Journal of Chromatography, 558 (1991) 328–332
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 476

Short Communication

Rapid identification of microbial starch degradation products from a complex nutrient medium by a thin-layer chromatographic method

LJUBICA VRBAŠKI* and ŽIKA LEPOJEVIĆ
Department of Pharmaceutical Technology, Faculty of Technology, 21000 Novi Sad (Yugoslavia)
(First received January 3rd, 1991; revised manuscript received May 21st, 1991)

ABSTRACT

An improved thin-layer chromatographic method has been established for the rapid identification of microbial starch degradation products from a complex nutrient medium. The method is based on spotting 10 μl of a diluted liquid starch medium containing microorganism cells on high-performance liquid chromatography plates precoated with silica gel 60 with a concentration zone for nano-thin-layer chromatography. The mobile phase is chloroform–glacial acetic acid–water (3:6:1, v/v/v). After the first development the plates were dried and again developed. The separated carbohydrates were detected with a 50% (w/v) solution of sulphuric acid in ethanol after heating at 150°C for 10 min. The duration of the assay was less than 2 h.

INTRODUCTION

With the progress of biotechnology it becomes important to have an effective method for screening strains of microorganisms with desired characteristics. Many of the problems associated with these studies arise from the difficulty of obtaining rapid, accurate and reproducible measurements, particularly from complex media. It is known that nutrient media contain a large number of organic and inorganic compounds of low, intermediate and high molecular mass that can interfere with each other and mask the desired metabolite. In such circumstances a variety of methods have been used for sample purification.

The microbiological problem of screening starch-positive strains of microorganisms requires the development of an adequate methodology, although numerous assays for the measurement of starch degradation products exists and the modern instrumental methods of chemical analysis are used. These methods are often technically difficult, time-consuming and do not permit use of a small volume of samples containing the microorganism cells.

The determination of products of metabolically degraded starch is of interest for

0021-9673/91/$03.50

many reasons, especially in monitoring the action under usual and unusual conditions of strains of microorganisms that can hydrolyse starch. A microbiological test for starch hydrolysis is afforded by flooding the plate with dilute (Lugol's) iodine after inoculation and growth. Absence of the bluish-purple colour characteristic of the starch–iodine complex indicates hydrolysis. For different microorganism strains the area of hydrolysis is of different width and colour. Problems occur when it is necessary to identify and determine the products of metabolically degraded starch and their relative proportions. It is generally agreed that thin-layer chromatography (TLC) offers the best potential for application in this microbiological analysis. Advantages include easy manipulation, shorter analysis times and use of small volumes of sample.

Over the past years a very large number of articles have been published on the chromatography of carbohydrates, among them two recent reviews [1,2]. However, from the microbiological point of view there are more articles dealing with separation of individual carbohydrates from model solutions than from complex culture media.

The method described in the present report is an adaptation of the procedure previously described [3] for the chromatographic separation of carbohydrates as possible products of starch metabolism. This paper [3] also describes the separation of individual carbohydrates from model solution. The principal advantage of the present procedure over those previously described is rapid identification of microbial starch degradation products together with a complex nutrient medium containing the cells of microorganisms. In addition, it is possible to obtain the spectra of carbohydrates after enzymatic hydrolysis of starch.

EXPERIMENTAL

Chemicals

All chemicals were of analytical-reagent grade. Glucose, soluble starch, acetone, chloroform and glacial acetic acid were purchased from Kemika (Zagreb, Yugoslavia) and maltose, raffinose and trehalose from Merck (Darmstadt, Germany). α-Cyclodextrin was acquired from Fluka (Buchs, Switzerland) and β-cyclodextrin from Chinoin (Budapest, Hungary).

Standards

The following standards were used: 1 mg/ml glucose, maltose, raffinose, trehalose, α- and β-cyclodextrin and soluble starch, separately and in a mixture containing 1 mg/ml of each.

Microorganisms

For this study four strains of bacteria were used as producers of various width and colour of zone hydrolysis on starch agar media, independently of their taxonomic status.

Medium and culture condition

One loopful of physiologically young cells was inoculated into a 100-ml Erlenmeyer flask containing 30 ml of nutrient broth: peptone 1-Torlak, 15 g/l; meat extract-Torlak, 3 g/l; sodium chloride, 5 g/l; dipotassium hydrogenphosphate 0.3 g/l (Torlak, Belgrade, Yugoslavia) with 10 g/l soluble starch, cultured at 25°C for 2 days.

