Influence of pH on the Dehydrogenase Activity in 
Fusarium graminearum Species Schwb.
Telemorph Gibberella Zeae (Schwb.) Petsch)
Production of Mycotoxins

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Dehydrogenases are enzymes that catalyzes the electron transfer reactions and act according to their redox potential (rH) from negative rH values to the highest positive ones. Depending on the chemical nature of the substrate which donates hydrogen, dehydrogenase bear appropriate names: α-ketoglutarate glucoso 6-fosfat dehidrogenase, isocitrate dehydrogenase, malate dehydrogenase, etc. The paper presents the influence of pH against the dehydrogenase activity, both if the mycelium and culture liquid, at 21 and 28 days from the inoculation.

Keywords: oxidoreductase, dehydrogenase, enzymes

The pH values, necessary for the growth of micro-organisms or for the production of certain substances biologically active ingredients, are parameters to be treated in the studies on the biology of the mold.

Most of the micro-organisms grows at pH values between 5 to 9, between these extremes each has an optimal pH of action [1]. Previously, the effect of pH carried out on the activity of oxidoreductazes were studied on other species, such as: Aspergillus niger and Aspergillus tereus species, (Trichoderma viride, Mirothecium verrucaria [2], Chaetomium globosum [3-5].

Studies on the effect of pH on the activity of enzymes at the level of the patosystem (Triticum aestivum-Fusarium graminearum) are few [6], establishing that the fungus needs for growing and developing pH values between 6 and 10. Fusarium graminearum requires, like all living organisms, chemical compounds necessary for the synthesis of cellular constituents, for the activity of enzymes and transport systems and for the energy supply of the entire organism [7,8]. The paper wants to highlight the influences of nature of hydrogen donor substrate and the pH against the enzymatic activity.

Experimental part

The determinations were made from the mycelium’s biomass resulting after the centrifugation of culture media. The determination method is based on the dehydrogenases capacity to transfer hydrogen from various substrates to the solution of 2,3,5-triphenyl tetrazolium chloride (TTC) which is reduced to red colored TTC formazan. The intensity of the color is proportional to the dehydrogenases concentration.

For the dehydrogenases determination with exogenous substrate, samples have been maintained at a temperature of 28°C for 17 h. As exogenous substrate, solutions of known normality of glucose [9-11], natrium isocytrate [12, 13], α-ketoglutaric acid [14] and malate dehydrogenase [15, 16] were used. The color intensity of the TTC formazan was measured with a Perkin Elmer spectrophotometer.

The enzyme activity was expressed as the amount of the resulting formazan, following the dehydrogenase action reported to the quantity of biological material of analysed (mycelium) [17]. The pH variations in the Fusarium graminearum culture media were studied in a range between 2.0 and 9.0. The Brown culture media was adjusted using NaOH 1N and HCl 0.1N. The determinations of biochemical parameters have been made at 21 and 28 days from seeding, in fungal mycelium and culture fluid. Al the date was expressed as μg formazan/g biological material. The data were statistically interpreted with the Student t test and the Pearson correlation coefficient (r), in order to determine whether there is an association between the two variables (pH and oxidoreductases activity) and how strong this association is [18].

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Results and discussion

The activity of glucose 6-phosphate dehydrogenase in mycelium under the influence of pH are shown in the figure 1. Thus, after 21 days from the inoculation, the highest value of the activity glucose 6-phosphate dehydrogenase has been recorded at pH 9 (0,0066 μg/g), followed by pH 4 (0,0044 μg/g), a.s.o. The lowest values were recorded at pH 2 and pH 3 (0,0012 μg/g and 0,0011 μg/g, respectively).

After 28 days from inoculation, the greatest enzyme activity has been recorded at pH 7 (0,0122 μg/g), and the lowest value of the enzyme activity has been recorded at pH 2 (0,0014 μg/g).

Following the dynamic in the activity of this enzyme there is evidence of an increase in the second period of study (after 28 days from inoculation) compared to the first period of time at most of the variants of work.

