Antioxidant Enzymes of Strains *Panax ginseng C.A.* Mey. and *Panax quinquefolius* L.

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Abstract

The strains of *Panax ginseng C.A.* Mey., *P. quinquefolius* L. and selected strains *P. ginseng-B, P.ginseng-A, P. quinquefolius-C* were investigated. Activities of SOD, catalase and peroxydase were determined by methods of Fridovich et al. (1979), Komov et al.(1975), Bovaird et al.(1982) respectively. Activities of SOD, catalase, peroxydase were investigated every day 5 in cycle of cultivation. For *P. ginseng* it was the 35 days, *P. quinquefolius* the 70 days, *P. quinquefolius-C* 90 days. *P. ginseng-B* 90 days, *P. ginseng-A* 60 days. The *P. quinquefolius*, *P. quinquefolius-C*, *P. ginseng-B* had clear differentiation and developed tracheid elements, which are absent in strain of P. ginseng.

The peaks of protein content for *P. ginseng* (4.5 units/g) and for *P. quinquefolius* (3.5 units/g) were on day 10 and remained unchanged till the last cultivation.The strain*P. ginseng-A* had two peaks of protein content (2.5 mg/g) on day 15 and on day 30. For*P. ginseng-B* strain these peaks were on day 5 and day 40 (3.5 mg/g).

Peroxydase activity peak (60 units/g) in P. ginseng strain was on day 10. This activity in*P. ginseng-B* had two peaks on day 15 and day 35 and reached 95 units/g , increasing to 150 units/g to day 80. In strain of *P. ginseng-A* was only one maximum of this activity -130 units/g on day 45. In *P. quinquefolius* peroxydase activity was 103 units/g on day 40, increasing to 135 units/g to day 90. For *P. quinquefolius-C* this activity peak was 136 units/g on day 60. Peroxydase activities for the upper and lower layers of biomass was different and varied considerably from 28-35 units/g in lower to 270-290 units/g for upper layer.

The SOD activity had two peaks in*P. ginseng* strain the 80 units/g and the 70 units/g on day 20 and day 35 respectively. Activity of SOD in*P. quinquefolius* strain reached 53 units/g on day 40 and increased up to 83 units/g to day 60.The similar increase of SOD activity was marked for*P. ginseng-B* to 85 units/g on day 90. In *P. ginseng* strain the 6 molecular isoforms SOD was

defined. One of them with Rf 0,6 was determined in all days of cycle, three other (Rf-0.43; 0.54; 0.80) only on day 10 and day 20. The isoform of SOD with Rf-0,29 was detected only on day 10 and with Rf-0,35 only on day 35. The catalase activity decreased in all strains to the last days of cultivation.

The changes of SOD, catalase and peroxydase activities reflect the differences between the strains of *Panax ginseng* and *Panax quinquefolius* and their selected forms. The correlation between maximum life span of strains and activities of their antioxydant enzymes were detected

Introduction

At present, plant cell cultures are beginning to be used as industrial sources for the production of different biologically active compounds and as objects for studying and modelling metabolic processes in plants on the cellular level.

Peroxidase is one of the most widely distributed enzymes in plants. It participates, together with superoxide dismutase and catalase, in oxygen metabolism and regulates oxidation of varius substances in the presence of H_2O_2. At present , the use of antioxidant enzymes as a marker for same physiological processes in plant cell culture is widely discussed (Gaspar et al., 1982) However, the date adduced as evidence of the defence role of peroxidase under stress are arguable, because until now in most investigations only peroxidase activity and its molecular heterogeneity have been estimated. These indices could not reflect certain alterations taking place in the plant cell in every case (Troitskaya et al., 1998). Thus, the aim of our research was to compare the activity of antioxidant enzymes (SOD, catalase, peroxidase) in the different strains of Panax L. in during of the period their cultivation.

Materials and Methods

Plant material

The strains of Panax ginseng C. A. Mey, *Panax quenquefolius* L. and selected strains Panax ginseng - A, Panax ginseng B, Panax quenquefolius C were investigated.

