INFLUENCE OF CARBON SOURCES ON SOME DEHYDROGENASES INVOLVED IN ENERGY METABOLISM OF *RHIZOPUS NIGRICANS* SPECIES

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Abstract

Fungi obtain energy and nutrients from organic matter degradation, using sugars for growth, which can range from simple hexoses up to complex polysaccharides. Cereals provide a rich source of nutrients for microbial growth and are at risk of infestation by fungi during storage. The objective of this study was to investigate the influence of carbon sources represented by grinded grains from three cereal species: wheat, corn and barley; on the activity of Krebs cycle dehydrogenases and on glucose dehydrogenase in saprophytic fungus *Rhizopus nigricans*. Enzymatic assays were performed in intervals of 5, 10 and 15 days from fungus mycelium, using Sîsoev method, modified by Artenie. The obtained results pointed out that dehydrogenases involved in Krebs cycle and in pentose phosphate pathway are influenced both by the amount and nature of carbon source and by the fungal culture age. Thus, in the first time interval values are maintained at moderate levels, in the second period enzymatic activity increases significantly and in the last time interval, along with nutritional resources depletion, enzymatic activity is extremely low in most experimental variants

Key words: *Rhizopus nigricans*, cereal caryopses, dehydrogenases.

Fungi are non-photosynthetic and widespread microorganisms, that play a vital role in the biodegradation of organic matter. They obtain energy and many of the nutrients to supply biosynthetic pathways trough the degradation of plant and other matter. Their role in the environment is recycling (Hanson J.R., 2009). Sugars are widely used for fungal growth, they can range from simple hexoses as glucose to polysaccharides as starch, cellulose and aromatic hydrocarbons, including lignin (Kavanagh K., 2005). Cereals are the most efficient source in human food, in terms of both energy supply and nutrition. Grains contain carbohydrates, protein, lipids, fiber, minerals and vitamins, in particularly D, E, and the B group, and have a near-neutral pH. Storage fungi invades cereal grains after harvest, causing loss of quality, weight, germinability and nutrient value (ICMSF, 2005). All living organisms require energy, which is ensured by cell respiration - a catabolic process that releases energy, usually in the form of ATP. It is vital that chemical reaction involved in cellular respiration takes place in a fast rate and optimal condition, enzymes playing an essential role in this process. Citric acid cycle is present in all aerobic organisms and represent a part of the metabolic pathways involved in converting carbohydrates, fats and protein into carbon dioxide and water to generate useful energy. The enzymes that are operating in tricarboxylic acid cycle are located in the mithocondrial matrix, except succinat dehydrogenase which is part of a protein complex belonging to internal mitochondrial membrane (Hederestedt L., Rutberg L., 1981; Hajjawi O.S., 2011). Mitochondria are refined and partly autonomous organelles that meet most cell energy requirements through oxidative metabolism. In most eukaryotes mitochondrial respiratory chain consist of a series of complex proteins that fulfil oxidative phosphorylation which leads in the end to ATP production, and fungi contain highly branched electron transporting respiratory chains (Videira A., Duarte M., 2002; Melo A.M., *et al*., 2004; Carneiro P., *et al*., 2012). Glucose dehydrogenase catalyzes the oxidation of β-D-glucose to β-D-glucono-1,5-lactone, while reducing cofactor NADPH to $NADP^+$ or, to a lesser extent NAD^+ to NADH (Weckbecker A., Hummel W., 2005).Tricarboxylic acid cycle and pentose phosphate pathway are essential for providing anabolic precursors (Rodrigues F., *et al*., 2006).

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This study aimed to investigate the influence of carbon sources represented by grinded grains from three cereal species: wheat, maize and barley, on the Krebs cycle dehydrogenase and glucose-dehydrogenase activity in fungus *Rhizopus nigricans*.

