CHROM. 5786

Starch gel electrophoresis of the peroxidase isozymes of the tea leaf

Starch gel electrophoresis has been used for the resolution of the peroxidases of the tea leaf. The occurrence of peroxidases in the tea leaf has been reported in ref. 1, a discontinuous system of buffers has been used[2] and the isozyme bands have been localized by a histochemical method based on the oxidation of benzidine in the presence of hydrogen peroxide.

Materials and methods

10 g of flush (clone TRI 777), 5 g of Polyclar (General Aniline and Film Corporation, New York, U.S.A.) and 0.5 g of sand were thoroughly mixed using a mortar and pestle. Tris–citric acid buffer of pH 8.5 (10 ml) was added and the tea juice was squeezed through chamois leather. The tea juice was centrifuged and the crude enzyme extract was used for starch gel electrophoresis. Hydrolyzed starch obtained from Connaught Laboratories, Ottawa, Canada, and Tris–citric acid buffer (pH 8.5)

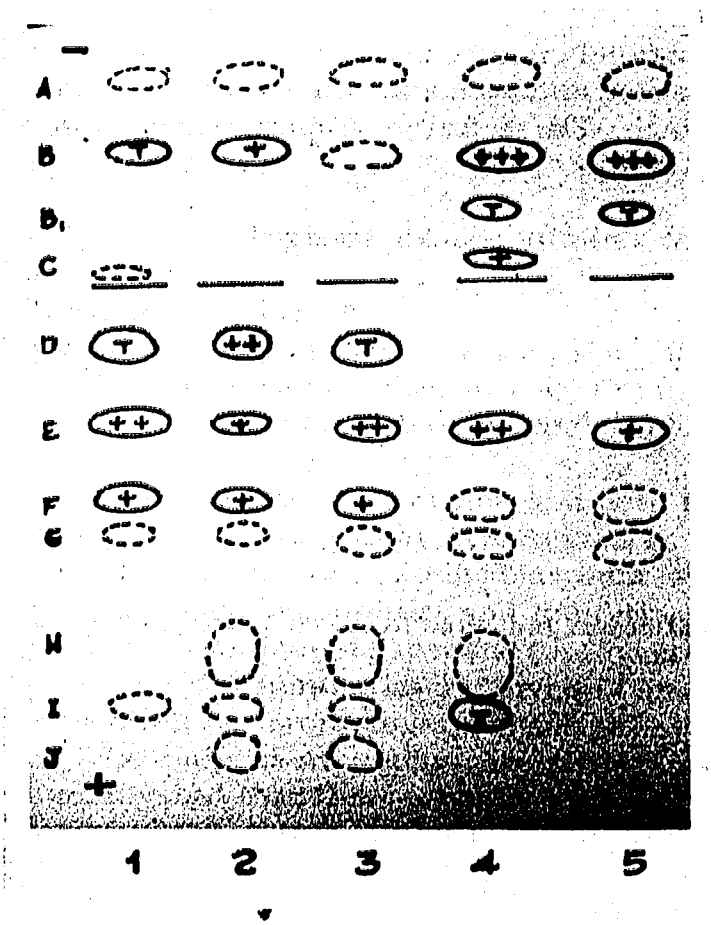

Fig. 1. Starch gel electrophoresis of the peroxidase isozymes in the tea leaf. 1 = TRI 9; 2 = DT 1; 3 = TRI 777; 4 = tea leaf after 1 h fermentation; 5 = tea leaf after 2 h fermentation. Activity of the isozyme bands: +++ = high; ++ = moderate; + = low; T = very low; dotted line = a trace.

were used for the preparation of the gel. The gel was chilled for 3 to 4 h before the insertion of pieces of Whatman 3MM filter paper, soaked in the enzyme extract. Sodium hydroxide–boric acid (pH 7.9) was used for the bridge.

The histochemical technique used for the localisation of the isozyme bands was essentially that of PANDEY[3]. The specific stain for peroxidase was prepared as follows. To 1 l of 7% acetic acid solution, 160 g of sodium acetate were added and then the solution was saturated with 1% EDTA solution and filtered. The mixture was then saturated with benzidine dihydrochloride and filtered again. Just before use, 1 ml of hydrogen peroxide was added to 100 ml of stock reagent. Violet bands appeared immediately at the regions of peroxidase activity. Intensification of colour occurred for approximately 30–45 min and then the bands gradually faded. Each sliced gel (14 × 8 cm) was incubated in the prepared solution.

Results and discussion

The results of the separation of the peroxidase isozymes are shown in Fig. 1. In the case of the fresh tea leaves (TRI 9, DT 1 and TRI 777), more than seven bands of enzyme activity were observed, of which at least three were positively charged. During the process of fermentation in the manufacture of black tea, remarkable changes in the activity of the isozyme bands were observed, as shown in Fig. 1.

The most notable observation was the rapid increase in activity of the isozyme band B during the first hour of tea fermentation, and even after 2 h fermentation the activity of this isozyme band remained high. The activity of most of the other isozyme bands decreased during fermentation.

The technical assistance of Mr. A. RANASINGHE is acknowledged.

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Received October 19th, 1971

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