THE INFLUENCE OF THE NITROGEN SOURCE ON SOME DEHYDROGENASES INVOLVED IN THE ENERGY METABOLISM OF THE FUNGUS *TRICHODERMA REESEI* QM9414

INFLUENȚA SURSEI DE AZOT ASUPRA ACTIVITĂȚII UNOR DEHIDROGENAZE IMPLICATE ÎN METABOLISMUL ENERGETIC CE CARACTERIZEAZĂ FUNGUL *TRICHODERMA REESEI* QM9414

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Abstract: *Fungi are a very diverse group of organisms which share a rather similar morphology and a significant metabolic diversity. This diversity is reflected in the variaty of substrates fungi can use as carbon sources. Trichoderma reesei is an industrial fungus largely exploited for its ability to produce valuable enzymes. For enzyme production, cheap substrates, usually cellulose rich are prefered. The objective of this study was to investigate the influence of carbon sources represented by bio residues resulting from different agricultural practices, namely wheat straws, barley straws and maize stalks on the activity of Krebs cycle dehydrogenases and on glucose dehydrogenase . Also, we analysed how the nitrogen source, represented by different amino acids affect the activity of the aforementioned enzymes. Our results indicate that enzymes involved in Krebs cycle are influenced by the nutritional substrate used, by the addition of certain amino acids and last but not least by the age of the fungal culture.*
Keywords: *Trichoderma reesei, Krebs cycle, agricultural residues*

Rezumat: *Fungii reprezintă un grup divers de organisme care împărtășesc o morfologie destul de similar și o mare diversitate metabolică. Această diversitate se reflectă în gama variata de substraturi pe care ciupercile le pot folosi ca surse de carbon. Trichoderma reesei este o ciuperca industrială, puternic exploatată pentru capacitatea sa de a produce enzime valoroase. Substraturile ieftine, bogate în celuloză sunt preferate pentru a produce enzime industiale. Obiectivul acestui studiu a fost de a investiga influența surselor de carbon, reprezentate de resturi provenite din diferite practici agricole, și anume paie de grâu, paie de orz și tulpini de porumb asupra activității dehidrogenazelor ciclul Krebs și asupra glucozo-dehidrogenazei. De asemenea, am analizat modul în care sursa de azot, reprezentată de diferiți aminoacizi afectează activitatea enzimelor menționate anterior. Rezultatele noastre, indică faptul că enzimele implicate în ciclul Krebs sunt influențate de substrat nutritiv folosit, de adiția anumitor aminoacizi și nu în ultimul rând de vârsta culturii fungice.*
Cuvinte cheie: *Trichoderma reesei, ciclul Krebs, deşeuri agricole*

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INTRODUCION

The species belonging to the genus *Trichoderma* are well known for their ability to degrade a variety of polysaccharides (cellulose, hemicellulose), and related polymers such as chitin (Harman and Kubicek, 1998). Throughout their evolution microorganims have adopted various metabolic pathways and regulation mechanisms to deal with different environments and different nutritional requirements. Through the process of respiration (aerobic) and fermentation (anaerobic) organisms get their energy from various substrates in the form of ATP. These processes allow the microorganims to produce ATP at rates and with different degrees of efficiency (Chambergo et. al., 2003). Respiratory process in fungi is similar to other aerobic organisms, and it consists of three interrelated processes, citric acid cycle, oxidative phosphorylation and electronic transport.

Trichoderma reesei is a metabolically versatile microorganisms able to use both simple and complex sources of carbon and nitrogen. Cellulosic waste from different agricultaral practices (e.g. sugar cane, corn cobs, barley and wheat straws), and the forest industry (sawdust), are promising substrates for cheap enzymatic hydrolysis. Nitrogen sources such as peptone are often used to reduce the lag phase of growth when using polymer substrates like cellulose. (Mandels and Andreotti, 1978). However, peptone is used both as a source of nitrogen and as carbon source by *Trichoderma reesei* when it is grown simultaneously with polysaccharides. Trichoderma reesie is able to use some amino acids as substrates for energy production, of which alanine, aspartic acid and glutamic acid (Danielson and Davey, 1973c; Jackson et. al., 1991). Thus, in this study we aimed to analyze how certain amino acids stimulate metabolic activity of the fungus *Trichoderma reesei* QM9414, especially the dehydrogenases involved in the Krebs cycle.