Cereal caryopses are not only a source of carbon through starch, cellulose, dextrin contained, but also a nitrogen source trough amino acids, a rich source of vitamins, pigments and minerals. The complexity of nutritional substrate strongly influences respiratory chain, thus some metals present in the cereal grains composition may inhibit or stimulate certain enzymes of the Krebs cycle. For example, zinc inhibit certain key enzymes of the Krebs cycle and disturb ATP production (Dinely K.E., *et al*., 2003; Strydom C., *et al*., 2006; Lemire J., *et al*., 2008), and iron has a positive effect on succinate dehydrogenase and isocitrate dehydrogenase activity and, also iron exposure increases NADH formation and therefore increases mitochondrialo oxygen consumption and formation of ATP by oxidative phosphorylation (Oexle H., et al., 1999). Vitamins also have an important role in energy metabolism. Thiamine (vitamin B1) functions as co-enzyme in the metabolism of carbohydrates, and when is not enough thiamine takes place an overall decrease in carbohydrate metabolism that could have serious consequences. Riboflavin (vitamin B2) enters into the structure of some dehydrogenases as FMN and FAD, contributing to redox reactions in metabolic pathways and in energy production through respiration. Nicotinamide is another form of niacin (vitamin B3) and exists within the redox active co-enzymes, nicotinamide adenine dinucleotide (NAD^+) and its phosphorylated form (NADP) (McCormick D.B., 1994, 1996).

MATERIALS AND METHODS

The study was conducted on saprophytic species *Rhizopus nigricans*. The fungus was isolated from germinated cereal caryopses. To isolate the fungus were used caryopses from three cereal species: wheat (*Triticum aestivum*), maize (*Zea mays*) and barley (*Hordeum vulgare*), *Rhizopus nigricans* being found in all three cereal types, representing the predominant species from culture plates. Wheat and corn grains came from the storage place of the Enterprise of Cereal Products from Chişinău, Republic of Moldova, and the barley grains came from a private household in Grebleşti village, from the Străşeni aria, Republic of Moldova. Pure culture was obtained after several cycles of growth on solid PDA medium (potato-dextrose-agar). *Rhizopus nigricans* species identification was based on morphological characteristics of mycelium from culture plates and on microscopic preparations. To conduct the experiments Leonian liquid medium was used (K_2HPO_4 1,25 g, $MgSO_4$. $7H_2O$ 0,625 g, peptone 1 g, glucose 20 g, distilled water 1000 ml) (Constantinescu O., 1974), from whose composition carbon source – glucose was replaced with grinded cereal caryopses. We obtained 4 medium variants: V1 – 10 g/l, V2 – 20 g/l, V3 – 30 g/l and a control variant in which the culture medium remained unchanged. Enzyme activity was assayed in intervals of 5, 10 and 15 days from fungus inoculation. The determination of Krebs cycle dehydrogenases and clucose dehydrogenase activity was performed through Sîsoev method, modified by Artenie, the five enzymes activity being reported to the quantity of soluble proteins determined trough Bradford method (Artenie Vl., *et al*., 2008).

RESULTS AND DISCUTIONS

Isocitrate dehydrogenase (IDH) is the branching point for carbon flux trough the Krebs cycle to produce energy and CO_2 and trough glyoxylate bypass to produce cellular constituents. IDH phosphorylation by kinase-phosphatase controls the division of isocitrate between the Krebs cycle and glyoxylate bypass (LaPort D.C., Koshland D.E., 1982; LaPorte D.C., Chung T., 1985; Klumpp D.J., *et al*., 1988; Ikeda T.P., *et al*., 1999). IDH catalyzes the oxidative decarboxilation of isocitrate to form α ketoglutarate, contributing to the synthesis of glutamate and other amino acids (Haselbeck R., McAlister-Henn L., 1993). In some cultures of microorganism, where were used different carbon sources, IDH levels depended on the growth phases of culture, with increased activity during exponential and early stationary phases, but after carbon source depletion decreases almost to zero. After nutrient substrate exhaustion IDH is not inactivated or degraded, but remains at low levels and in state of alert in order to facilitate recovery when nutrition conditions become again favorable (Roy S.O., *et al*., 1999).

Isocitrate dehydrogenase activity assayed in saprophytic fungus *Rhizopus nigricans*, grown on media with different carbon sources, is illustrated in Figure 1. At 15 days after inoculation enzymatic activity recorded moderate values in medium variants with wheat caryopses (V1-0.5864 μg formazan/g biomass/mg protein, V2 – 0.4239 μg formazan/g biomass/mg protein, V3 – 0.412 μg formazan/g biomass/mg protein), the obtained values being very close between them. The values found in medium variants supplemented with corn and barley caryopses are slightly higher. IDH activity reached maximum in V2 version with corn, and the minimum value in

variant V3 with wheat (0,413 μg formazan/g biomass/mg protein).

