COMPARISON OF COMMERCIAL AND LABORATORY PREPARED CITRATE AGAR AS A DIAGNOSTIC MEDIUM

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ABSTRACT

The ability to utilize citrate as sole carbon source using 75 bacterial stock cultures was tested in a citrate medium formulated from basic chemical ingredients in the laboratory. A commercial Simmons citrate medium obtained from Biotec Laboratories (Surrey, United Kingdom) was employed as a control. Both the laboratory-prepared medium and the commercial medium were inoculated simultaneously with each of the 75 bacterial cultures supplied as coded unknowns. Results of citrate utilization were recorded after 24 and 48 hours of incubation at 360C ± 10C. Forty-six (46) strains gave positive citrate utilization results in both the laboratory prepared medium and the commercial Biotec medium after 24 hours of incubation. Nineteen (19) strains gave equivocal or intermediate results in both media after 24 hours of incubation but re-incubation for an additional 24 hours resulted in clearly positive citrate utilization results. Ten (10) strains gave negative results in both media and these results remained negative after re-intubation for an additional 24 hours. There were no discrepant results of citrate utilization and a 100% correlation of test results was obtained. The identities of the test strains were revealed at the end of the study and the citrate utilization test result for each strain was shown to be what was expected of it given its identity and the predictions of diagnostic tables. This blind study indicates the equivalence of a laboratory-prepared citrate medium and the one obtained commercially. However, more extensive studies would need to be carried out to confirm this. Considering the fact that the cost of one unit of a laboratory-prepared citrate medium, is far less than that of a commercial medium, it is suggested based on the outcome of this investigation that laboratory preparation of the citrate medium from basic ingredients should be more cost-effective.

Key Words: Citrate medium, Enterobacteriaceae, Laboratory-prepared, utilization
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INTRODUCTION

Many tests used in the identification of members of the Enterobacteriaceae can be roughly classified into first line tests and second line or back up tests. For all practical purposes, the front-line tests are crucial for provisional identification and screening of isolates. Their proper choice and use enable fairly accurate identification of commonly encountered organisms at minimum cost [1]. Since front line tests are the most frequently performed tests in the process of routine identification of Enterobacteriaceae, the media for carrying out these tests are the most heavily consumed. If the cost of routine identification of Enterobacteriaceae using conventional media is to be curtailed, then attention must be directed at cutting the cost of media for first line tests [2]. One of the most important first line tests for enteric identification is Simmons citrate agar [3]. The Simmons citrate medium has a fairly simple composition. The component of the medium is relatively cheap and can be very readily procured. A laboratory-prepared citrate agar medium prepared from basic chemical ingredients could be a means of cutting down the cost of performing the citrate utilization test in a busy routine laboratory, provided the laboratory-prepared medium is shown to exhibit satisfactory performance when compared with a reliable commercial stock of Simmons citrate agar [4, 5]. This investigation therefore seeks to evaluate a laboratory-prepared citrate agar medium using commercial Biotec Simmons citrate agar as the reference standard.

MATERIALS AND METHODS

Media preparation

The commercial Simmons citrate agar medium was prepared from dried (dehydrated) stock purchased from Biotec Laboratories (Surrey, United Kingdom). The Instruction of the manufacturers was followed in the preparation of the medium. After sterilization had been carried out, the bottles were removed from the autoclave, screwed tight and allowed to cool. The bottles carrying the slants of the commercial Simmons citrate medium were labeled Biotec citrate agar.

Following Laboratory instructions and using the laboratory formula, citrate agar medium was prepared from basic chemical ingredients as detailed in

table 1. Approximately, 5ml of the medium was dispensed in small 7ml capacity bijou bottles, closed loosely and autoclave at 1150°C for 15 minutes. When the sterilization was completed, the bottles were removed from the autoclave while still hot and were screwed tight. They were slanted and allowed to cool. The bottle carrying the slants of the laboratory-prepared medium were labeled laboratory citrate agar.

Bacterial strains

Table 1: Composition of Citrate Agar Medium (g/l)

Medium Component	Biotec Citrate Agar Medium(B C A)	Laboratory Prepared Citrate Agar Medium (L C A)
Magnesium Sulphate	0.2	0.4
Ammonium Dihydrogen Phosphate	0.2	0.2
Trisodium Citrate	2.0	3.0
Sodium Ammonium Phosphate	0.8	-
Dipotassium Dydrogen Phosphate	-	1.0
Sodium Chloride	5.0	1.0
Ammonium Sulphate	-	3.0
Agar	14.0	15.0
Bromothymol Blue	0.080	0.08
pH Approximation	7.0	7.2

Table 2: Comparisons of Citrate Utilization Test Results Using Commercial Biotec and Laboratory Prepared Simmons Citrate Agar Media

Number of agreement			Number of disagreement		% Agreement
BCA+	BCA(+)	BCA-	BCA+	BCA-	
LCA+	LCA(+)	LCA-	LCA-	LCA+	
46	19	10	-	-	100

BCA = BIOTEC (Commercial) Simmons Citrate Agar Medium

LCA = Laboratory Prepared Simmons Citrate Agar Medium

+= Positive Reaction after 24hours of Incubation

(+) = Delayed Positive: Intermediate Reaction after 24hours of Incubation and Positive Reaction after 48hours of Incubation.

= Negative Reaction after 24hours and 48Hours of Incubation.

Table 3: Strain Designation, Strain Identity and Citrate Utilization Results of Organisms Used in the Blind Evaluation of a Laboratory-Prepared Citrate Medium.