Different strains of tissue culture of *Panax* L . were collected on modifications of Murashige and Skoog medium. The biomass of selective cells were produced by recultivated the cells of Panax ginseng and Panax quenquefolius on the medium with different concentrations Germany-

organic compounds 2 carboxiethyl- germanysesquioxan (strain A) and the other a 1-hydroxygermatran- monohydrat (strain B and C respectively). The strains were cultivated by the surface methods on selective media. The cycle of cultivation was the 35 90 days, in darkness and the temperature 26°C.

Methods

Protein was estimated using the method of Lowry et al. (1951). Abcorbance was measured at 750 nm and bovine serum albumin was used as a standart .

Superoxide dismutase (SOD) enzymic activity was determined by photochemical assay. SOD increase the rate of the aerobic photooxidation of dianisidine, sensitized by riboflavin, to a pigment whose accumulation could be followed at 460 nm. (Misra, Fridovich, 1977).

Catalase activity was determined by titrometric method (Komov, 1978).

Peroxidase activity was determined by spectrophotometric assay, o-phenylendiamine was used as a substrate (Bovaird, 1982).

Activities of SOD, catalase, peroxydase were investigated every 5 day in cycle of cultivation

Results and Discussion

In our previous experiments we studied activity and some chemical and physical properties of SOD in process of growth of cell culture Panax ginseng. Dynamics of level of activity of SOD were examined for 30 days of cell growth in every 4-5 days. It was established, that there are two peaks of enzyme activity on 20 and 35 days of growth, and that correlates with mitotic activity of cells *P. ginseng*. It was obtained two forms of enzymes from cells of different age. On the early stage of cell development and after 30 days of cell growth two isoforms had same electroforetic mobility (Rf 0.28, and Rf 0.72). However, on 20 days there was a lock of isoform 2 and apperance isoform3 of this enzyme (Rf 0.94) (Kirillova et al., 1997).

In these experiments the cycle of cultivation for Panax ginseng was 35 days, Panax quinquefolius it was the 70 days, and for selective strain *P. quinquefolius* C and Panax ginseng-B it were the 90 days, and for Panax ginseng A it was 60 days (Fig. 1).

The The Panax quinquefolius, *P. quinquefolius*- C and *P. ginseng* B had clear differentiation and developed tracheid elemens, which are absent in strain of *P. ginseng*. It was established, that maximum time of life the cells in vitro are correlated with maximum differentiation tracheid cells

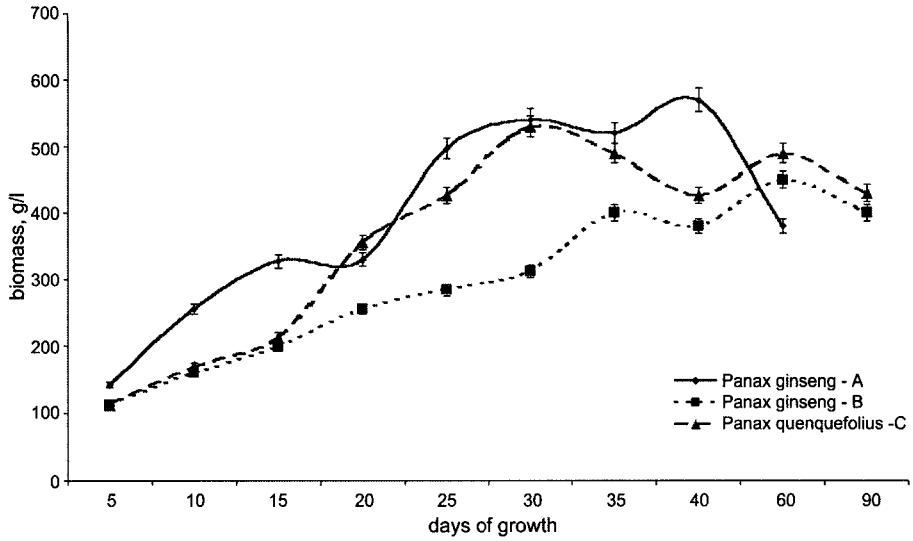

Fig. 1. Changes of biomass during the growth of differrent strains of Panax.

in selective strains.