MATERIAL AND METHOD

The fungus *Trichoderma reesei* was grown on PDA medium at 28°C, for 7 days. Enzymatic determinations were performed by inoculating the microorganism in Mandels liquid medium (Ferreira et. al., 2009). The nitrogen sources represented by ureea, peptone and ammonium sulfate were replaced by various amino acids: alanine, asparagine, glutamic acid, histidine, methionine, valine and serine at a concentration of 1g/L. A control variant was made in which the nitrogen source was absent. The carbon source-glucose was replaced by 30 g/L wheat straws, barley straws and corn stalks. Prior to the addition in the medium, these substrated were chopped with a power grinder. The liquid cultures were incubated for 14 days at a constant temperature of 28°C. Enzymatic determinations were performed at 7 and 14 days. The enzymatic activity was assayed using the metod described by Cojocaru (2008).

RESULTS AND DICUSSIONS

Isocitrate dehydrogenase (IDH, E.C 1.1.1.42) is an enzyme that catalysed the oxidative decarboxylation of isocitrate, leding to the formation of α-ketoglutarate and CO_2. Three isomorphic genes are currently know for this enzyme: IDH3 catalyzes the third step of the Krebs cycle, converting NAD^+ to

NADH in the mitochondria, while IDH1 and IDH2 are located both in the cytosol and in the mitochondria and peroxisome. The influence of amino acids on the activity of isocitrate-dehydrogenase is illustrated in Figure 1. Its activity was stimulated by valine (2,6682 µg formazan/g biomass) and glutamic acid (1,6498 µg formazan/g biomass), in the media with wheat straws, by glutamic acid (2,0584 µg formazan/g biomass), alanine (1,4731 µg formazan/g biomass) and asparagine (1,2597 µg formazan/g biomass), in the variants with barley straws and finally by glutamic acid (3,731 µg formazan/g biomass), valine (3,2845 µg formazan/g biomass) and serine (2,2557 µg formazan/g biomass) in media with corn stalks. The activity of isocitrat-dehydrogenase is inhibitate by the addition of methionine.

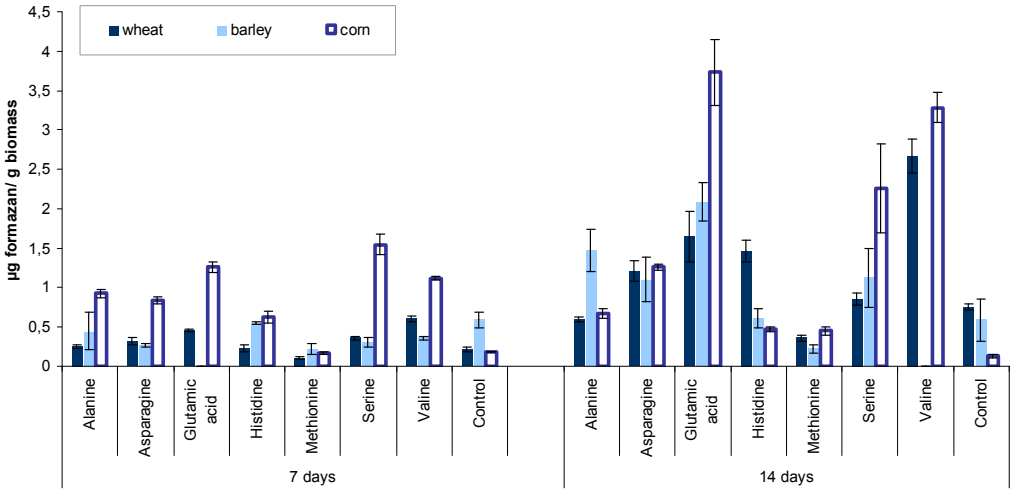

Fig. 1 - The influence of amino acids on isocitrate dehydrogenase in *Trichoderma reesei* grown on media with wheat straws, barley straws and corn stalks.

