Available online at www.elixirpublishers.com (Elixir International Journal)

Bio Technology

Elixir Bio Tech. 69 (2014) 23706-23709

ISSN: 2229-712X

Detection of protease from latex producing plant by X-ray film by DOT-BLOT method

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ARTICLE INFO

Article history:

Received: 8 November 2013;

Received in revised form:

20 April 2014;

Accepted: 28 April 2014;

Keywords

Plant latex,

Protease assay,

X-ray film,

Proteinestimation.

ABSTRACT

Many plants contain latex that exuded when leaves are damaged & number of protein & enzymes have been found in it. The latex of some plant families such as *Asclepiadaceae, Apocynaceae, Caericaceae, Euphorbiaceae, Moraceae, Meliaceae, Sopodilla* contains endopeptidase. In presence study fourteen various latex producing plants were identify for the presence of proteolytic activity by dot-blot X ray film method. The *Euphorbia synudenium, Caloteopisprocera, Thevetia Peruviana, Ficusreligiosa, Caricapapaya, Azarirachta indica, Ficusbengalensis, Manikarazopota, caloteopisgigantea*, degrade the gelatin on the x-ray film & the clear zone is formed at the site of application on x-ray film which indicates the presence of protease in the sample. The *Jatrophacurcus, Plumeriarubera, Euphorbia triucalli, Ficusracemosa, Ricinuscommanis* show no zone of clearances at the site of sample on x-ray film which shows absence of protease. The protein estimation of a various latex containing plants were done by lowery's method. The *Euphorbia synudenium ,Caloteopisprocera, Thevetiaperuviana, Ficusreligiosa, Caricapapaya ,Azarirachtaindica, Ficusbengalensis, Manikarazopota, caloteopisgigantea, Jatrophacurcus, Plumeriarubera, Euphorbia triucalli Ficusracemosa, Ricinuscommanis*in show protein concentration in between the rang of 45µg-390µg/ 0.1ml respectively. The proteolytic activities of enzymes preparation isolated from latex containing plants were estimated by using casein as substrate.In the present paper we described a simple and inexpensive procedure to detect protease of latex containing plants by the X- ray film dot-blot method.

Introduction

Latex is widely distributed in plant more than 12000-35000 species have been reported to contain it. Many proteases from plant latex have been isolated and their properties extensively investigated, e.g., ficin from *Ficuscarica*, euphorbains from Euphorbia spp., papain and related proteases from *Carica papaya*(Arnon R. Papain, 1970, Liener IE, Friedensen B. Ficin, 1970, Pal G, Sinha NK, 1980) and calotropain from *Calotropis gigantean(*Abraham KJ, Joshi PN,1979). Proteases have also been purified and characterized from oat, wheat flag, maize, *Phaseolus vulgaris*, *Onopordum turcicum*, *Spinacia oleracea* and *Petroselinum crispum* leaves (Jiang WB, Lers A, Lomaniec E, Aharoni N, 1999). Proteases are important enzymes of plant metabolism and are instrumental in regulating senescence (Lauriere C. 1983). They are responsible for the degradation of proteins. Proteolytic enzymes are used extensively inindustrial and medical applications (WardO P. 1985**).** As latex often contains toxic compounds against herbivorous insects (e.g. cardenolide in milk weed and alkaloid in poppy) (Dussourd 1993; Farrel et.al., 1991; Harborne, 1993),and as large amount of fluid intensely exudes immediately after an insect attack at the point of damage in spite of the relatively small total amount of latex suggested to exist in whole plant (Dussourd & Denno, 1991; Farrel et.al. 1991),some biologists hypothesized that latex provides plants with an ideal defense mechanism against insect herbivores (Dussourd ,1993,Dussourd & Denno, 1991; Farrel et.al., 1991; Harborne, 1993; Dussourd & Eisner,1987). However, neither apparent toxicity nor toxins have been reported from the majority of latex producing plants. For e.g., no apparent toxins have been reported from the latex of papaya, Ficus species, dandelion, mulberry, or the rubber tree, although these plants are well known latex producing plants. In such cases, the defensive role of latex has usually been attributed partly to its sticky nature, which would enable the plants capture and immobilize the mouth parts of insects (Dussourd, 1993,Dussourd& Denno, 1991; Farrel et.al., 1991). However, the absence of apparent toxicity from such plants appears to be inconsistent with and even undermining the widely accepted defense hypothesis. Meanwhile, latex is known to be a rich source of enzyme such as proteases (Arima et al.2000; Arribere et.al., 1998; Cohen et al., 1986; Kimmel &Smith et al., 1954; Kramer& Whitaker, 1964; Sgarbieri et al.,1964), chitinase (Azarkan, 1997; O'Riordain et al., 2002) etc. In particular, cysteine proteases are found in the latex of several plants, such as papaya and fig, in great abundance (Arribere et.al., 1998; Cohen et al., 1986; Kimmel&Smith et al., 1954; Kramer& Whitaker, 1964; Sgarbieri et al., 1964), although their physiological roles remain unknown.The enzymes that cleave peptide bonds of a protein are referred to as proteolytic enzymes or proteases. In the present paper, we described a simple and inexpensive procedure to detect protease of latex on the X- ray film by dot-blot method.Gelatine, a denatured form of collagen is a substrate commonly used to detect proteolytic activity [D.E.kliener 1994]. [A.L.cheung et.al.1991] have demonstrated the use of gelatin coating present on X-ray film as a substrate for detecting aggregate proteolytic activity in a dot-blot assay. With

