A DIP-STICK ASSAY FOR DEXTRAN ANALYSIS

By

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Abstract

With the goal of developing a rapid, inexpensive method for measuring dextran concentrations in cane juice, we selected a dextran specific phage that can be used as an assay reagent. Phage antibody libraries express the universe of antigen binding fragments (Fab) as protein ligands. After serial panning, monoclonal phages for dextran were chosen, using a Sephadex agarose electrophoresis system (SAES). Dextran binding affinity was determined and a paper dip-stick assay was constructed. This assay, when tested on core lab juice, showed good correlation with the Midland Antibody test for dextran. It is a method that is both quick enough and cheap enough for potential use in screening for dextran in loads of cane arriving at the sugar mill.

Introduction

Dextran produces a number of significant problems during sugar production. It can be found in sugar mill process streams at greater than 5000 ppm on volume in juice and at higher than 750 ppm in raw sugar.

The presence of dextran in sugar process juices is normally indicative of stale cane, as much of the dextran is generated in damaged plant (sugarcane) material.

Stale juice is immediately obvious as it requires more lime to raise the pH and longer heating times for adequate clarification (Chung, 2000). If the dextran levels are high, it may not clarify.

Dextran in syrups causes production losses due to increased viscosity, lowered crystallisation rates and changes in crystal morphology (which affects purging in centrifuges and can lead to increased sucrose losses on separation).

Because dextran in sugar is not removed by refining, financial penalties are imposed on the seller of raw sugar for sugar containing dextran above 250 ppm (Clarke and Godshall, 1988).

A rapid, low-cost method for dextran can be applied to screen loads of sugarcane prior to delivery to the mill yard.

The isolation of an anti-dextran phage suitable for use in a simple assay format, such as a paper-dip stick was targeted as an appropriate reagent for dextran analysis in this application.

Phage display antibody is an efficient production system that provides an alternative to standard antibody methodology.

A phage library (Fab 2loz) was screened for dextran binding phages using five different methods: thin layer chromatography (TLC), Sephadex column, enzyme-linked immunosorbent assay (ELISA) screening, a combination of ELISA and Sephadex column screening, and a technique developed in the course of this research, Sephadex bead agarose electrophoresis (SBAE).

Immunonephelometric assays were used to determine the dextran binding affinity of all new phage preparations.

Phages recovered from lower dextran band

Phages recovered from upper dextran band

**Fig. 1—Immunonephelometric assays of two phage preparations selected from TLC plates.** An immunonephelometric assay was used to monitor phage affinity for dextran T-2000. The assay measures turbidity using scattered light of the lattice complex formed between dextran and phages and is based on the MCA-SucoTest\textsuperscript{TM}, using the same nephelometer. \( \Delta \text{Turbidity} (\Delta N) \) was calculated by subtracting the initial reading \( (N_0) \) from the reading \( (N) \) obtained at specific time intervals. The error bars represent standard deviation of triplicate experiments.

Protein coated polyvinylidene difluoride membranes (PVDF) dipped into dextran solution (T2000) and dried at room temperature were used as the matrix.

Polyvinylidene difluoride membranes (PVDF) are recommended over cellulose acetate membranes because PVDF is more efficient in blocking non-specific binding of antibody on membranes (Dewey et al., 1989).

Test solution is applied to the membrane and it is dried at room temperature. Then phage (5 \( \mu \)L) is applied to the paper, incubated for 1 min, washed once by dipping in 1% PBST (PBS + 1% Tween 20) and dried. A diluted \( (1: 10000) \) solution (1.0 \( \mu \)L) of HRP-anti-M13 in 5% MPBST (PBS + 1% Tween 20 + 5% skim milk) is applied and then after 5 sec washed three times by dipping into 1% PBST. TM Blue\textsuperscript{TM} substrate (0.5 mL) was applied to develop colour.

The intensity of the spots that were formed was determined using scanning densitometry. Dextran concentrations greater than 50 ppm in sugar juices could be visually detected using a paper-dip assay.

This assay was independent of phage concentration between \( 4.4 \times 10^5 \) t.u./mL and \( 4.4 \times 10^7 \) t.u./mL.

Saturation with dextran was achieved below 500 ppm of T2000, the colour intensity did not increase with concentrations above 500 ppm of dextran. Saturation of antigen on the polyvinylidene difluoride membranes (PVDF) limited the upper range of the assay.

The intensity increased with molecular size of dextran because large dextran has more epitopic sites to react with a paratope, antigen binding sites, than small size dextran.

