Activity of 1,3-β-D-Glucanases in Soil
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ABSTRACT. Air-dried soil did not exhibit any measurable 1,3-β-D-glucanase activity. The enzyme activity was observed after a previous wetting of the soil or after supplementing it with glucose or casein hydrolyzate. The addition of 1,3-β-D-glucan resulted in a significant increase of the enzyme activity in the variant with water and casein hydrolyzate but not in the variant treated with glucose.

The study of enzymic lysis of cell walls of hyphae and fungal spores by microorganisms contributes to the understanding of the mechanism of decomposition of the walls in soil. The results obtained constitute a basis for the application of lytic enzymes in the control of some fungal soil pathogens (Mitchell and Alexander 1963; Mitchell 1963). The lytic activity is characteristic for microorganisms producing 1,3-β-D-glucanases and chitinase (EC 3.2.1.14). In addition, on plant roots 1,3-β-D-glucanases can release the so-called elicitor from cell walls of plant pathogenic microorganisms which can further stimulate the production of phytoalexins by plants thus playing an important role in the protection against pathogens (Keen and Yoshikawa 1983). In order to clarify these phenomena it is necessary to know the activity of 1,3-β-D-glucanases as well as the conditions required for their production and the occurrence of microorganisms producing these enzymes in soil and on plant roots. In spite of the fact that the production of 1,3-β-D-glucanases was described in microorganisms isolated from the soil (Tanaka and Phaff 1965) and that the enzyme activity was demonstrated directly in soil (Jones and Webley 1968), conditions of the enzyme production in soil are not yet known. In the present communication the effect of some nutritional factors on the activity of 1,3-β-D-glucanases in chernozem soil was investigated. Samples of chernozem soil were taken from the surface arable layer (0—100 mm) and used in the form of air-dried structural aggregates 0.2—2.0 mm in diameter. The soil contained 2.12 % C, 0.17 % N (C : N ratio 12.1), the pH of the aqueous suspension (1 : 2.5) was 7.8. Soil cultivation (27 °C, 60 % full water capacity) and determination of CO₂ production in soil were described before (Ryšavý and Macura 1972a). The activity of 1,3-β-D-glucanases
was determined according to Lethbridge et al. (1978), however, instead of laminarin, 1,3-β-D-glucan from *Saccharomyces cerevisiae* cell walls served as substrate (Holan et al. 1984). In the supernatant of the incubation mixture glucose concentration was determined with glucose-oxidase (EC 1.1.3.4)—peroxidase (EC 1.11.1.7) (Bio-La-test, Lachema, Czechoslovakia). The enzyme activity was expressed as pkat per 1 g soil. This assay fulfills the basic conditions for the enzyme determination in soil (Skujins 1967). The assay of the activity of 1,3-β-D-glucanases with the aid of coloured substrate (Zithing and Linko 1971) could not be employed as the coloured reaction product was strongly adsorbed to the soil particles.

![Graph showing CO₂ production and 1,3-β-D-glucanase activity during cultivation of soil](image)

**Fig. 1.** Total CO₂ production (*top*) and 1,3-β-D-glucanase activity (*bottom*) during cultivation of soil; *empty circles* — wetting only; *empty rectangles* — supplemented with glucose (6 mg/g soil); *empty triangles* — supplemented with casein hydrolyzate (6 mg/g soil). *Full symbols* designate values obtained in soil supplemented with additional 1,3-β-D-glucan (2 mg/g soil).

Air-dried soil did not exhibit any measurable enzyme activity. After wetting of the soil with water the enzyme was active as early as in a single day (Fig. 1 *bottom*). A similar situation was observed when the soil was supplemented by glucose or casein hydrolyzate (both 6 mg/g soil). The increased