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XEROPHYTIC PLANT O. VULGARIS AND *C. PTEROGONUS* A NOVEL SOURCE OF ALPHA-AMYLASE (EC.3.2.1.1) FOR PAS-DIASTASE STAINING IN HISTOPATHOLOGY LABORATORY

Life Science & Biotechnology

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ABSTRACT

Thermodynamic knowledge applied to visualization of tissue elements coupled with conversion of image version into explainable text in the histopathology is using different stains and enzymes to demonstrating elements in tissues that assist to diagnosis various disease. In the Periodic Acid-Schiff-Diastase technique is used to differentiation of diastase sensitive and resistant glycogen to diagnose and choice effective treatment in clinical practice. Present investigation undertaken to evaluation of Opuntia vulgaris and Cereus pterogonus plant alpha-amylase effects on the Periodic Acid-Schiff-Diastase special stain. In Vitro and liver tissues analysis of diastase activity showed that maximum diastase activity on 600, 700, 800, 900, 1000 IU/mL for Opuntia vulgaris. In Cereus pterogonus diastase showed maximum activity on 400, 500, 600, 700 and 800 IU/mL respectively. However, present investigation shows that both xerophytic plants have diastase potential to use Periodic Acid-Schiff and diastase staining.

KEYWORDS

Xerophytes, Amylase, PAS-Diastase, Histopathology.

INTRODUCTION

Life begins to synthesis and utilization of energy under thermodynamic process. Hence, thermodynamic knowledge applied to visualization of tiny particles coupled with conversion of image text into explainable text in the field of histopathology using to the different stain to demonstrating elements in biological tissues that assist to diagnosis and treatment of various disease in clinical practice. Periodic Acid-Schiff-Diastase (PAS-D) a histochemical technique refers that diastase enzyme digestion for polymer of glycogen into sugar that washed away and diastase resistant mucins and lipoprotein remain in the samples that will give magenta colour with Periodic Acid-Schiff stain (1). Primary aim of PAS-D staining is to differentiation of glycogen from other PAS positive components in various histopathological samples such as lover, intestine, kidney tissues and blood cells. PAS-D highly useful to identification of alpha-1 antitrypsin globules in liver, *Tropheryma whipplei* a bacterial present in macrophages of small intestine mucosa and cell wall of clinically important fungi was resistant to diastase digestion and positive for PAS (2,3). In the clinical practice highly important to differentiation of diastase sensitive and resistant polymer of glycogen like element to choice effective treatment.

Opuntia vulgaris and *Cereus pterogonus* comes under the family of xerophytic plants adapted to grow in high temperature regions and xerophytes plant enzymes are thermo stable naturally. Nevertheless, *Opuntia vulgaris* and *Cereus pterogonus* producing thermo stable enzymes cane be use industrial and clinical laboratory like thermostable fungi and bacterial enzyme (4). Present investigation undertaken *Opuntia vulgaris* and *Cereus pterogonus* alpha-amylase effects on the Periodic Acid-Schiff-Diastase special stain. A novel, thermostable and cost effective alpha-amylase enzyme in histopathological laboratory application is presently highly needed and in the study undertaken in this aspect.

MATERIALS AND METHODS

Materials

Xerophytic plants *Opuntia avulgaris and Cereus pterogonus* were collected from semi-arid region of Puducherry, India. The stains and chemicals used in the present investigation were purchased from Sigma-Aldrich (St. Louis, MO, USA). The reagents and stains preparation using doubled distilled water in the study.

Extraction, precipitation of enzyme

Plant surface cuticular layer was gently removed in sterile condition followed by cladode are chopped into pieces and homogenized to prepare 20 % (w/v) in 0.1M KH_2PO4/Na_2HPO_4 phosphate buffer in pH 7.0. Homogenate was filtered using muslin cloth and centrifuged at 10000xg for 15 min to obtain clear supernatant used for enzyme source. Followed by ammonium sulfate was slowly added to extract and 80% saturation protein precipitate collected after centrifugation and protein precipitate re-dissolved in 0.1M KH_2PO_4/Na_2HPO_4 phosphate buffer in pH 7.0 and used for alpha-amylase enzyme source (5,6).