In the second study period the IDH activity increased significantly in samples supplemented with wheat and barley caryopses, but decreased in those with corn. There is a correlation between nutrient background concentration and enzyme activity, thus in variants with grinded wheat caryopses enzyme activity increases with the concentration of carbon source (V1 – 0.8629 μg formazan/g biomass/mg protein; V2 – 0.9666 μg formazan/g biomass/mg protein; V3 – 1.165 μg formazan/g biomass/mg protein), and in those with corn (V1 – 4.2227 μg formazan/g biomass/mg protein; V2 – 3.1305 μg formazan/g biomass/mg protein; V3 – 3.1205 μg formazan/g biomass/mg protein) and barley (V1 – 0.8883 μg formazan/g biomass protein; V2 – 0.5342 μg formazan/g biomass/mg protein; V3 – 0.35 μg formazan/g

biomass/mg proteină) caryopses increases inversely with the carbohydrate amount.

At 15 days from inoculation IDH activity increases significantly in variants with corn caryopses (V1 – 6.0655 μg formazan/g biomass/mg protein; V2 – 5.491 μg formazan/g biomass/mg protein; V3 – 7.2687 μg formazan/g biomass/mg protein), but decreases drastically in those with wheat grains (V1 – 0.0103 μg formazan/g biomass/mg protein; V2 – 0.0362 μg formazan/g biomass/mg protein; V3 – 0.0652 μg formazan/g biomass/mg protein), registred values being very close to zero. At samples with barley caryopses was observed a slight increase in enzyme activity (V1 – 1.1046 μg formazan/g biomass/mg protein; V2 – 1.0133 μg formazan/g biomass/mg protein; V3 – 0.8401 μg formazan/g biomass/mg protein).

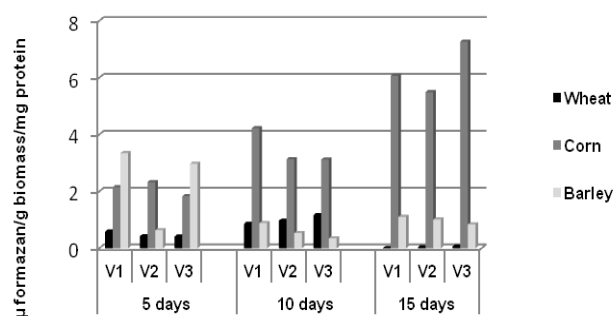Figure 1 **The influence of carbon sources on isocitrate dehydrogenase activity in *Rhizopus nigricans***
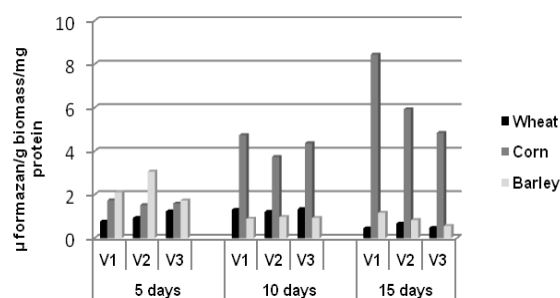
Figure 2 **The influence of carbon sources on α-ketoglutarate dehydrogenase activity in *Rhizopus***

α-Ketoglutarate dehydrogenase (EC 1.2.4.2.) functions in the Krebs cycle as a anzymatic complex of three subunits: oxoglutarate dehydrogenase (E1), hihydropoyl succinyltransferase (E2), dihydropoyl dehydrogenase (E3), that is responsible for converting α ketuglutarate to succinil CoA and production of NADP, providing electrons directly to respiratory chain. This enzyme is sensitive to reactive oxygen species and its inhibition could be critical. In addition α-ketoglutarate dehydrogenase is not only a target for ROS, but is able to generate reactive oxygen species during its catalytic activity (Tretter L., Adam-Vizi V., 2004; Tretter L., Adam-Vizi V., 2005). Monitoring in time of α-ketoglutarate dehydrogenase activity is plotted in Figure 2. In the first time period from inoculation (5 days) recorded values remain in the moderate range, except medium variant with barley caryopses (V1 – 2.1129 μg formazan/g biomass/mg protein; V2 – 3,0615 μg formazan/g biomass/mg protein; V3 – 1,7347 μg formazan/g biomass/mg protein). There is no definite correlation between the concentration of carbon sources and the evolution of enzyme activity.

