Changes in soil enzyme activities following additions of cyanobacterial biomass and exopolysaccharide

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Abstract

The aim of this research was to establish changes in the overall activity of extracellular enzymes: β-glucosidase, phosphomonoesterase, arylsulphatase, protease and urease and the intracellular enzyme dehydrogenase produced by the addition of Tolypothrix tenuis and Microchae tenera (Cyanobacteria) exopolysaccharide (EPS) and biomass to a silty clay loam soil. Both biomass and EPS of M. tenera significantly increased β-glucosidase activity by 0.1 and 29.3%, respectively. Both cyanobacteria species significantly increased urease activity by 15–1%.

Cyanobacterial biomass and EPS from T. tenuis and M. tenera produced significant increases in protease (76–90%, 101–136%), phosphomonoesterase (19–27%, 13–22%), arylsulphatase (148–167%, 406–174%) and dehydrogenase activity (16–32%, 43–30%). © 2000 Elsevier Science Ltd. All rights reserved.

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Decomposition of residues in soil releases nutrients such as nitrogen, phosphorus and sulphur required for plant and microbial growth. Various processes are involved in this nutrient cycling including reactions caused by inorganic catalysts (Conti, 1998) and those catalysed by intracellular enzymes (e.g. dehydrogenase) and extracellular enzymes such as β-glucosidase, protease, phosphomonoesterase and arylsulphatase. The extracellular enzymes are derived from microorganisms, plant roots and soil animals (Martens et al., 1992).

Incorporation of organic materials into soil promotes microbial and soil enzyme activity. Soil enzymes are thought to be largely of microbial origin and some are obviously associated with viable cells; however many enzymes can remain catalytic in cell debris, in soil solution or complexed with clay or organic colloids. Although the ecological role of cell debris and extracellular complexed enzymes is yet to be completely explored, Burns (1982) hypothesized that humic–enzyme complexes may benefit some organisms by hydrolyzing substrates that are too large or insoluble for microbial uptake. Soils that have been managed to promote soil quality (e.g. minimum tillage, organic amendments, crop rotations) should have higher biological activity than intensively used soils, and this should be reflected in greater enzyme production and possibly greater potential to stabilize and protect extracellular enzymes by forming soil enzyme complexes (Dick et al., 1996).

Cyanobacteria, such as Nostoc muscorum and Tolypothrix tenera, produce extracellular enzymes that decompose organic residues (Hussein et al., 1989a,b). They are also used as inoculants to increase the polysaccharide content and microbial activity of soil (Storni de Cano et al., 1997; Zulpa de Caire et al., 1997; Zaccaro de Mulé et al., 1999).

The aim of this research was to identify changes in the activity of soil extracellular enzymes β-glucosidase, phosphomonoesterase, arylsulphatase, protease and urease and the intracellular dehydrogenase following the addition of cyanobacterial exopolysaccharide and biomass to soil.

Strains of T. tenera (Nº 40d) and Microchae tenera (Nº 13a) were used. These were maintained in Allen and Stanier (1968) medium and are from the culture collection of the Biology of Cyanobacteria Laboratory, University of Buenos Aires. They were chosen from a number of species by comparing their growth on soil by dry weight assessment at day 30. To obtain the exopolysaccharide (EPS) and sufficient biomass, the strains were cultured in 2-L Erlenmeyer flasks with Allen and Stainer modified medium (without sodium nitrate) and incubated at 28 ± 1°C under fluorescent
light at 45 \mu E m^{-2} s^{-1}. At the end of the exponential growth phase (25 days) the cyanobacterial biomass was separated by centrifugation (8000 x g). EPS was isolated from the growth medium (i.e. supernatant after centrifugation) according to Nakagawa et al. (1987), but without a dialysis step. The wet weight harvested was 4.6 g l^{-1} for T. tenuis and 2.65 g l^{-1} for M. tenera. The pH of the final solutions were 8.6 and 8.4 for T. tenuis and M. tenera, respectively.

A poorly drained soil (Typic Argialboll USDA, 1994) with silty clay loam texture from Viyetes, Province of Buenos Aires, Argentina, was collected at 0–10 cm depth.