Malate dehydrogenase (MDH, L-malat: NAD oxidoreductase, EC 1.1.1.37) catalyzes the reaction NAD/NADH-dependent, of interconversion of malate and oxaloacetate. In eukaryotic cells are at least two forms of MDH, an isomorphic is located in the mitochondria, the other in the cytosol (Minárik et. al., 2002). A third form was found in yeasts, and is involved in the conversion of malate product of glyoxal in the cicle of glyoxylate (Minard and McAlister-Henn, 1991). Some amino acids introduced into the culture medium as a source of nitrogen can stimulate the activity of this enzyme. The increse in MDH activity is dependant on the complex poysaccharide used as a carbon source (figure 2). Thus, in media with wheat straws, the activity is stimulated by histidine (0,554 µg formazan/g biomass) and alanine (0,2181 µg formazan/g biomass), in media with barley straws by alanine (1,1749 µg formazan/g biomass) and serine (1,04 µg formazan/g biomass), and in variants with corn stalks by glutamic acid (3,2331 µg

formazan/g biomass), serine (2,2074 µg formazan/g biomass) and valine (2,316 µg formazan/g biomass).

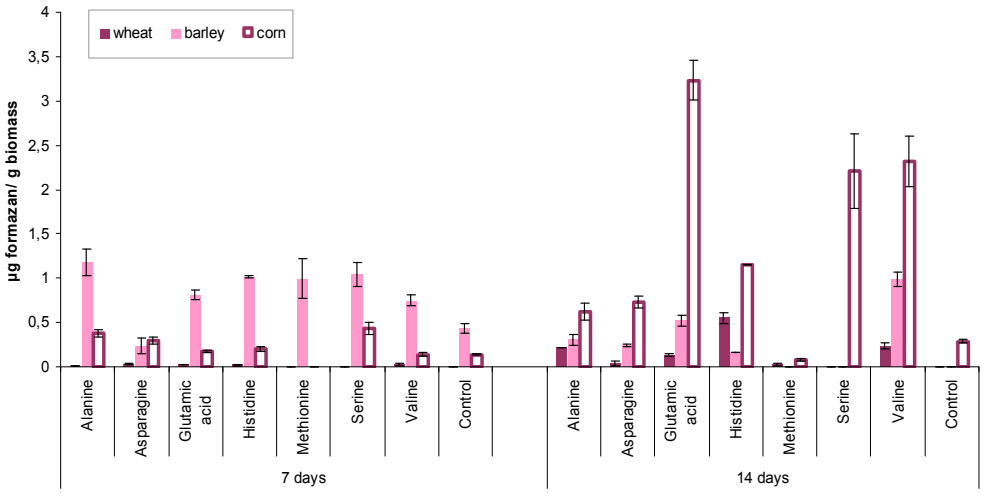

Fig. 2 - The influence of amino acids on malate dehydrogenase in *Trichoderma reesei* grown on media with wheat straws, barley straws and corn stalks.

Succinate dehydrogenase (SDH, E.C.1.3.99.1, succinate-oxidoreductase, Complex II), is a dehydrogenase present in all aerobic organisms and is involved in donating electrons from succinate oxidation to the respiratory chain. Succinate dehydrogenase activity is modulated by specific activators and inhibitors (Hederstedt L. & Rutberg L., 1981). Amino acids stimulate the activity of SDH as follows (Figure 3): in the variants with wheat straws activity is stimulated by valine (2,6682 µg formazan/g biomass), glutamic acid (1,6498 µg formazan/g biomass) and histidine (1,4674 µg formazan/g biomass), in variants with barley straws by alanine (0,4471 µg formazan/ g biomasă) and histidine (0,5546 µg formazan/g biomass), and in variants with corn stalks SDH is stimulated in the presence og glutamic acid (3,731 µg formazan/g biomass), valine (3,2845 µg formazan/g biomass) and serine (2,2557 µg formazan/g biomass). Low levels of SDH activity have been reported especially in media with methionine compared to control, regardless of the agricultural waste introduced into the media.

Alpha-ketoglutarate dehydrogenase (alpha-KGDH, E.C 1.2.4.2) is an enzyme that catalyzes the oxidation of α-ketoglutarate to succinyl-CoA, producing NADH and CO_2, supplying electrons to the respiratory chain. By cultivating the fungus *Trichoderma reesei* on media with different amino acids, this enzyme was stimulated by the addition of histidine (0,8903 µg formazan/g biomass) and glutamic acid (0,4394 µg formazan/g biomass), in media with wheat straws, by serine (1,5737 µg formazan/g biomass) and glutamic acid (1,289 µg formazan/g biomass), in media with barley straws and by glutamic acid (0,4648

µg formazan/g biomass) and asparagine (0,3738 µg formazan/g biomass), in media with corn stalks (Figure 4). Low values in activity were recorded in the variants with methione compared to control.

