

## Production of Peroxidase from The Cell Suspension Cultures of Horseradish (*Armoracia rusticana*)

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### ABSTRACT

Horseradish (HR) plants are considered the main source of peroxidase enzyme. The enzyme is extracted from the roots of field-grown plants which require at least six months to reach maturity. Tissue culture technique was used in the current study as an alternate method to obtain fresh horseradish peroxidase (HRP) throughout the year. The ability of ten cultivars of horseradish (ILH1590, ILH1722, ILH1005, ILH28A, ILH7586, ILH316-3, ILH647, ILH22C, ILH1069 and ILH1038) to produce peroxidase enzyme *in vitro* was investigated. To optimize callus induction, leaf blade, petiole and root segments from the ten cultivars were cultured on MS medium supplemented with 0.5, 0.75, 1 or 5 mg/l 2, 4-D. Virus-free and virus-infected plants of the ten cultivars were used in this study, both grown in either light or dark conditions. HRP activity was determined using spectrophotometric colour changes based on the oxidation of phenol-aminoantipyrine solution in the presence of hydrogen peroxide. In order to standardize the assay, a regression equation was calculated from a standard curve of known HRP activity (Sigma, St. Louis). Activity was expressed in two ways:  $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$  FW and as specific activity in  $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{mg}^{-1}$  protein. Protein concentration was determined with a Pierce BCA protein assay kit. The best 2, 4-D concentration used to obtain callus from either leaf blades or petioles was 0.5 mg/l. The results of the current study showed that horseradish cultivars varied in their peroxidase production and that virus-free HR suspension cultures produced more peroxidase than either virus-infected cultures or control. The results also showed that light is essential to maximize the production of peroxidase. In addition the study showed that six weeks old suspension cultures can produce at least as much peroxidase activity as six months old mature field grown roots, thus the *in vitro* system has the capability to speed the manufacture of fresh peroxidase enzyme and facilitate several production cycles through the year.

**Key words:** horseradish, peroxidase, callus, suspension culture, explants, virusfree plants, cultivars.

### INTRODUCTION

Horseradish (*Armoracia rusticana* P. G. Gaertn., B. Mey. et Scherb.) is a perennial crop that belongs to family Brassicaceae. Horseradish roots are a rich source of the peroxidase enzyme Kushad *et al.*, (1999). Peroxidase (present in peroxisomes) prevents the toxic accumulative effects of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), a strong oxidizing agent produced as an end product of oxidative metabolism (Oneonta University, 2004). HRP is a well-known enzyme used as an indicator for various chemical reactions that produce peroxide. The enzyme is used within enzyme-based immunoassay systems by conjugating it to antibodies. Peroxidase has been also used in bio-bleaching Harazono *et al.*, (1996), in degrading of wood pulp into fuel (Macek *et al.*, 1993), and in removal of carcinogenic pollutants from industrial wastewater Peive *et al.*, (1972); (Klibanov and Morris, 1981); (Dec and Bollag, 1994); Roper *et al.*, (1996); (Krel 1991) reported that the world production of horseradish peroxidase was estimated by 30 billion kilo-units, and he expected demand to double in 2010. The three major HRP producers in the United States are Sigma<sup>1</sup> Chemical Co., St. Louis; ICN Pharmaceuticals<sup>2</sup> Inc., Costa Mesa, California<sup>3</sup> and Boehringer Mannheim Corp., Indianapolis, Indiana Kushad *et al.*, (1999). Peroxidase is present in various plants, however, the

highest known concentration is found in the roots of horseradish (Scripps laboratories, 2003). Although, peroxidase activity has been reported in peanut, tobacco, and soybean (Buttery and Buzzell, 1968); Cairns *et al.*, (1980); Lobarzewski and Van Huystee, 1982), the highest peroxidase activity in soybean seed is about 1% of that in horseradish Kushad *et al.*, (1999).

Horseradish roots are the most economically important part of the plant and are the source for the enzyme peroxidase. HRP is usually harvested from fresh field-grown HR roots. *In vitro* culture could provide an alternate method to obtain fresh HRP throughout the year. In this study, the yields of HRP from suspension cultures were compared to those of fresh roots.

### MATERIALS AND METHODS

This research was carried out at the University of Illinois at Urbana – Champaign in 2008. The ability of ten cultivars of horseradish (ILH1590, ILH1722, ILH1005, ILH28A, ILH7586, ILH316-3, ILH647, ILH22C, ILH1069 and ILH1038) to produce peroxidase enzyme *in vitro* was investigated.

#### Preparing plant material.

For these experiments *in vitro* plants were produced in two ways: from field grown (mosaic