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the help of X-ray film assay variety of proteolytic enzymes including serine proteinases, Metalloproteinase,thiolproteinases, and acid proteinases have been demonstrated [A.L Cheung., 1991].The present study was conducted to detect the protease from fourteen various latex producing plants by dot-blot X -ray film method.

Material & method:

Collection of latex from the sample:

The plants were obtained from the rural area around the Vaijapur village. The latex was collected in a sterile container by breaking of the leaves while the other parts of the plant were obtained by up-rooting the plant.

Protease activity of latex by the dot- blots method:

10µl of latex sample, spotted on to strip of X-ray film .The protease present in latex degrade the gelatin on the X-ray film and the clear of zone is formed at the site of sample applied on X-ray film [Vinod Borde et.al.2012].[Fig.1]

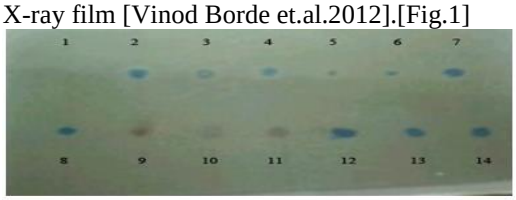

Fig.1: Dot-blot assay on x-ray film

Jatrophacurcus, Plumeriarubera, Euphorbia triucalli, Ficusracemosa, Ricinuscommanis show no zone of clearances is formed at the site of sample on x-ray film, in fig.1,5,9,10,11.The *Euphorbiasynudenium, Caloteopisprocera, Thevetia Peruviana, Ficusreligiosa, Caricapapaya, Azarirachtaindica, Ficusbengalensis, Manikarazopota, caloteopisgigantea*, degrade the gelatin on the X-ray film & show the clear zone at the site of sample on X-ray film, in fig.2,3,4,6,7,8,12,13,14.

Protein Estimation:

Protein concentration in the enzyme extract was determined using Folin Ciocalteu reagent as per the procedure of Lowry et al. (1951), Crystalline Bovine Serum Albumin used as standard protein for preparation of standard curve. The different aliquots of protein standard allowed reacting with Folin phenol reagent. The absorption of the blue color developed was measured at 540 nm using spectrophotometer. [Table: 1]

Determination of protease activity:

Protease activity was assayed by a modified method of [Tsuchicla et.al.1986] by using casein as substrate.100µl 0f enzymes solution was added to 900 µl of substrate solution [2% casein in 10mM. Tris-Cl buffer pH 8.0] the mixture was incubated at 50°C for 20 min. Reaction was terminated by the addition of an equal volume of 10% chilled Trichloro acetic acid (TCA) then the reaction mixture was allowed to stand in ice for 15 min to precipitate the insoluble protein.The supernatant was separated by centrifugation at 10,000 rpm for 10 min at 4^0c,the acid soluble product in the supernatant was neutralized with 5ml of 0.5M Na_2CO_3 solution .The colour developed after adding 0.5 ml of 3 fold diluted Folin ciocalteau reagent was measured at 660 nm.All assay were done in triplicate .One protease unit is defined as the amount of enzymes that release 1µ mol of tyrosine per ml per minute under the above assay condition. The specific activity is expressed in unit of enzymes activity per milligram of protein. [Table: 2]

Results:

The fourteen samples collected were analyzed for protease activity. *Euphorbia synudenium, Caloteopisprocera Thevetia Peruviana, Ficusreligiosa, Caricapapaya, Azarirachta indica, Ficusbengalensis, Manikarazopota, caloteopisgigantea* degrade the gelatin on the X-ray film & a clear of zone is formed at the site of sample on X-ray film. The *Jatrophacurcus, Plumeriarubera, Euphorbia triucalli, Ficusracemosa, Ricinus communis* show no zone of clearance at the site of sample on X-ray film. Total protein concentration of *Euphorbia synudenium, Caloteopisprocera, Ficusreligiosa, Ficusracemosa, Jatrophacurcus, Thevetiaperuviana, Plumeriarubera, Euphorbia triucalli, Carica papaya, Azarirachta indica ,Ficusbengalensis, Manikarazopota, caloteopisgigantea, Ricinuscommanis* were found in range of 45µg-390 µg/0.1ml respectively. The specific activity of crude enzymes preparation isolated from latex containing plants were estimated by using casein as substrate and is in rang of 0.61827 to 9.444 units. *Euphorbia synudenium* show highest specific activity 9.444 unit/mg, *Jatrophacurcus* 6.75 unit/mg, *Manikarazopota* 6.51786 unit/mg, *Ricinuscommanis* 5.9375 unit/mg, *Euphorbia triucalli* 3.20513 unit/mg, *Ficusreligiosa* 2.1794 unit/mg, *Thevetiaperuviana* 1.70833 unit/mg, *Azarirachtaindica* 1.70458 unit/mg, *Ficusracemosa* 1.40036 unit/mg, *Plumeriarubera* 1.03571 unit/mg, *Caricapapaya* 0.93537 unit/mg, *Caloteopisprocera* 0.81675 unit/mg, *Ficusbengalensis* 0.80357 unit/mg, respectively, were as *Caloteopis gigantea* show the lowest specific activity 0.61827 unit/mg .

Conclusion:

From the above result it was concluded that, all the selected plant containing latex from varies family show the proteolytic activity as common biological activity. India has a large tribal population, which is regularly using plant latex for the treatment of various diseases. Though so many utilities plant latex are known but their overall ethnobotanical use is still unknown that might be more helpful for development novel antibiotics from plant latex. However, before its clinical medicinal and industrial uses its phytochemical analysis is highly needful.Most of these properties are need to be explored. No doubt, plant latex is an industrially important raw material that can be made easily available for production of valued products such as much cheaper antibiotics for common microbial infections. In addition, there is a possibility to generate many more commercialized products by using plant latex especially fires, glues, adhesives, paints, flourings, films ,contraceptives, finger stalls, teats and immunodiagnostic materials. More specifically, use of latex and its products are environmentally much safer and these are easily recyclable or biodegradable in nature.Today proteases have become an integral part of the food and feed industry, and plant latex could be a potential source of novel proteases with unique substrate specificities and biochemical properties. And hence the present study was conducted to detect the protease from fourteen various latex producing plants by dot-blot X -ray film method.

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Table 1: Total protein concentration in latex

SR.NO	SAMPLE NAME	CONCENTRATION IN OF PROTEIN µg/0.1ml
1	*Euphorbia synudenium*	112.5
2	*AzadirachtaIndica*	390
3	*Ricinuscommanis*	367.5
4	*Carica papaya*	290
5	*Manikarazapota*	195
6	*Jatrophacurcas*	100
7	*Thevetia peruviana*	120
8	*Calotropis gigantea*	70
9	*Ficusreligiosa*	70
10	*Ficusbengalensis*	140
11	*Ficusracemosa*	87.5
12	*Calotropisprocera*	45
13	*Plumeriarubera*	85
14	*Euphorbia triucalli*	110

Table 2: Total proteolytic activity of latex containing plant

SR.NO	SAMPLE NAME	TOTAL VOLUME (ml)	TOTAL ACTIVITY (unit)	TOTAL PROTEIN (mg)	SPECIFIC ACTIVITY (unit/mg)
1	*Euphorbia synudenium*	7.5	796.875	84.375	9.444
2	*AzadirachtaIndica*	7.5	140.623	82.5	1.70458
3	*Ricinuscommanis*	7.5	534.375	90	5.9375
4	*Carica papaya*	7.5	257.8125	275.625	0.93537
5	*Manikarazapota*	7.5	684.375	105	6.51786
6	*Jatrophacurcas*	7.5	506.25	75	6.75
7	*Thevetia peruviana*	7.5	576.5625	337.5	1.70833
8	*Calotropis gigantea*	7.5	403.125	652.025	0.61827
9	*Ficusreligiosa*	7.5	637.5	292.5	2.1794
10	*Ficusbengalensis*	7.5	421.875	525	0.80357
11	*Ficusracemosa*	7.5	304.6875	217.5	1.40036
12	*CalotropisProcera*	7.5	520.3125	637.05	0.81675
13	*Plumeriarubera*	7.5	543.75	525	1.03571
14	*Euphorbia triucalli*	7.5	468.75	146.25	3.20513

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