Sample preparation

In the first experiment culture broth samples were deproteinized by adding 4 ml of acetone to 2 ml of broth. The mixture was held in the cold (4 ± 1°C) for 24 h, and then centrifuged for 10 min (1600 *g*). The supernatant was evaporated *in vacuo* (27°C). The remaining solid was redissolved in 2 ml of double-distilled water and applied as sample. In the second experiment culture broth containing cells of microorganisms was diluted with double-distilled water in the ratio 1:1.

Thin-layer chromatography

High-performance thin-layer chromatographic (HPTLC) plates precoated with silica gel 60 (10 × 20 cm) with a concentration zone (2.5 × 20 cm) for nano-TLC (Merck) were used. From the prepared samples and standards, 10 μl of each were spotted onto the plate. After the samples had been added and dried, the plate was placed onto a chromatographic chamber (Desaga, Heidelberg, Germany) with 70 ml of chloroform–glacial acetic acid–water (3:6:1, v/v/v) in the bottom. The chamber was covered with a lid, and solvent allowed to move up the plate. When the solvent reached the top, the plate was removed and dried for 10 min under hot air. The plate was returned to the chamber and ascent made on fresh solvent of the same composition. The dried plate was sprayed with a 50% (w/v) solution of sulphuric acid in ethanol and heated at 105°C, which allowed the coloured spots to develop in about 10 min.

RESULTS AND DISCUSSION

It is accepted [4–7] that proteins in nutritive media interfere with direct determination of carbohydrate. Therefore the optimal conditions for their elimination were investigated, the best results being obtained under the conditions described above. Results given by this method are shown in Fig. 1.

Separation of carbohydrates from microbial starch degradation products

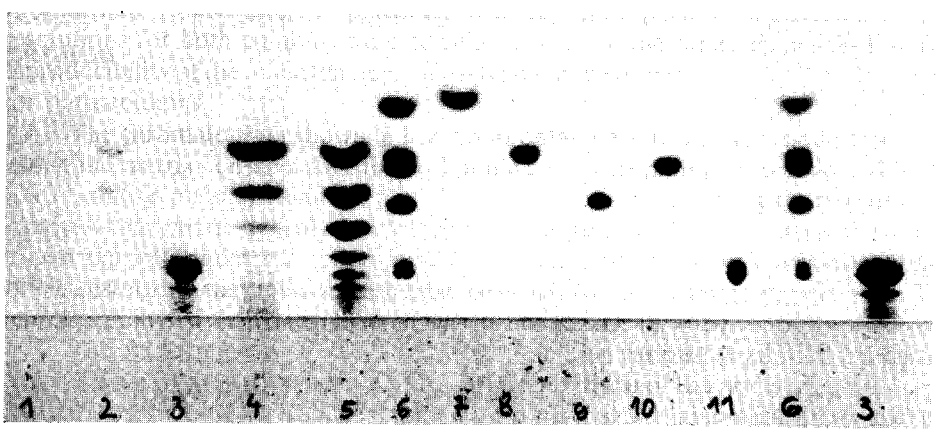

Fig. 1. TLC of carbohydrates after deproteinization of the starch nutrient medium. Spots: 1 = nutrient medium; 2 = strain of bacterium a_5; 3 = strain of bacterium a_6; 4 = strain of bacterium a_{12}; 5 = strain of bacterium a_{952}; 6 = mixture of carbohydrate, 10 μl; 7 = glucose, 10 μl; 8 = maltose, 10 μl; 9 = raffinose, 10 μl; 10 = trehalose, 10 μl; 11 = α- + β-cyclodextrin, 10 μl. For conditions see Experimental section.