At 10 days after inoculation of media α-ketoglutarate dehydrogenase activity decreases drastically in barley samples (V1 – 0.8988 μg formazan/g biomass/mg protein; V2 – 0.9778 μg formazan/g biomass/mg protein; V3 – 0,9299 μg formazan/g biomass/mg protein), increases slightly in the wheat samples (V1 – 1.3019 μg formazan/g biomass/mg protein; V2 – 1.2084 μg formazan/g biomass/mg protein; V3 – 1.3322 μg formazan/g biomass/mg protein), and nearly doubles in corn variants (V1 – 4.7361 μg formazan/g biomass/mg protein; V2 – 3.7394 μg formazan/g biomass/mg protein; V3 – 4.3641 μg formazan/g biomass/mg protein). In the last time period of experiment α-ketoglutarate dehydrogenase values increases significantly in corn variants (V1 – 8.4439 μg formazan/g biomass/mg protein; V2 – 5.9232 μg formazan/g biomass/mg protein; V3 – 4.837 μg formazan/g biomass/mg protein) and decreases in those with wheat (values ranging between 0.4512 and 0.6687 mg formasan / g biomass / mg protein) and barley (V1 – 1.1682 μg formazan/g biomass/mg protein; V2 – 0,8345 μg formazan/g biomass/mg protein; V3 – 0,5658 μg formazan/g biomass/mg protein).

Succinate dehydrogenase (SDH, E.C. 1.3.99.1) is the only tricarboxilic acid cycle enzyme that is linked to mitochondrial membrane and therefore it may participate in respiration beside the function that performs in the Krebs cycle (Ruiz-Herrera J., Garcia L.G., 1972). SDH is a flavoprotein that catalyzes the succinate oxidation to fumarate and transfers the reduced resulting equivalent directly to respiratory chain. Changes in growth conditions can affect the respiratory activity and Krebs cycle development.

SDH activity assayed in mycelium of saprophytic species *Rhizopus nigricans* is graphically presented in Figure 3. In the first study period SDH activity recorded modest values in all experimental variants regardless of the carbon source nature and amount. Maximum activity was found in variant V3 with barley (2.2082 formazan mg/g biomass/mg protein), and the minimum value in variant V1 supplemented with wheat (0.6559 µg formazan/g biomass/mg protein).

At 10 days of incubation in wheat samples (V1 – 1.2302 µg formazan/g biomass/mg protein; V2 – 1.3531 µg formazan/g biomass/mg protein; V3 –

1.2401 µg formazan/g biomass/mg protein) and corn samples (V1 – 3.0462 µg formazan/g biomass/mg protein; V2 – 2.4528 µg formazan/g biomass/mg protein; V3 – 2.8 µg formazan/g biomass/mg protein) enzymatic activity increases slightly, but in those with barley decreases drastically (V1 – 0.7283 µg formazan/g biomass/mg protein; V2 – 0.6246 µg formazan/g biomass/mg protein; V3 – 0.9147 µg formazan/g biomass/mg protein). There is no major differences between versions wit different concentrations of the same nutrient substrate.

In the last time period after inoculation takes place a significant increase in SDH activity in corn variants (values ranging between 5.426 and 10.1738 µg formazan/g biomass/mg protein), while in wheat variants activity strongly decreases, recorded values being close to zero. In medium variants with barley recorded values are slightly higher than those recorded in the second period, except variant V3 (0.3696 µg formazan/g biomass/mg protein) where the enzyme activity strongly decreases.
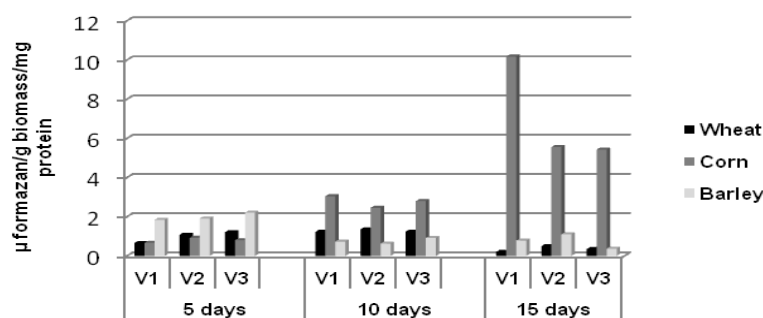

Figure 3 **The influence of carbon sources on succinate dehydrogenase activity in *Rhizopus nigricans***