The peaks of protein content for strain *Panax ginseng* (4.5 mg/g) and for Panax quinquefolius (3.5 mg/g) were on day 10 and remained unchanged till the last days of cultivation. The selective strains Panax ginseng A had two peaks of protein content (2.5 mg/g) on day 15 and on day 30. For selective strain *Panax ginseng* B these peaks were on day 5 and day 40 (3.5 mg/g) (Fig. 2).

Peroxydase activity peak (60 units/g) in stain of *Panax ginseng* was on day 10. This activity in Panax ginseng B had two peaks on day 15 and day 35 and reached 95 units/g, increasing to 150 units/g to day 80. In strain of *Panax ginseng* A was only one maximum of this activity 130 units/g on day 45. In *Panax quinquefolius* peroxidase activuty was 103 units/g to day 90. For *Panax quinquefolius* C this activity peak compose 136 units/g on day 60 (Fig. 3). Peroxydase activities for the upper and lower layers of biomass was different and varied considerably from 28-35 units/g in lower to 270-290 units/g for upper layer. The maximum of traxeides elementas was formed in lower layers of biomass but maximum activity peroxydase was connected with the parenchmatic cells in upper layer.

We showed that activation of the antioxidant system both in the strain closely related to the Panax species and in the strains cultivated on the medium containing germanium (strain-A, strain- B and strain C) could be evidence for the alterations in oxygen consumption by the cells of these cultures. For *P. quinquefolius*, this may be caused by the greater differentiation of this

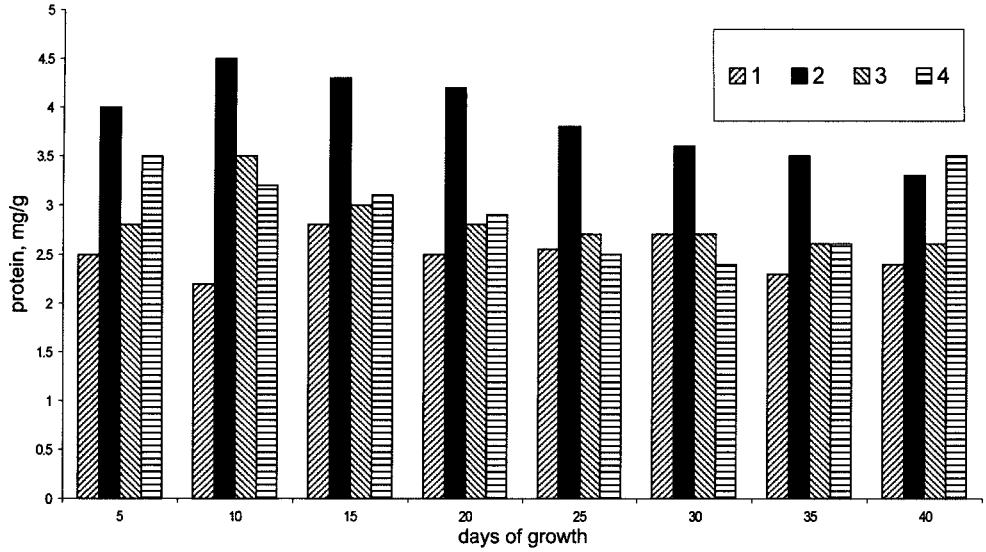

Fig. 2. Protein content during the growth of Panax callus cell culture.

