Journal of Chromatography, 232 (1982) 160—164
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 1344

Note

Rapid identification of *Klebsiella* by gas chromatography

K.S. MANJA* and K.M. RAO

Department of Microbiology, Defence Research and Development Establishment, Gwalior - 474002 (India)

(First received January 12th, 1982; revised manuscript received May 17th, 1982)

Klebsiella, an opportunistic pathogen, is found in cases of burns, pneumonia [1, 2], urinary tract infections [3] and a variety of other infections in man and animals [4]. Recently, a primary *Klebsiella* identification method has been reported [5] employing MacConkey inositol carbenicillin agar, which takes 24 h for identification. A quicker method would help in the early diagnosis and therapy of these infections. A possible application of gas chromatography (GC) in diagnostic microbiology has been reported by many workers [6—9]. The GC identification of *Klebsiella* based on its characteristic products is done by the analysis of glycoprotein components in liver samples of infected rats [10]. GC analysis of acids and amines produced by *Klebsiella* in a chemically defined medium has also been made use of [11, 12]. Derivatisation of the sample is required in these procedures.

We report here a simple GC method for rapid identification of *Klebsiella*. Adonitol, a sugar alcohol, is fermented by all the strains of *Klebsiella* [13], yielding ethanol [14] which can be detected by GC.

MATERIALS AND METHODS

The bacteria used in this study were isolated from the natural water sources and urine samples of patients suffering from urinary tract infections. The identity of the culture specimens was confirmed using the procedure reported in ref. 13. The bacteria were cultured in M-9 salt mixture medium as described earlier [15], the lactose being replaced by adonitol (Difco). Carbenicillin, 50 μg/ml, was also incorporated in the medium. Organisms, $1 \cdot 10^5$, of 62 strains comprising *Klebsiella*, *Salmonella*, *Escherichia coli*, *Citrobactor* and *Arizona*

0378-4347/82/0000—0000/$02.75

were inoculated to the modified M-9 salt mixture medium and incubated at 37°C for 5 h. Six strains of *Klebsiella* were incubated with the medium for 18 and 24 h.

Gas chromatography

A Toshniwal gas chromatograph Model ROL 4 with a flame-ionisation detector was used. Stainless-steel tubing (180 cm × 0.31 cm I.D.) was packed with 15% EGS on Chromosorb W HP 80—100 mesh. Nitrogen at a flow-rate of 40 ml/min was the carrier gas. The operating temperatures of the column, injection port and detector were 110, 150 and 170°C, respectively. The electrometer amplifier was operated at $1 \cdot 10^{-9}$ A. A Heath-kit multispeed servo chart recorder was operated at 1 mV sensitivity and a chart speed of 7.5 cm/min. Five microlitres of 62 5-h old strains of various cultures of bacteria, and of 18- and 24-h old cultures of six strains of *Klebsiella* were directly injected into the chromatograph. The peak height recorded for ethanol in the medium was measured. Twelve lots of culture media were injected into the GC system to determine if there were any background peaks produced by the medium. Standard peaks were also recorded for ethanol of various concentrations, ranging from 50 to 900 ppm in the medium (Fig. 1).

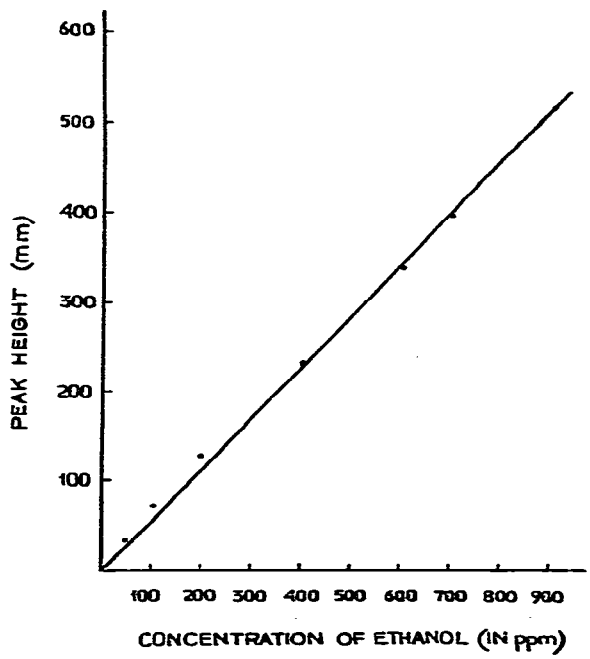

Fig. 1. GC response of various concentrations of ethanol.

RESULTS

Fig. 2 shows the GC peak for ethanol produced by *Klebsiella* in the medium and the absence of the ethanol peak in other bacterial cultures. The results are presented in Table I. The GC peak for ethanol was very sharp, making it

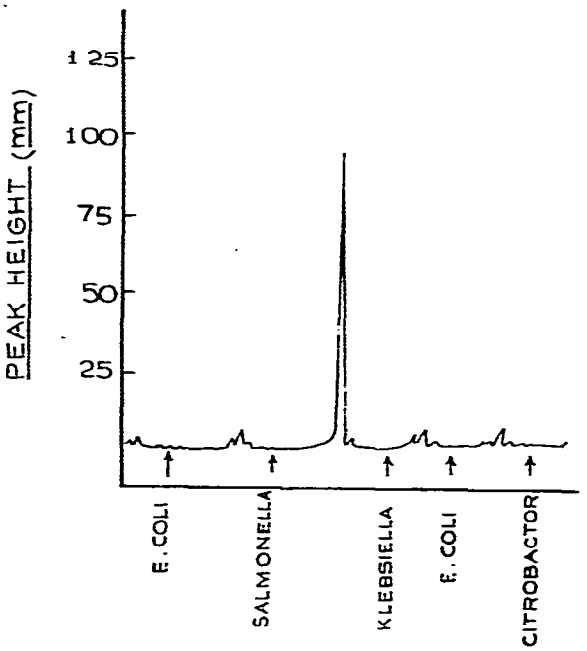

Fig. 2. GC profiles of culture media.