In order to establish the effect of biomass and exopolysaccharide additions on the enzyme activities of the soil, two treatments were performed:

(i) Inoculation with biomass. Non-sterile soil samples dried, sieved (2 mm pore diam.) and 120 g dispensed in each of 30 plastic boxes (13 x 8 x 5 cm^3). The soil was saturated with distilled water (\equiv 160 g final weight). The boxes were kept at 25 \pm 1^\circ C and with constant moisture content throughout the experiment and placed under 45 \mu mol photon m^{-2} s^{-1} irradiance, 12 h photoperiod.

(ii) Addition of exopolysaccharide. Twenty boxes were prepared, as above. Ten boxes were amended with 40 ml T. tenuis EPS solution and 10 with 40 ml of M. tenera EPS solution. Corresponding controls were set up.

After 90 days, the activities of \beta-glucosidase, urease, protease, phosphomonoesterase, arylsulfatase and dehydrogenase were determined using the methods described by Alef and Nannipieri (1995).

An analysis of variance using a randomized complete block design was performed. The homogeneity of experimental error mean squares for treatment was examined using Bartlett’s test for homogeneity of variances. The Duncan Test was used to compare means (Steel and Torrie, 1985).

The biomass in the inoculated soil treatments grew during 90 days producing a film that covered 60–90% of the surface with mean thickness of 2–3 mm.

The \beta-glucosidase, urease, protease, phosphomonoesterase, arylsulfatase and dehydrogenase activities increased in most cases following cyanobacterial inoculation and EPS amendment (Tables 1 and 2). A small (p < 0.05) 4% increase in \beta-glucosidase activity was measured when M. tenera biomass was added but there was no effect with T. tenuis. As the inoculants do not contain cellulose, there was no obvious addition of inducer molecules and cyanobacterial addition did not result in an increase in the biosynthesis of \beta-glucosidase by the indigenous soil microflora. The addition of M. tenera EPS did result in an increase in \beta-glucosidase probably because it provided organic nutrients for \beta-glucosidase-producing soil microflora thereby stimulating the synthesis of this enzyme.

The increase of urease activity produced following inoculation by both strains was significant (p < 0.05), but the low values obtained were probably due to the low substrate concentration in the original soils without fertilization and after treatment.

The addition M. tenera, T. tenuis and their EPS led to large increases (100–140%) in soil protease activity, which may reflect the excretion of enzyme by the actively growing cyanobacteria although not all cyanobacteria excrete proteolytic enzymes. However, cyanobacterial EPS is known to exert a stimulating effect on microbial enzyme production (Dmitrovskii and Sadchikov, 1994).

Both the cyanobacterial inoculants and EPS increased acid phosphatase activity. T. tenuis probably produced extracellular cell-bound phosphatase as enzyme activity increased by 27%, while EPS addition produced an increase of 13%. M. tenera biomass and exopolysaccharide produced similar effects: 19 and 22% increase in enzyme activity.
respectively. These results imply that *M. tenera* excreted, which is generally induced by P deficiency, is widespread among cyanobacteria; in several species it is optimal between pH 8.0 and 10.0 coinciding with the pH of Typic Argialboll (Healy, 1982).

Both strains produced significantly high increases in aryl-sulphatase activity. The values were highest with EPS addition, especially for *T. tenuis* when a 400% increase was recorded. This increase may have been due to a combination of enzyme secretion and the stimulation of biosynthesis by other microorganisms.

An increase of dehydrogenase activity was stimulated by addition of both strains either as biomass or as EPS (16–43%). Although dehydrogenase has been shown to be sensitive to soil management effects (Martens et al., 1992), it is less useful as a measure of long-term changes in soil quality because it cannot accumulate in a complexed form in soils and is best used as an indicator of the viable microbial populations (Dick et al., 1996).

In general, EPS addition (especially *T. tenuis*) produced a higher increase of the enzymatic activity than biomass addition. The difference between the increments produced by biomass or EPS addition was caused in part by the necessity for the biomass to undergo cellular lysis prior to the liberation of intracellular enzymes as the results indicate very low excretion of extracellular enzymes. EPS solution contained proteins some of which could have exoenzymatic activity and other substances that could behave as inducer molecules that stimulate exoenzyme synthesis and secretion by indigenous soil microorganisms.

According to our previous experience and the present results, addition of cyanobacteria could be a promising cultural practice for the amelioration of degraded soils.

References