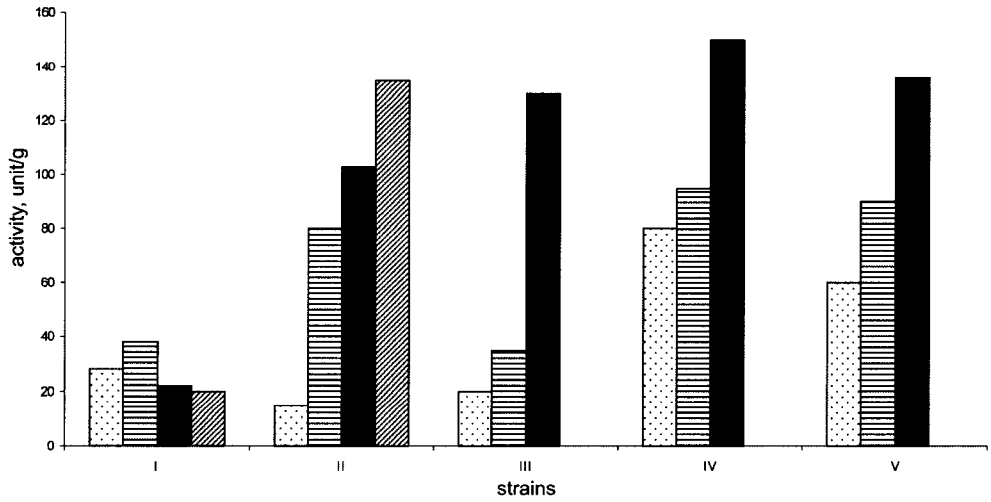

Fig. 3. Peroxidase activites during the growth of Panax cells culture.

biomass compared with *Panax ginseng*. It was reported that low amounts of germanium oxide intensified oxygen uptake by animal cells (Gar and Mironov, 1982). These data allow us to suggest that addition of organic germanium to the growth medium could also lead to the same process in plant cell.

We observed that the SOD activity had two peaks in Panax ginseng strain the 60 units/g and 70

units/g on day 20 and day 35 respectively.

1-Panax ginseng **A**; 2-Panax ginseng ; 3-Panax quenquefolius; 4-Panax ginseng.

Activity of SOD in Panax quinquefolius strain reached 53 units/g on day 40 and increased to 83 units/g

Days of research : Panax ginseng (I) 5, 10, 30, 40; Panax quenquefolius (II) 5, 10, 40, 90; Panax ginseng **A** (III) 15, 35, 45, Panax ginseng **B** (IV) 15, 35, 80; Panax quenquefolius **C** (V) 30, 50, 60.

The similar increase of SOD activity was marked for Panax ginseng B to 85 units/g on day 90. (Fig. 4).

Days of researh: Panax ginseng (I) 20, 35, 45; Panax ginseng **B** (II) 20, 40, 90; Panax quenquefolius (III) 20, 40, 60; Panax quenquefolius **C** (IV) 20, 40, 60.

In Panax ginseng strain the six molecular isoforms SOD was defined. One of them with Rf 0,6 was determined in all days of cycle cultivation, three other (Rf-0.43; Rf- 0.54; and Rf -0.80) were determined only on day 10 and day 20. The isoform of SOD with Rf- 0, 29 was detected only on day 10 and with Rf- 0.35 only on day 35.

Catalase activity was observed with maximum on day 20 and day 30 for all studying strains of Panax. At this growth period the catalase activity increase with the growth of the biomass and whereas catalase activity decreased about 10-15 fold in cells after the 80-90-days growth period.

Conclusions

The activity of antioxidant enzymes (peroxidase, SOD and catalase) for the different strains of Ginseng with germanium organic were studied for the first time. The maximum of activity of enzymes was appear in different period of cultivation. The correlation between maximum life span of strains and activites of their antioxydant enzymes were detected. We observed a high activity antioxidant enzymes in the different strains of Panax within an active growth period (20-35 days). These date allow us to suggest that this fact seems to be connected with the processes of biological oxidation and intens respiration of the dividing cells. More significant changes (more increase enzymatic activity) in peroxidase and superoxide. Dismutase it was reported at the end of growth period (80-90 days). This occurs simultaneously with the end of rapid increase the biomass. This fact can be due to aging of tissue when toxic peroxydes and superoxydes are accumulated in the tissue.

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