The speed of the enzymatic reactions is influenced by the pH of the culture media, its campaign as a general rule, within a narrow range of pH, bearing the name of the optimum pH of action [19], a statement confirmed, in general, also in our results; the optimum pH of the glucose 6-phosphate dehydrogenase activity is 8,5. Following of the activity of the enzyme glucose 6-phosphate dehydrogenase under the influence of the pH of the contents from 2 to 9, two time intervals (at 21 days and 28 days from inoculation), it has been found that the lowest activity has been obtained under the influence of a very acid pH (2 and 3), and the highest activity has been obtained for a pH value close to the optimum pH value (pH 8.5).

The results on the effect of pH on the activity of the isocitrate dehydrogenase in mycelium are shown in the figure 2. 21 days after the inoculation the highest activity has been recorded at pH 5 (0,0157 μg/g) and the lowest at pH 3 (0,0012 μg/g).

After 28 days, the lowest value of the activity of the isocitrate dehydrogenase has been recorded at pH 5 (0,0045 μg/g) and the highest activity of the enzyme at pH 8 (0,0955 μg/g).

The activity of the isocitrate dehydrogenase under the influence of the various pH values (2-9) on the two periods of time taken in the study, indicates a poor correlation with the pH of the environment; in 28 days after the
incubation, the maximum value of the activity of the isocitrate dehydrogenase has been recorded on the variant with pH 7.0 and pH 8.0, similar with the pH of the optimal activity for this enzyme of the Cycle Krebs, confirming the results obtained by other authors [6].

All these data show that the activity of the α-ketoglutarate dehydrogenase from both the time intervals concerned (21 and 28 days after the inoculation) had a higher value on the environment with pH 7.0 results that support and the data obtained from the other species of fungi under the influence of the same factor, which shows that the activity of the enzyme may be optimal at a neutral pH [5, 20].

The results concerning the activity of malate dehydrogenase in miceliul final under the influence of pH are shown in the figure 4. At 21 days of from the inoculation, the highest value of the activity of the enzyme has been recorded at pH 5.0 (0.0118 μg/g) and the lowerst enzyme activity at pH 2 (0.0016 μg/g).

After 28 days from inoculation it has been found that the lowest activity of the enzyme has been recorded at pH 2 (0.0020 μg/g), followed, in ascending order, by 0.0026 μg/g (pH 3.0), 0.0031 μg/g (pH 4.0), 0.0039 μg/g (pH 9.0), 0.0070 μg/g (pH 8.0), 0.0077 μg/g (pH 6.0), 0.0090 μg/g (pH 5.0) and 0.0094 μg/g (pH 7.0).

Following the dynamic in the activity of malate dehydrogenase dehydrogenase it has been found that, after 28 days from inoculation, compared with the first 21 days, the values of the enzyme activity decreased (figure 4).

At pH values of 2, 3 and 4, the activity of this enzyme had small values at both 21 and 28 days from inoculation, while the values recorded on the medium with pH 5.0 - pH 9.0 activities were significantly higher.

**Conclusions**

The analysis of the obtained results in the dynamics of Krebs Cycle’s dehydrogenases (at 21 and 28 days after inoculation), indicates that the pH variation, influenced in different ways metabolic activity, namely acid pH values has led to a sharp drop in the enzymatic semnificatica values, whereas values of pH around neutral stimulated the studied biochemical parameters.

The calculus of Pearson’s correlation coefficient (r) between the enzymes acivity and the pH values indicates that the metabolic activity of the fungus *Fusarium graminearum*, has a precise tolerance interval.
Thus, in the case of glucose 6-phosphate dehydrogenase, at 21 days the correlation was poor ($r = 0.436$), as opposed to the interval of 28 days when the correlation was high ($r = 0.756$).

In the case of isocitrate dehydrogenase, at 21 days the correlation was null ($r = 0.050$), as opposed to the interval of 28 days when the correlation was high ($r = 0.756$).

The α-ketoglutarate dehydrogenase had a null correlation at 21 days ($r = 0.230$) and a high correlation at 28 days ($r = 0.874$).

For the malate dehydrogenase the Pearson correlation coefficient was null at 21 days (0.282) and moderate at 28 days (0.539). All the data were calculated for a significance level of 0.01.

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