culture. Using sol-gel technique, we synthesized and analyzed four types of matrices: TEOS +5% substrate; TEOS-NH_2 +5% substrate; TEOS+10% substrate +10% PEO; TEOS-NH_2 +10% substrate +10% PEO.

Cellulase activity

Endo-1,4-β-glucanase activity was detected on sodium carboxy-methyl cellulose (Na-CMC) as substrate according to Wood and Bhat (Wood and Bhat, 1988). Reaction mixtures containing 0.5 ml 1% solution of Na-CMC in 0.05 M Sodium-acetate buffer (pH 4.8) and 0.5 ml enzyme solution, were incubated at 50°C for 30 min.

Results and Discussion

In this study, we investigated the cellulase profile of strain *Trichoderma viride* SL-45 by using silica organic compounds. Vegetative culture of this strain was immobilized in four variations of sol-gel hybrid silica materials, composed of tetraethylorthosilicate as an inorganic precursor and different organic compounds - PEO and carboxymethylcellulose. Cellulase production by free and immobilized cultures was investigated in shake flasks up to 840th h during submerged fermentation process (Figure 1A, B).

According to the results cellulase activity of 18.97 U/ml was obtained from culture immobilized in sol-gel matrix composed of TEOS and 5% Na-CMC (as substrate) at 384th h of the fermentation process (Figure 1A). Expression of cellulase activity of this culture is almost two fold higher compared with the enzyme activity in free cell cultures (8.99 U/ml) at 120th h. Higher cellulase activity is maintained throughout the whole process until 840th h. On that base it was confirmed the positive effect of the immobilization of a strain from genus *Trichoderma*. The similar results were reported in other studies. (Duff, 1988; Attalla and Salleh, 2010).

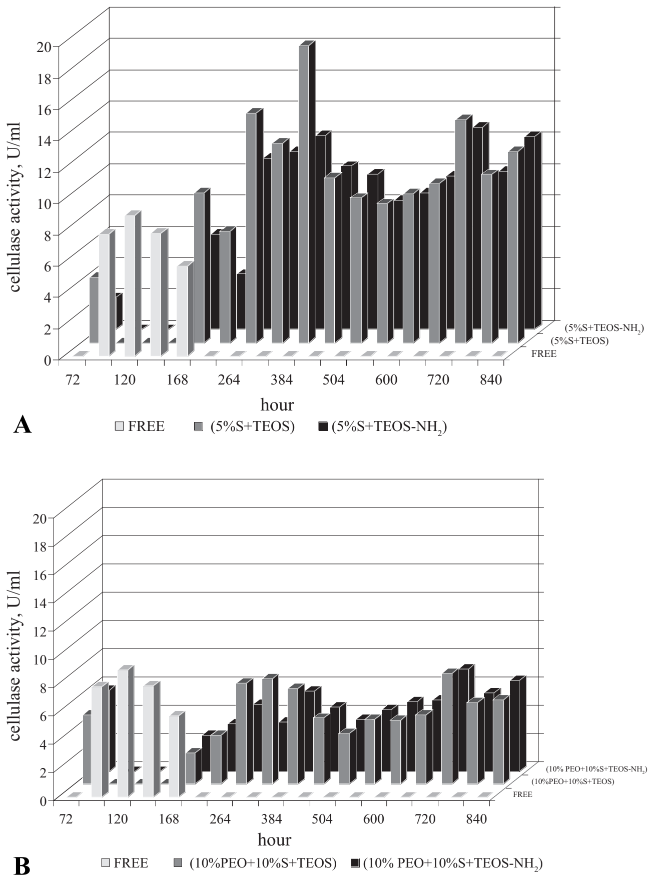

Fig. 1. Cellulase activity of free and immobilized cultures of strain *Trichoderma viride* SL-45 in hibrid matrix composed of:
A – 5% substrate + tetraethylorthosilicate;
B – 10% PEO + 10% substrate + tetraethylorthosilicate

In contrast, vegetative culture, immobilized in hybrid matrix containing 10% substrate and 10% PEO, showed cellulase activity of 7.44 U/ml at 336th h, which is comparable with the activity of free cells (8.99 U/ml at 120th h). Similar results were obtained in immobilized culture in sol-gel matrix (10% Na-CMC, 10% PEO and TEOS-NH_2) (Figure 1B).

We have observed that the cellulase activity of strain immobilized in both synthesized matrices (Figure 1B) was similar to that of free culture (at 840th h). Probably these results are due to large amount of organic components as to Na-

CMC and PEO, included in sol-gel matrices. The high quantity of organic compounds in the media and in sol-gel matrix is favorable for the growth of culture and manifestation of the cellulase activity during batch fermentation process.

In the study, we have obtained immobilized cultures of strain *Trichoderma viride* SL-45 by the use of hybrid matrices which increase cellulase activity two times, compared with free vegetative culture cells. It was found that the method of immobilization had positive effect on strain activity.

Conclusions

In summary: the fungal strain *Trichoderma viride* SL-45 was successfully immobilized in hybrid matrices, composed by TEOS as an inorganic precursor and 5% Na-CMC as substrate. We investigated the relationship between strain immobilization, cellulase activity and matrix compositions. This method and the new sol-gel hybrid matrix enabled synthesis of cellulolytic enzyme in natural medium, containing soluble substrate as the only carbon source and inductor of biosynthesis. Enzymes accumulated in the medium could be used for effective hydrolysis of cellulose-containing raw materials.

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