EXTRACTION OF ACID PHOSPHOMONOESTERASE FROM SOIL: TESTING OF VARIOUS EXTRACTANTS

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Abstract


The aim of this work was to investigate the suitability of various types of extractants for extraction of acid phosphomonoesterase from soil using various types of extractants. Succinate-borate buffer of pH 4.8 or 0.1 M K₂SO₄ were the most efficient to extract this enzyme compared to 0.1 M glutamic acid or 0.005 M salicylic acid. Extraction using 0.1 M glutamic acid gave significantly (P<0.05) lowest extraction yield. The following extracts were obtained: clear K₂SO₄ and glutamic acid extracts, succinate-borate buffer extracts were of slight coloration, and in some cases, rose-colored extracts of salicylic acid. The results of this work are in accordance with low extraction yields of phosphomonoesterase reported in other studies.

acid phosphomonoesterase, extracts, glutamic acid, K₂SO₄, salicylic acid, soil

Activity of extracellular enzyme acid phosphomonoesterase (orthophosphoric monoester phosphohydrolase, E.C. 3.1.3.2.) in soil is commonly determined to evaluate release of available phosphorus from soil organic matter. Besides the commonly determined total soil acid phosphomonoesterase activity, several attempts were performed to extract some parts of this activity. For this purpose, various types of extractants were used. For example, extraction using sodium pyrophosphate (0.14 M, pH 7.1) is based on solubilization of soil organic matter when enzyme-humic substances complexes are extracted. In this case, activity of acid phosphomonoesterase may be inhibited by humic substances (Rejšek et al., 2011, sent to journal). Sodium pyrophosphate extracts were brown with significant amount of humic substances displaying enzymatic activity mostly below the detection limit, in some cases extraction yield 0.1% of total soil acid phosphomonoesterase activity was reported. Tris-HCl, Tris-HCl with bovine serum albumine (BSA) and Triton X-100 separately or together gave colorless to light yellow extracts indicating very low solubilization of humic substances. Treatment with Tris-HCl buffer (50 mM, pH 7.5) gave acid phosphomonoesterase extraction yield from 0 to approx. 1% (see Fornasier and Margon, 2007). Tris-HCl buffer plus 1% Triton X-100 extraction may have a loosening effect of the enzymes and soil organic matter complexes and as in case of extraction using Tris-HCl buffer with 4% BSA when protein exchange is supposed, 2–8 times higher extraction yield of enzymes compared to Tris-HCl extraction was reported (Fornasier and Margon, 2007). When both Triton X-100 and BSA were added into Tris-HCl buffer, more than additive extraction yield reaching 2–13% was obtained (Fornasier and Margon, 2007). Other types of extractants used are mentioned in works of Batistic et al. (1980), Nannipieri et al. (1980), Hayano (1988), Pascual et al. (2002) etc. Besides extraction of fraction of acid phosphomonoesterase from soil, other types of treatments were tested. For example, sonication was reported to increase acid phosphomonoesterase activity of soils (De Cesare et al., 2000).

In this work we have attempted to extract acid phosphomonoesterase from soil using various...
type of extractants including 0.1 M K₂SO₄, 0.1 M glutamic acid, 0.005 M salicylic acid and succinate-borate buffer of pH 4.8. The aim of this study was to find the best extractant to extract fraction of acid phosphomonoesterase from soils. The results may be used for various types of ecological studies.

**MATERIAL AND METHODS**

The soil used for the experiments was obtained from the “Bílý Kříž” (“White Cross”) experimental research station, located in the Moravian-Silesian Beskids Mts. in the northeastern part of the Czech Republic. The region has a sub-continental climate with a typical mean annual air temperature of 4.9 °C and mean annual precipitation of 1100 mm. The number of days with snow cover is 160 per year.