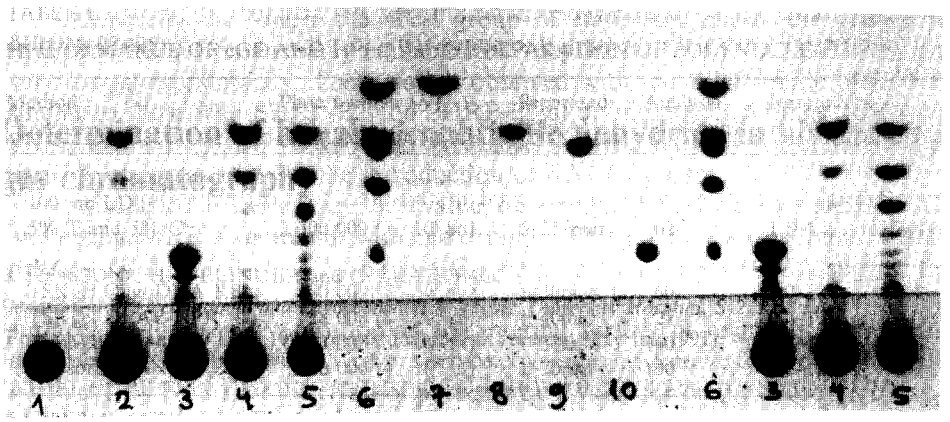

Fig. 2. TLC of carbohydrates a diluted starch medium. Spots: 1 = nutrient medium; 2 = strain of bacterium a_5; 3 = strain of bacterium a_6; 4 = strain of bacterium a_{12}; 5 = strain of bacterium a_{952}; 6 = mixture of carbohydrate, 10 μl; 7 = glucose, 10 μl; 8 = maltose, 10 μl; 9 = trehalose, 10 μl; 10 = α- + β-cyclodextrin, 10 μl. For conditions see Experimental section.

proved to be comparable to that of the components of the standard mixture-Maltotriose in standard mixture was substituted by raffinose, as, according to the experimental conditions, they have the same R_F values [3]. The purification period is too long, so that a relatively small number of samples could be analysed in a normal working day. This initiated the idea of spotting the plates with nutritive media that contained the microorganism cells. The first results indicated that the media should be diluted, the best dilution being in the ratio 1:1. Results are shown in Fig. 2. In this case raffinose was not used as a standard.

When the results obtained after deproteinization (Fig. 1) are compared with the results obtained on the plate with cell sample (Fig. 2), it can be seen that the spots clearer in the second case, that is when sample contains the cells. It seems that some loss occurred in the course of purification.

The advantage of this method, in our opinion, is that the spots of carbohydrates from the nutritive media containing bacteria cells are clear and distinctly separated (Fig. 2). It is quite easy to determine the number of starch metabolic products and thus to characterize the particular strain. The results are reproducible when conducted

TABLE I

R_F VALUES OF CARBOHYDRATES ON HPTLC PLATES (n = 5)

Carbohydrate	$R_F \times 100$
Glucose	62
Maltose	51
Trehalose	49
Raffinose	39
α-Cyclodextrin	24
β-Cyclodextrin	23

repeatedly under the same experimental conditions. A particular strain will give the same number of spots with the same R_F values. This fact has not yet been reported in the accessible literature.

As from the aspect of biotechnology it is extremely important to obtain a very large quantity of material, the sensitivity of the proposed method was not important in the present investigation.

R_F values for the pure substances used as standards for products of metabolically degraded starch are given in Table I. R_F values were determined from the arithmetic means of five runs.

CONCLUSION

Advantages of the method described in this paper include the simplicity of the procedure, the short chromatographic run time and full reproducibility for small samples. The assay does not require time-consuming purification or extraction procedures. The method can also be recommended as a valuable screening procedure for detection of desired microbial starch degradation products from a complex nutrient medium.

ACKNOWLEDGEMENT

This study was financially supported by the Research Council of Vojvodina, Yugoslavia.

REFERENCES

1 A. M. Siouffi, E. Mincsovics and E. Tyihak, *J. Chromatogr.*, 492 (1989) 471.
2 S. C. Churms, *J. Chromatogr.*, 500 (1990) 555.
3 Lj. Vrbaški and Ž. Lepojević, *Zb. Prir. Nauk Matica Srpska*, 77 (1989) 129.
4 G. Zweig and J. Sherma (Editors), *Handbook of Chromatography*, Vol. 2, CRC Press, Cleveland, OH, 1972, p. 204.
5 J. A. DePinto and L. L. Campbell, *Science*, 146 (1964) 1064.
6 J. A. DePinto and L. L. Campbell, *Arch. Biochem. Biophys.*, 125 (1964) 1064.
7 R. D. McDowall, *J. Chromatogr.*, 492 (1989) 3.