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RAPID DETECTION OF ALPHA-AMYLASE INHIBITORS

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Enzyme inhibitors of various origin have been intensively studied for years. To find new inhibitors, especially those produced by microorganisms, a large screening procedure is necessary. A rapid and simple procedure would be a considerable asset in detecting the presence of a required inhibitor. Such a procedure for the rapid detection of alpha-amylase inhibitors is described here. It is a modification of a previously described method for rapid detection of amylases, which is based on amylase hydrolysis of a thin layer of starch lying on a glass plate[1].

The starch plates were prepared as described previously[1]. Briefly, potato-starch (Sigma, USA; 1 g) was suspended in 100 ml of water and the suspension was boiled in a water bath for 15 min. Portions of the hot starch solution were poured on the glass plates (8 × 8 cm); usually 3 ml were used to cover the plates evenly. Water was allowed to evaporate at ambient temperature overnight so that a thin layer of firmly attached starch was formed on the plates. The solutions to be tested (e.g. culture media after cultivation of microorganisms, extracts from various tissues, fractions after liquid chromatography, solutions of various chemicals) were mixed, in the wells of a microtiter plate using a microwell plate shaker, with buffered alpha-amylase solution in the ratio ranging from 1:1 to 3:1; after 10–30 min incubation, 10–40 μl of the mixture were pipetted onto the surface of the starch plate to form a small drop. The concentration of alpha-amylase, which caused complete hydrolysis of starch layer in 10 min at room temperature (using 10 μl drop), was chosen for these purposes. The incubation was carried out at ambient temperature or in a thermostat for 20–30 min after the application of the last mixture drop. The plate was then thoroughly rinsed with running water so that the starch fragments originating from the action of non-inhibited alpha-amylase were washed away. The plate was then overlaid with an iodine solution (1 g of iodine and 3 g of potassium iodide in 100 ml of water) to form the blue starch-iodine complex. The plate was rinsed with running water again and evaluated. Colourless spots on a blue background indicated the absence of alpha-amylase inhibitors. The presence of inhibitors in the solutions tested caused the starch layer where the drops had been applied to remain unhydrolyzed. Where the inhibitor had low activity, partial hydrolysis of the starch layer occurred.

Three alpha-amylase preparations were used for model experiments; alpha-amylase from *Bacillus licheniformis* (type XII-A. No. A-3403, Sigma, USA; used as a 1:1000

dilution), alpha-amylase from *Bacillus sp.* (type XI-A, No. A-1278, Sigma, USA; used at a concentration of 1 mg/ml) and unstimulated human saliva (used after centrifugation as a 1:99 dilution). Succinate buffer (0.1 M), pH 6.0, containing calcium chloride (5×10^{-3} M), was used for dilution.

The compounds tested as potential inhibitors of alpha-amylase were silver nitrate, copper(II) sulfate, lead(II) nitrate, disodium salt of ethylenediaminetetraacetic acid (EDTA), oxalic acid, iodoacetic acid, sodium sulfate (20 mmol/l in water) and culture medium after cultivation of *Tolypocladium terricola* (an entomopathogenic fungus; obtained from dr. V. Matha, Institute of Entomology, České Budějovice, Czechoslovakia), Distilled water was used for comparison in control mixtures.

The tested solutions and water were mixed with alpha-amylase solutions or with buffer in the ratio 3:1 so that the final concentration of compound in the mixture was 5 mmol/l. After 30 min incubation at room temperature, aliquots (20 μl) were pipetted on a starch plate. The plate was incubated for 30 min at ambient temperature and then treated as described above.

The results are shown in Figure 1. It can be seen that aqueous solutions not containing amylases did not affect the starch layer (column D). By contrast, the starch layer must be completely hydrolyzed for the control alpha-amylase mixtures applied on the plate (row 1). When these two pre-requisites are fulfilled, the ability of various compounds to inhibit alpha-amylase can be readily seen. All three alpha-amylases were completely (or almost completely) inhibited by silver nitrate, copper(II) sulfate and oxalic acid. Lead(II) nitrate and iodoacetic acid completely inhibited human saliva alpha-amylase and almost completely bacterial amylase from *B. licheniformis*. EDTA and culture medium after cultivation of *T. terricola* inhibited only human saliva alpha-amylase. Sodium sulphate did not inhibit any of the tested alpha-amylases.

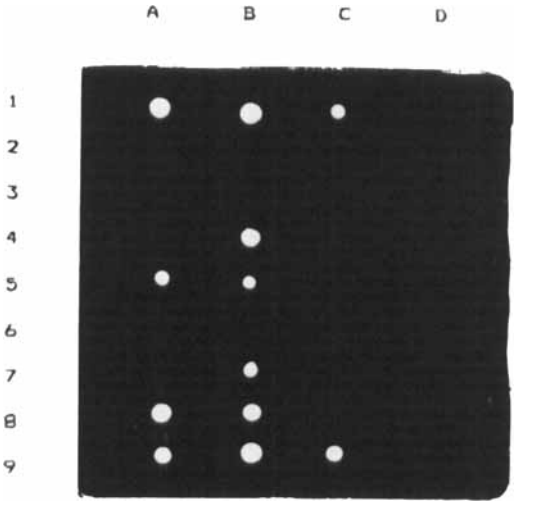

FIGURE 1 Starch plate after detection of alpha-amylase inhibitory activity of various samples. Lines: 1 – water (control); 2 – silver nitrate; 3 – copper(II) sulfate; 4 – lead(II) nitrate; 5 – disodium salt of ethylenediaminetetraacetic acid; 6 – oxalic acid; 7 – iodoacetic acid; 8 – culture medium after cultivation of *T. terricola*; 9 – sodium sulfate.
Columns: A – alpha-amylase from *B. licheniformis*; B – alpha-amylase from *Bacillus sp.*; C – alpha-amylase in human saliva; D – buffer.

saliva alpha-amylase. Sodium sulphate did not inhibit any of the tested alpha-amylases.

The described procedure enables rapid screening of a great number of samples for the presence of alpha-amylase inhibitors. Samples showing inhibition activity using this simple procedure can be further assayed by more refined methods.

Reference

1. Šafařík, I. (1989) *J. Biotechnol.*, **9**, 153.