TABLE I

RESULTS OF GC ANALYSIS OF VARIOUS BACTERIA IN ADONITOL MEDIUM

Bacteria screened	No. of strains	No of strains giving peak for ethanol	Peak height (mm)
Klebsiella	30	30	78*
E. coli	15	—	<5
Citrobactor	10	—	<5
Salmonella	5	—	<5
Arizona	2	—	<5
Control medium lots	12	—	<5

*Average.

TABLE II

PRODUCTION OF ETHANOL BY *KLEBSIELLA*

Klebsiella strains	Peak height (mm)		
	5 h	18 h	24 h
MB/14	65	475	525
MB/19	73	500	500
MB/20	87	500	538
MB/21	100	500	500
MB/22	85	475	535
MB/23	70	500	535

difficult to measure the peak area; the peak height was found to be directly proportional to the amount of ethanol produced in the medium. *Klebsiella* produced sufficient ethanol to give a peak height of 78 mm in 5 h, corresponding approximately to 100 ppm ethanol. The amount of ethanol in the medium increased to 900 ppm when cultures were incubated for 18 h, but not beyond that time (Table II). None of the other bacteria produced ethanol from adonitol (Fig. 2). Twelve lots of culture media and cultures of other bacteria gave no significant peak for ethanol.

DISCUSSION

Virtually all *Klebsiella* strains ferment adonitol [13], and ethanol in the adonitol-based medium in which bacteria are grown indicates the presence of *Klebsiella*. Further, carbenicillin in the medium selectively suppresses the growth of other bacteria and permits the multiplication of *Klebsiella* [5]. In the conventional method for primary identification of *Klebsiella*, inositol is used by many workers [5, 16, 17]. A few strains of *Salmonella* and *Citrobactor* also ferment inositol but they do not attack adonitol [13]. Hence adonitol as substrate for *Klebsiella* is superior to inositol.

The GC procedure described above could help as a rapid identification method for *Klebsiella*. Another advantage of the method is that only 0.5 ml of culture medium is required as compared to 15 ml in the conventional method. We injected 1500 samples directly without the gas chromatograph losing any analytical efficacy.

In this study we used 10^5 organisms as the initial inoculum. A typical urinary tract infection as described by Kass [18] is the presence of bacteriurea in a concentration of 10^5 or more organisms per ml of urine. It is evident that bacteriurea caused by *Klebsiella* can be diagnosed by the GC method in 5 h. We are investigating on these lines and the results will be reported later.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. P.K. Ramachandran, Director, for the encouragement given in this study. We also thank Mr. K.N. Kaul for secretarial assistance.

REFERENCES

1 L.P. Jervey and M. Hamburger, Arch. Int. Med., 99 (1957) 1.
2 R.H. Wylie and P.A. Korschner, Amer. Rev. Tuberc., 61 (1951) 465.
3 R.R. Gillies, in R. Cruickshank, J.P. Duguid and R.H.A. Swain (Editors), Medical Microbiology, E.& S. Livingstone Ltd., London, 1965, p. 258.
4 W. Burrows, in W. Burrows, J.W. Moulder, R.M. Lewert and J.W. Rippon (Editors); Textbook of Microbiology, Saunders Co., Philadelphia, PA, 1968, p. 488.
5 S.T. Bagley and R.J. Seilder, Appl. Environ. Microbiol., 36 (1978) 536.
6 J.B. Brooks, W.B. Cherry, L. Thacker and C.C. Alley, J. Infect. Dis., 126 (1972) 142.
7 E. Reiner, J.J. Hicks, M.M. Ball and W.J. Martin, Anal. Chem., 44 (1972) 1058.
8 J.B. Brooks, G. Choudhary, R.B. Craven, C.C. Alby, J.A. Liddle, D.C. Edman and J.D. Converse, J. Clin. Microbiol., 5 (1977) 625.
9 N.J. Hayward, and T.H. Jeavons, J. Clin. Microbiol., 6 (1977) 202.

10 B.M. Mitruka, in C.G. Heden and T. Illeni (Editors), New Approaches to the Identification of Microorganisms, Wiley, New York, 1975, p. 135.
11 J.B. Brooks, D.S. Kellogg, Jr., M.E. Shepherd and C.C. Alley, J. Clin. Microbiol., 11 (1980) 45.
12 J.B. Brooks, D.S. Kellogg, Jr., M.E. Shepherd and C.C. Alley, J. Clin. Microbiol., 11 (1980) 52.
13 P.R. Edwards and W.H. Ewing (Editors), Identification of Enterobacteriaceae, Burgess Publishing Co., Minneapolis, MN, 1962.
14 J.R. Porter, Bacterial Chemistry and Physiology, Wiley, New York, 1950, p. 949.
15 J.S. Newman and R.T. O'Brien, Appl. Microbiol., 30 (1975) 584.
16 T.J. Davis and J.M. Matson, J. Infect. Dis., 130 (1974) 402.
17 N.J. Legakis, J.T. Papavassillior and M.E. Xilinas, Zentralbl. Bakteriol. Parasitenk. Infektionskr. Hyg. Abt. 1 Orig. Reihe A, 235 (1976) 453.
18 E.H. Kass, Trans. Ass. Amer. Physicians, 69 (1956) 56.