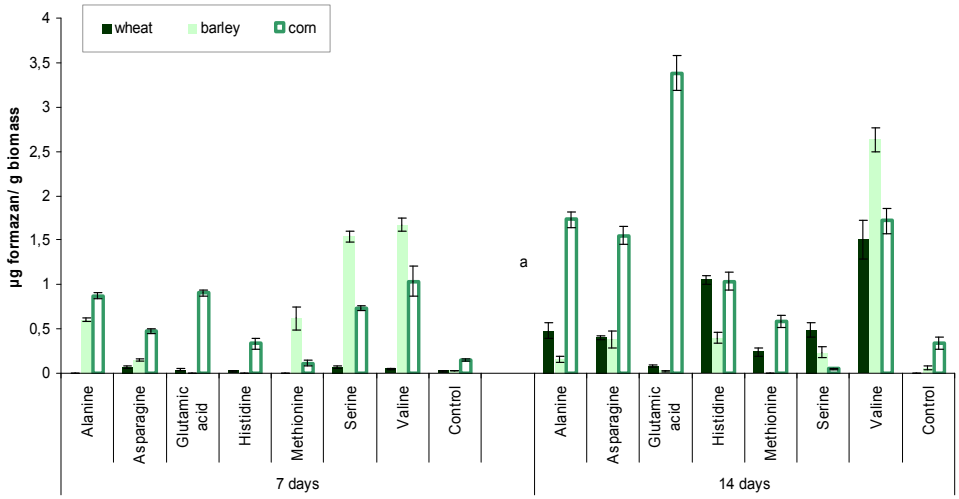

Fig. 3 - The influence of amino acids on succinate dehydrogenase in *Trichoderma reesei* grown on media with wheat straws, barley straws and corn stalks.

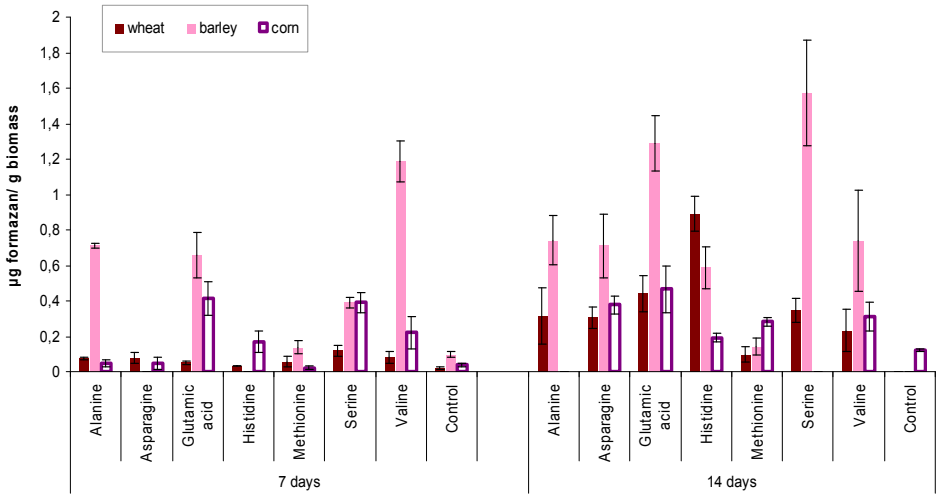

Fig. 4 - The influence of amino acids on α-ketoglutarate dehydrogenase in *Trichoderma reesei* grown on media with wheat straws, barley straws and corn stalks.

CONCLUSIONS

1. The energy metabolism of *Trichoderma r*eesei is modulated by a number of nutritional factors, such as the carbon and the nitrogen source of the culture medium. Thus, the use of cellulosic material derived from different agricultural practices can stimulate the activity of Krebs cycle dehydrogenases. Corn stalks proved to be more appropriate nutrtional substrate for the activity of dehydrogenases.

2. The addition of amino acids such as glutamic acid, valine and serine in the growth medium improves the activity of dehydrogenases, while adding methionine causes the opposite effect.

3. The age culture is also an important factor that shapes the metabolism of Trichoderma reesei species. Thus, at 7 days, the activity of dehydrogenases is lower, while at 14 days there is a significant increase in activity.

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