Strain Designation	Strain Identity	Result of Citrate Utilization	
		BCA	LCA
A_1	*Bacillus megaterium*	+	+
$A_{2, C13}$	*Bacillus cereus*	-	-
A16	*Bacillus cereus*	+	+
$A_{3, D15}$	*Serratia mercescens*	+	+
C_{14}	*Escherichia coli* NCTC 10418	-	-
$A_{4, D6}$	*Escherichia coli*	-	-
D_9	*Escherichia coli*	+	+
A5- A6, A17-A18, $B_{1-B4, B8, B10-B19, B21-B22}$	*Klebsiella pneumonia*	+	+
$B_{5-B7, B9, B20}$	*Klebsiella pneumonia*	(+)	(+)
$A_{7-A8, D3}$	*Pseudomonas aeruginosa*	+	+
D_2	*Pseudomonas aeruginosa* NCTC 6749	+	+
C1-C2, C4, C7, C9-C11	*Proteus mirabilis*	+	+
C3-C6, C8, C12	*Proteus mirabilis*	(+)	(+)
D_{13}	*Proteus vulgaris*	+	+
C	*Salmonella samara*	(+)	(+)
D_5	*Salmonella sp.* Group B	(+)	(+)
D_7	*Salmonella sp.*	+	+
D_8	*Shigella flexneri*	-	-
A_9	*Enterobacter cloacao*	+	+
D_{14}	*Enterobacter sp.*	+	+
A_{15}	*Acinetobacter calcoaceticus*	(+)	(+)

A_{14}	*Providentia stuartii*	+	+
D_{10}	*Providencia alcalififaciens*	+	+
D_{12}	*Providencia nettgeri*	+	+
D_{11}	*Aeromonas hydrophila*	(+)	(+)
A_{10}	*Micrococcus luteus*	+	+
A_{13}	*Enterococcus faecalis*	(+)	(+)
A_{11}	*Staphylococcus aureus*	+	+
D_{19}	*Staphylococcus aureus*	-	-
A_{12}	*Staphylococcus sp* (coagulase negative)	(+)	+
$A_{19, D4}$	*Staphylococcus sp* (coagulase negative)	+	+
A_{20}	*Staphylococcus sp* (coagulase negative)	(+)	(+)
D_{16-D17}	*Staphylococcus sp* (coagulase negative)	-	-

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+ = Positive Reaction after 24hrs of Incubation

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= Negative Reaction after 24hrs and 48hrs of Incubation

DISCUSSION

Utilization of citrate was one of the main pillars of the first panel of tests used for the laboratory identification of members of the Enterobacteriaceae. [4, 7, 9, 10]. For many years, the IMViC panel was considered as standard tool for the differentiation of members of the Enterbacteriaceae. Increased knowledge about the metabolism of members of this group, refined definition of genera, species and variants within the group, necessitated the used of additional biochemical tests for proper identification of isolates in this family [11, 12, 13]. In spite of the wide range of tests that have replaced the IMViC system as useful diagnostic tests, utilization of citrate remains a very important component in any panel of tests for identifying Enterobacteriaceae whether by conventional methods or by commercial diagnostic kit, [4, 12,14, 15]. The usefulness of the citrate utilization test has increased in value beyond the borders of the Enterobacteriaceae family [4, 16, 17,18] Identification schemes for Gram positive organisms such as Staphylococcus sp. and Bacillus sp. regularly include the citrate utilization tests [3, 5, 19, 20, 21]. In developing countries where commercial identification kits are not likely to be routinely used because of high cost, most of the identification work on the bench would still be relying on the conventional media [4, 14, 22].

It is therefore not out of place to investigate ways of lowering the costs of citrate utilization test using conventional media. Even in developed countries, the desire to lower cost by using laboratory-prepared medium as opposed to commercial kits is already becoming evident [1, 14, 15,]. Researchers have demonstrated the value of laboratory-prepared media in lowering the cost of the identification of isolates with respect to commonly used tests [11, 7]. Another researcher in 1991 demonstrated with clear facts and figures, using fairly current retail prices of media components, that laboratory-prepared lysine-decarboxylase broth which gives the same degree of accuracy in test for lysine decarboxylase when compared to commercial Talor-lysine medium, actually cost about one fifth the price for unit test performed with the commercial medium [12, 9].

It is clear that a similar relationship cost-wise can be established between many laboratory-prepared media and commercial media. It is therefore clear that a laboratory-prepared Simmons citrate agar should be much cheaper than the one prepared from a commercial dehydrated stock. The results of this investigation clearly indicate the equivalence of BCA and LCA with respect to accurate detection of citrate utilization among members of the Enterobacterioceae and organism not belonging to this family. Given the advantage of lower cost, the laboratory-prepared medium is to be preferred since the ingredients for this medium do store better under tropical conditions compared to pre-formulated dehydrated stock, this is also a factor in favour of the laboratory-prepared medium.

However, further evaluation of LCA using larger number of isolates and wider spectrum of species should help to further validate its usefulness as cost-saving device in the laboratory investigation of citrate utilization.

CONCLUSION

In view of the fact that the laboratory investigation evaluated the laboratory-prepared citrate agar medium using commercial Biotech Simmons citrate agar as the reference standard and obtained 100% correlation there by exhibiting satisfactory performance and being of low cost compare to the commercially prepared Simmons citrate agar medium, hence this can be used for the isolation and identification of members of the enterobacterioceae. Considering time and cost of importation, it is cost effective and therefore better to use laboratory-prepared citrate agar medium for the isolation and identification of members of the enterobacterioceae during routine investigation and research laboratories in Nigeria

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