The chosen phages, AE-M1114-m74-2R, showed high specificity against dextran but not corn starch, sucrose, glucose or chitin.
Table 1—Specificity of phage (AE-M1114-m74-2R) for polysaccharides. A phage (AE-M1114-m74-2R) based paper dip assay was tested for specificity against dextran T2000, corn starch, sucrose, glucose, and chitin at 1000 ppm. Phosphate buffered saline (PBS) was used as a control. The developed colour was scanned by NucleoVision scanning densitometry system. Standard deviations were obtained from triplicate experiments (n = 6).

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Normalised intensity (N.I.)</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextran</td>
<td>27</td>
<td>7.1</td>
</tr>
<tr>
<td>Corn starch</td>
<td>6</td>
<td>8.5</td>
</tr>
<tr>
<td>Sucrose</td>
<td>1.5</td>
<td>2.1</td>
</tr>
<tr>
<td>Chitin</td>
<td>1.5</td>
<td>0.7</td>
</tr>
<tr>
<td>Glucose</td>
<td>6</td>
<td>8.5</td>
</tr>
<tr>
<td>PBS (Control)</td>
<td>3.5</td>
<td>4.9</td>
</tr>
</tbody>
</table>

Mixed juice solutions were diluted with distilled water prior to assay. After dipping each test paper into a serially diluted mixed juice sample and drying at room temperature, 5 µL of a phage solution containing between $6.5 \times 10^4$ to $5 \times 10^5$ t.u./mL were applied to the papers.

After 1 minute, the papers were washed by dipping in 1% PBST (PBS + 1% Tween 20) and dried. One µL of a dilution (1:10000) of HRP-anti-M13 in 5% MPBST (PBS + 1% Tween 20 + 5% skim milk) was applied for 5 sec and then washed by dipping three times into 1% PBST. TM Blue$^\text{TM}$ substrate (0.5 mL) was applied to develop colour.

The test strips were compared to standard papers, which had previously been dipped into known dextran solutions ranging from 0 to 125 ppm.

Dextran concentration in mixed sugar juices was estimated by multiplying the dilution factor of the sample by the concentration of a standard that showed similar colour intensity. The results were compared to those obtained with the Midland SucroTest$^\text{TM}$ (Anon. 2002).

![Graph](image-url)
Analysis of dextran in mixed juices from four different sugar mills showed high correlation with Midland SucroTest™. With some simplification, this paper-dip assay system has potential to become a method suitable for routine screening of sugarcane coming to the mill.

REFERENCES


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LE DOSAGE RAPIDE DU DEXTRAN PAR BANDELETTES TESTS

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MOTS CLEFS: Dextran, Phage Visuel, Méthode Rapide, Bandelettes Test.

Résumé

On a choisi un phage spécifique au dextrane qui peut servir pour sa détermination par bandelette test, pour développer une analyse rapide et peu coûteuse donnant la concentration du dextran dans les jus de cannes. La littérature concernant les antigènes de phages montre que les fragments d’antigènes (Fab) s’attachent comme des ligands de protéines. On a choisi des phages monoclonaux pour le dextran, et un système électrophorétique Sephadex. L’affinité du dextran a été déterminée et on a développé une méthode utilisant des bandelettes tests. Cette méthode, qui montre une bonne corrélation avec les résultats obtenus en se servant de la technique développée par Midland Antibody, est rapide et peu coûteuse ; elle peut donc servir pour analyser les chargements de canne à l’entrée de la sucrerie.

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UNA VARILLA PROBADORA DE ENSAYO PARA EL ANÁLISIS DE DEXTRANA

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PALABRAS CLAVE: Dextrana, Despliegue de Fagos, Varilla de Ensayo.

Resumen

Con el objeto de desarrollar un método rápido y económico para medir las concentraciones de dextrana en el jugo de caña, seleccionamos un fago específico para la dextrana que puede usarse como reactivo de ensayo. Las bibliotecas de anticuerpos de los fagos expresan el universo de fragmentos de antígenos que se tienden a unirse (Fab) como ligamentos proteicos. Después de un lavado para su separación en serie, se eligieron los fagos monoclonales para la dextrana, usando un sistema de electrophoresis de agarosa Sephadex (SAES por sus siglas en inglés). La afinidad de unión de la dextrana se determinó con una varilla con papel de ensayo que se construyó al efecto. Este ensayo, al probarse en jugo de laboratorio, mostró una buena correlación con la prueba de Anticuerpos Midland para la dextrana. Se trata de un método que es tanto suficientemente rápido como suficientemente económico como para darle un uso potencial en el cernido de la dextrana en las cargas de caña que llegan al ingenio azucarero.