The experimental 23-year-old Norway spruce stand is located at 908 m a. s. l. (N 49°30’10”, E 18°32’20”). The stand comprises approximately 99% Norway spruce (*Picea abies* (L.) Karsten) and 1% fir (*Abies alba* Mill.). The first mixed sample composed from 5 sub-samples of H horizon was taken there. The second mixed sample was obtained from Ah horizon of the experimental meadow at the same locality. The moderately mown meadow plant association belongs to the *Nardo-Callunetea* class (Formánek et al., 2008).

After sieving through a 5 mm sieve, the soil samples were stored at 6 °C until the analyses. Acid phosphomonoesterase was extracted using 0.1 M K₂SO₄, 0.1 M glutamic acid, 0.005 M salicylic acid or succinate-borate buffer of pH 4.8. In all cases, 5 g of wet soil were treated with 25 mL of each of the solution, shaken (20 min., 300 rpm), and filtered. Consequently, 4 mL of filtrate were incubated with 6 mL p-NPP in succinate-borate buffer of pH 4.8. Determination of acid phosphomonoesterase was performed according to Rejšek (1991). All data were processed using the Statistica 9 software (α = 0.05, n = 3). One Way Anova plus Fischer’s LSD test were used.

**RESULTS AND DISCUSSION**

The results of this study showed that succinate-borate buffer of pH 4.8 or 0.1 M K₂SO₄ were the most efficient agents to extract acid phosphomonoesterase from soil (Fig. 1). While the extracts using K₂SO₄ were colorless, succinate-borate buffer extracts were of slight coloration, but still virtually colorless. Other types of extractants like salicylic acid produced rose-colored extract, when Ah horizon soils were extracted. In case of H horizon of forest soil, colorless extracts were obtained using salicylic acid. Colorless extracts were produced also by glutamic acid treatment giving the lowest extraction yield (P<0.05). The results obtained in this work correspond with low extraction yields when acid phosphomonoesterase or other types of enzymes were extracted from soils within other works. In various studies, extraction of phosphatase was performed using water, Chelex 100-Na⁺ active resins and distilled water, 4 mM CaCl₂, sodium pyrophosphate (e.g. 0.14 M, pH 7.1), phosphate buffer (e.g. 0.2 M, pH 8) in the presence of EDTA, Tris-HCl buffer (50 mM, pH 7.5), Tris-HCl buffer plus 1% (w/v) Triton X-100 (permeabilizing
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agent), Tris-HCl buffer plus 4% (w/v) BSA, Tris-HCl buffer plus both 1% Triton X-100 and 4% BSA (Batistic et al., 1980; Nannipieri et al., 1980; Hayano, 1988; Pascual et al., 2002; Fornasier and Margon, 2007). Increased activity of extracted phosphatases appeared after ultrafiltration (Nannipieri et al., 1982). Activity of acid phosphomonoesterase in soil extracts obtained in this work corresponds with those reported by e.g. Fornasier and Margon (2007) or Margon and Fornasier (2008). Nevertheless, more research is necessary to better evaluate occurrence of easily extractable acid phosphomonoesterase in various soils.

CONCLUSIONS

Various extractants were tested to extract acid phosphomonoesterase fraction from soil. The testing was performed to better understand phosphorus transformation in soils of various types of ecosystems. The results presented in this work show that succinate-borate buffer of pH 4.8 or 0.1 M K$_2$SO$_4$ are the most efficient extractants compared to solutions of 5 mM salicylic or 0.1 M glutamic acid. The advantage of treatment with 0.1 M K$_2$SO$_4$ is obtaining colorless extracts. Nevertheless, more research is necessary to find the best method how to extract fraction of acid phosphomonoesterase from soil. The study was supported by the grant MSM6215648902 / Forest and Wood: the support of functionally integrated forest management and use of wood as a renewable raw material phase 4/2/3, part II “The management strategy of nature conservation areas”, and the project “Activity of acid phosphomonoesterase in soil of mountain ecosystems: elaboration of the methods and their application” (Complementary activities of the Mendel University in Brno).

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