Malate dehydrogenase (MDH, EC 1.1.1.37) is a key enzyme that plays an important metabolic role in aerobic pathway for producing energy and in malate/aspartate shuttle. The enzyme catalyzes in tricarboxilic acid cycle the NAD/NADH dependent conversion of malate and oxaloacetate (Tayeh M.A., Madigan, M.T., 1988; Labrou N.E., Clonis Y.D., 1997; Minark P., et al., 2002). In some bacterial culture, where were used different carbon sources MDH activity was influenced by nutrient substrate used for growth, its concentration and growth phases of culture, so MDH was synthesized during all growth phases, highest levels being recorded during exponential ant stationary growth phases (Mendoza P., et al., 2009). Evolution of MDH activity measured at three time intervals in *Rhizopus nigricans* mycelium is represented in Figure 4. In first time interval MDH activity is maintained at low levels in all work variants that contain grinded cereal caryopses. Highest values were obtained from

medium samples with corn caryopses (V1 – 3.5667 µg formazan/g biomass/mg protein; V2 – 3.0548 µg formazan/g biomass/mg protein; V3 – 3.0284 µg formazan/g biomass/mg protein), and the lowest in those with wheat grains (values ranging between 0.9322 and 1.1492 µg formazan/g biomass/mg protein). No significant differences were noted between work variant regardless nutrient substrate amount.

In the following interval chosen for study (10 days) MDH activity increases slightly in variants supplemented with corn and wheat caryopses, but decreases in those with barley. This time we can point out a correlation between concentration of grinded caryopses and evolution of MDH activity, thus in corn samples enzyme activity increases with the concentration of carbon source (V1 – 4.3797 µg formazan/g biomass/mg protein; V2 – 3.1039 µg formazan/g biomass/mg protein; V3 – 3.0269 µg formazan/g biomass/mg protein), and in those with wheat increases inversely with

concentration of grinded caryopses (V1 – 1.1021 µg formazan/g biomass/mg protein; V2 – 1.2948; V3 – 1.6047 µg formazan/g biomass/mg protein).

In the last time period (15 days) takes place a significant increase in MDH activity in corn samples (values ranging between 7.2684 and 10.527 µg formazan/g biomass/mg protein). It is preserved the same correlation between enzyme activity and concentration of wheat caryopses observed in the previous period.
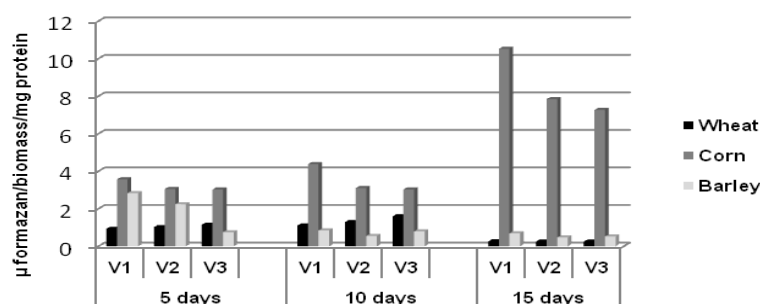

Figure 4 **The influence of carbon sources on malate dehydrogenase activity in *Rhizopus nigricans***

The activity of glucose dehydrogenase (GDH) is depicted in Figure 5. In the first study period enzymatic activity recorded moderate values in medium variants supplemented with wheat caryopses (V1 – 0.7596 µg formazan/g biomass/mg protein; V2 – 0.929 µg formazan/g biomass/mg protein; V3 – 1.228 µg formazan/g biomass/mg protein) and corn caryopses (V1 – 0.606 µg formazan/g biomass/mg protein; V2 – 0.597 µg formazan/g biomass/mg protein; V3 – 0.4649 µg formazan/g biomass/mg protein), but in those with barley recorded values are quite high compared with the rest of medium variants (V1 – 4.1793 µg formazan/g biomass/mg protein; V2 – 7.302 µg formazan/g biomas/mg protein; V3 – 1.5263 µg formazan/g biomass/mg protein).

In the second time period enzyme activity drastically decreases in samples with barley (V1 – 0.8681 µg formazan/g biomass/mg protein; V2 – 0.8827 µg formazan/g biomass/mg protein; V3 – 1.0242 µg formazan/g biomass/mg protein), decreases slightly in those with wheat (V1 – 0.9048 µg formazan/g biomass/mg protein; V2 – 0.7944 µg formazan/g biomass/mg protein; V3 – 0.7725 µg formazan/g biomass/mg protein), and increases in those with corn (V1 – 2.6257 µg formazan/g biomass/mg protein; V2 – 1.6089 µg formazan/g biomass/mg protein; 1,6774 µg formazan/g biomass/mg protein).