Determination of enzyme activity

The α-amylase activity was determined using Hemy and Chiamori (1964) method by analysed amount of sugar released in reaction using the substrate polysaccharide starch. In the assay mixture 1.0mL starch, 1.5mL buffer, 100µL of isolated plant extract. Followed by incubated the reaction mixture for 30min at end of reaction stopped by 0.5mL NH_2SO_4 and sodium tungstate, after that centrifuged 5000xgfor 10min. The 1.0mL of resulted solution and 1.0mL of alkaline copper solution added and incubated for 10min followed by 1.0mL of phosphomolybdic acid added and incubated for 5min. The end of assay blue colure formed was analysed using double beam UV-Visible spectrophotometer against the blank. The plant enzyme activity was expressed in IU/mg protein (7).

In Vitro Periodic Acid-Schiff and Diastase staining

Three groups are A, B and C consist of four comparable tubes as follow tube 1, distilled water, tube 2, glucose solution (0.5mg/mL), tube 3, starch solution (0.5mg/mL), tube 3, BSA solution (0.5mg/mL). In the 2.4mg protein of plant extract contain 240 IU α-amylase activity used in the experiment. In the first group 5 drops of Schiff reagent added all the four tube followed by incubated for 5min. Group two, 1mL of 0.5% of freshly prepared Periodic Acid added and allowed to react for 15min followed by 5 drops of Schiff reagent added all the tubes and incubated for 5min. In the group C, 0.5mL of α-amylase solution added all the tube and incubated for 15min followed by 1mL of 0.5% of freshly prepared Periodic Acid added and allowed to react for 15min followed by 5 drops of Schiff reagent added all the tubes and incubated for 5min. Similarly, different concentration, 100 to 1000 IU/mg of plant α-amylase was employed to demonstration of Periodic Acid-Schiff and diastase staining, and after the incubation colour changes visually recorded (8).

Periodic Acid-Schiff and Diastase staining for liver

The deparaffinisation of thin rat liver sections (4µm) was carried out by start with xylene treatment for 15min, after that rehydration using different percentage of alcohol in each step given 2min followed by slides was washed in running tap water for 10-15min. 0.5mL of different concentration, 100, 200, 400, 600, 800 to 1000 IU/mL of plant α-amylase solution added all the slides and incubated for 15min, followed by carefully washed in running tap water for 10min. Than slides was treated with 0.5% Periodic acid solution and incubated for 5min than washed in distilled water for 6 time, followed by slides treated with Schiff reagent for 15min than washed in tap water for 5min and counter stained with Hematoxylin for 1min, washed with tap water for 10 min followed by dehydration employed with different percentage of alcohol finally clearing with xylene and DPX mounted (9).

RESULTS

The *O. vulgaris* and *C. pterogonus* plants cladodes homogenates showed α-amylase activity of 8 ± 2 and 9 ± 3 IU/mL. Though ammonium sulfate precipitated protein of *O. vulgaris* and *C. pterogonus* exhibited 22 ± 4 and 26 ± 6 IU/mL α-amylase activity (Fig 1). In the isolated enzyme was used PAS-diastase staining.

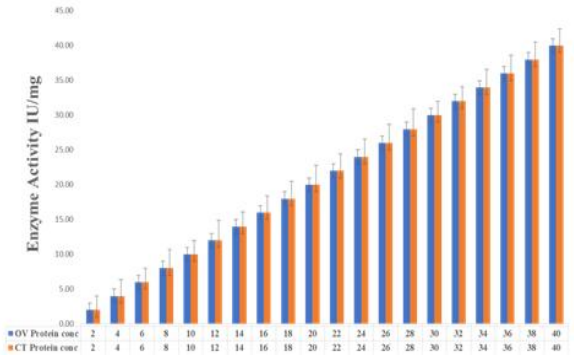

Figure 1. α-amylase activity at different concentration of *Opuntia vulgaris* and *Cereus pterogonus*.

In Vitro analysis of groups A comprised the distilled water, glucose, starch and BSA reacted with only Schiff reagent resulted to mild pink colour observed. But the group B, starch treated with 0.5% periodic acid followed by Schiff reagent given purple colour with starch solution positive PAS staining and reaming water, glucose and BSA observed negative for PAS. The group C, starch solution was initially treated with plant α-amylase followed by 0.5% periodic acid and Schiff reagent observed negative PAS staining and water, glucose and BSA shown pink colour (Fig 2).

Figure 2. PAS-Diastase staining demonstration without tissue sections, Group A. Solutions were treated only Schiff dye, Group B. pretreated with 0.5% periodic acid then Schiff reactive, Group C, Solutions were digested with α-Amylase (700 IU/mg) followed by treated with 0.5% periodic acid then Schiff reactive.