In the third period under study, there is a significant increase in enzyme activity in variants with corn caryopses, but in those with wheat and barley it decreases slightly. Maximum of GDH activity was obtained in version V1 supplemented with grinded corn caryopses (8.3792 µg formazan/g biomass/mg protein), and the minimum in medium variant V1 supplemented with wheat grains (0.4451 µg formazan/g biomass/mg protein).
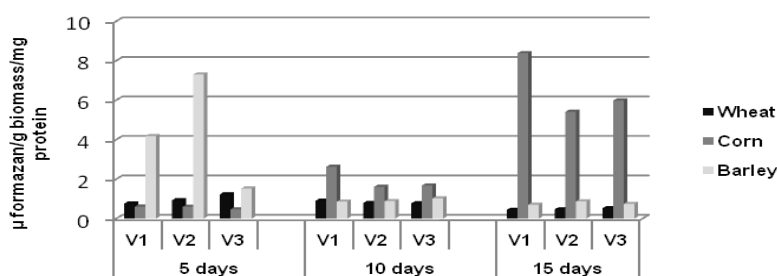

Figure 5 **The influence of carbon sources on glucose dehydrogenase activity in *Rhizopus nigricans***

CONCLUSIONS

● The nature of nutrient substrate had a strong impact on Krebs cycle and pentose phosphate cycle enzymes activity, so the highest values were recorded in medium variants supplemented with grinded corn caryopses, and the lowest values were recorded in those with wheat grains.

● The age of fungal culture also had a decisive influence on the enzymatic activity, thus most of enzymes had an extremely low activity, close to zero, in last time interval, except for medium versions supplemented with grinded corn caryopses, where at 15 days from fungus inoculation, all studied enzyme recorded very high values.

● The amount of carbon source did not significantly influence the activity of dehydrogenases involved in energy metabolism in *Rhizopus nigricans*, not being recorded notable differences in the experimental variants.

REFERENCES

Carneiro P., Duarte, M., Videira, A., - *Distruption of alternative NAD(P)H dehydrogenases leads to decreased mitochondrial ROS in Neurospora crassa*, Free Radical Biology &Medicine. 52: 402-409.

Cojocaru, D.C., 2009 – *Enzimologie practică*. Editura Tehnopress, p. 141-143.

Constantinescu, O., 1974 - *Metode şi tehnici în micologie*, Editura Ceres, Bucureşti, p. 105-106.

Dinely, K.E., Votyakova, T.V., Reynolds, I.J., 2003 – *Zinc inhibition of cellular enrgy production: implication for mitochondria and neurodegeneration*, J. Neurochem., 84: 563-570.

Hajjawi, O.S., 2011 – *Succinate dehydrogenase: assembly, regulation and role in human desease*, European Journal of Scientific Research, Vol. 51 No. 1, pp. 133-142.

Hanson, J.R., 2009 – *The chemistry of fungi*, Royal Society of Chemestry, Cambridge, UK, pp 2-3.

Haselbeck, R., McAlister-Henn, L., 1993 – *Function and expresion of yeast mitochondrial NAD- and NADP-specific izocitrat dehydrogenases*, The Journal of Biological Chemestry, Vol. 268, p. 12116-12122.

Hederstedt, L., Rutberg, L., 1981 – *Succinate dehydrogenase – a comparative review*, Microbiol Rev., 45(4): 542-555.

Ikeda, T.P., Houtz, E., LaPorte, D.C., 1992 – *Isocitrate dehydrogenase kinase/phosphatase: identification of mutations which selectively inhibit phosphatase activity*, Journal of Bacteriology, p. 1414-1416.

International Commission on Microbiological Specifications for Food (ICMSF), 2005 – *Microbial Ecology of Food Commodities, Microorganism in Foods 6*, Second edition, Kluwer Academic/Plenum Publishers, New York, pp 392-396.

Kavanagh, K., 2005 - *Fungi: biology and applications*, John Wiley & Sons Ltd, Chichester, p. 22-24.

Klumpp, D.J., Plank, D.W., Bodwin, L.J., Stueland, C.S., Chung, T., LaPorte, D.C., 1988 – *Nucleotide sequence of aceK, the gene enconding isocitrate dehydrogenase kinase/phosphatase*, J. Bacteriol., 170: 2763 – 2769.