Hence, the plant α-amylase breakdown the starch resulted to negative for PAS. Similarly, different concentration, 100 to 1000 IU/mg of plant α-amylase treated with starch solution followed by 0.5% periodic acid followed by Schiff reagent was employed to demonstration of Periodic Acid-Schiff and diastase staining, α-amylase concentration depended PAS colour changes noted in both plant α-amylase (Fig 3).

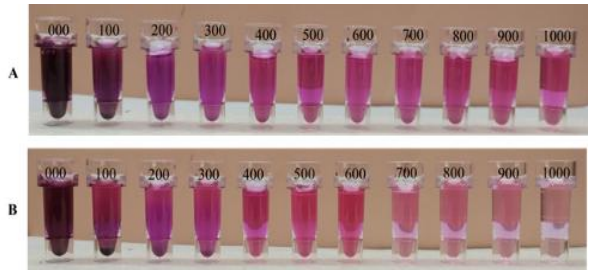

Figure 3. PAS-Diastase staining at different concentration of, A. Opuntia vulgaris and B. Cereus pterogonus α-Amylase demonstration without tissue section.

The result obtained from In Vitro Periodic Acid-Schiff and diastase staining with starch reflated same result with liver tissue glycogen, Both, O. vulgaris and C. pterogonus plants α-amylase extract used present investigation for the PAS-diastase staining in 100, 200, 400, 600, 800 to 1000 IU/mL different concentration noted significant diastase activity in the liver section compared to positive control (Fig 4). In the present investigation demonstrated the potential novel source of diastase present in xerophytic plant O. vulgaris and C. pterogonus. Here with reporting that crude extract of xerophytic plant O. vulgaris and C. pterogonus can be used in histopathology laboratory special stain.

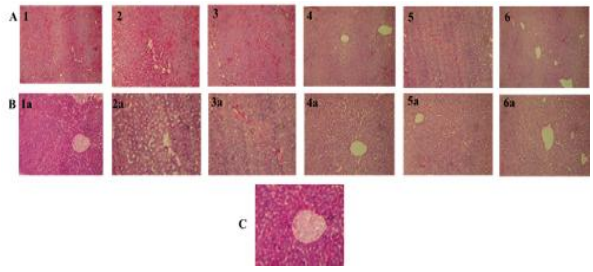

Figure 4. Shows the 10x magnification of PAS-Diastase stained liver tissue at the different concentration of A. *Opuntia vulgaris* (1-6) and B. *Cereus pterogonus* (1a-6a) α-amylase was treated with 100, 200, 400, 600, 800 to 1000 IU/mL along with C. positive control.

DISCUSSION

Use of α-amylase increased various histopathology laboratory globally increased significantly, subsequently research focused attention on thermally stable α-amylase from new source such as bacterial and plants. Therefore in the current investigated α-amylase from the xerophytic plants *O. vulgaris* and *C. pterogonus* for demonstration of Periodic Acid-Schiff and diastase staining. Present investigation find advantages to use plant α-amylase such as isolation, storage, cost effective, least contamination, if compared with commercial supply of α-amylase (10).

The effects of different concentration 100 to 1000 IU/mg of α-amylase from *Opuntia vulgaris* and *Cereus pterogonus* observed diastase activity (Fig 3 and 4). In Vitro and liver tissues analysis of diastase activity showed that maximum diastase activity on 600, 700, 800, 900, 1000 IU/mL for *Opuntia vulgaris* (Fig 3A and Fig 4A). Similarly, *Cereus pterogonus* diastase activity analysed showed maximum activity on 400, 500, 600, 700 and 800 IU/mL respectively (Fig 3B and Fig 4B). However, present investigation shows that both xerophytic plants have diastase potential to use histopathology laboratory Periodic Acid-Schiff and diastase staining, nevertheless, Cereus pterogonus had more diastase activity (11).

CONCLUSION

Our study is the first to demonstrated xerophytic plants *Opuntia vulgaris* and *Cereus pterogonus* a potential novel source of α-amylase for PAS-diastase staining in histopathology laboratory. Also the xerophytic plant derived α-amylase a thermal stable enzyme source for investigation glycogen content on in biological samples in histopathological laboratory. Alternative to using high cost commercial preparation of α-amylase. Cost effective and natural source of α-amylase using in the histopathology laboratory highly appreciated.

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Conflicts of Interest

The authors declare that there is no conflict of interest.

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