Labrou, N.E., & Clonis, Y.D., 1997 - *L-Malate dehydrogenase from Pseudomonas stutzeri: purification and characterization*, Arch Biochem Biophys, 337: 103-114.

LaPorte, D.C., Chung, T., 1985 – *A single gene codes fos the kinase and phosphatase which regulate isocitrat dehydrogenase,* J.Biol. Chem., 260: 15291-15297.

LaPorte, D.C., Koshland, D.E., 1983 – *Phosforilation of isocitrate dehydrogenase as a demonstration of enhanced sensitivity in covalent regulation*, Nature, 305: 286-290.

Lemire, J., Mailloux, R., Appanna, V.D., 2008 – *Zinc toxicity alters mitochondrial metabolism and leads to decreased ATP production in hepatocytes*, J. Appl. Toxicol., 28: 175-182.

McCormick, D.B., 1996 – Co-*enzymes, Biochemistry of*, Encyclopedia of Molecular Biology and Molecular Medicine, Ed. Meyers R.A., Vol. 1, p. 396-406.

McCormick, D.B., 1997 – *Co-enzymes, Biochemistry*, Encyclopedia of Human Biology 2nd edition, Academic Press, San Diego, p. 847-864.

Melo, A.M., Bandeiras, T.M., Teixeira, M., 2004 – *New insights into type II NAD(P)H: quinine oxidoreductases*, Microbiol. Biol. Rev. 68: 603-616.

Mendoza, P., Servin-Gonzales, L., Flores, M.E., 2009 – *Malate dehydrogenase of Saccharopolyspora erytraea CA340: purification and effect of carbon source on its synthesis*, Rev Latinoam Microbiol, 51: 18-22.

Minarik, P., Tomaskova, N., Kollarova, M., Antalik, M., 2002 – *Malate dehidrogenase – Structure and function*, Gen. Physiol. Biophys., 21: 257 – 265.

Oexle, H., Gnaiger, E., Weiss, G., 1999 – *Iron-dependent changes in cellular energy metabolis: influence on citric acid cycle and oxidative phosphorylation*, Biochim. Biophys. Acta, 1413: 99-107.

Rodrigues, F., Ludovico, P., Leao, C., 2006 - *Sugar metabolism in yeasts: an overview of aerobic and anaerobic glucose catabolism*, Biodiversity and ecophysiology of yeasts, Springer-Verlag Berlin Heidelberg, pp 101-105.

Roy, S.O., Packard, T.T., Berdalet, E., St-Amand, L., 1999 – *Impact of acetate, pyruvate, and physiological state on respiration and respiratory quotiens in Pseudomonas Nautica*, Aquat Microb Ecol, 17: 105-110.

Ruiz-Herrera, J., Garcia, L.G., 1978 – *Regulation of succinate dehydrogenase in Escherichia coli*, Journal of General Microbiology, 72: 29-35.

Strydom, C., Robinson, C., Pretorius, E., Whitcutt, J.M., Marx, J., Bornman, M.S., 2006 – *The effect of selected metals on the central metabolic pathways in biology: A review*, Water SA, Vol. 32, No. 4, pp. 543-554.

Tayeh, M.A., Madigan, M.T., 1988 – *Malate dehydrogenases in phototrophic purple bacteria – Thermal stability, amino acid composition and immunological properties*, Biochem. J., 252: 595-600.

Tretter, L., Adam-Vizi, V., 2004 – *Generation of reactive oxygen species in the reaction catalyzed by α-ketoglutarat dehydrogenase*, The Journal of Neuroscience, 24: 7771-7778.

Tretter, L., Adam-Vizi, V., 2005 – *Alpha-ketoglutarate dehydrogenase: a target and generator of oxidativ stress*, Phil. Trans. R. B, 360: 2335-2345.

Videira, A., Duarte, M., 2002 – *From NADH to ubiquinone in Neurospora Mitochondria*, Biochim. Biophys. Acta 1555: 187-191.

Weckbecker, A., Hummel, W., 2005 – Glucose dehydrogenase for the regeneration of NADPH and NADH, Microbial enzymes and biotransformations, Methods in Biotehnology, Vol. 17, 225-238.

Zhu, Y., Chen., X., Chen, T., Shi, S., Zhao, X., 2006 – *Over-expression of glucose dehydrogenase improves cell growth and riboflavin production in Bacillus subtilis*, Biotehnol Lett, 28: 1667- 1672.