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A SELECTIVE MEDIUM FOR THE ISOLATION OF THE ACINETOBACTER GENUS BACTERIA

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A selective and differential medium was developed for the isolation of Acinetobacter *genus bacteria. This* Acinobacter Agar Medium *(p. H = 7.4) contains in grams per litre: thiotone, 10; yeast extract, 3; NaCl, 5; saccharose, 10; mannitol, 10; sodium citrate, 0.5; sodium desoxycholate, 0.1; crystal violet, 0.00025; phenol red, 0.04 and agar-agar 15. This medium has the advantage of inhibiting the growth of cocci and Gram-positive bacilli, by the use of sodium citrate and sodium desoxycholate associated with the crystal violet; and of differentiating the Gram-negative bacilli from the* Enterobacteriaceae, *through the fermentative activity upon the saccharose and/or mannitol, contrasting with the complete inactivity of the* Acinetobacter *genus bacteria over those substances.*

In the last decades the members of the so called *Mima-Herellea* group (Debord, 1940), at present classified as *Acinetobacter* (Lautrop, 1974) and named by several French researchers as *Moraxella* (Andureau, 1940; Pièchaud, Pièchaud & Second, 1956) have been isolated from infectious lesions in pure culture (Faust & Hood, 1949); Svihus et al., 1961; Solé-Vernin, 1968) or, more commonly, in mixture with other microorganisms (Travassos, 1960; Mandel, Wright & McKinnon, 1964).

Randall & Linegar (1970) noted a significant increase in the number of isolations of this bacteria from 1957 to 1968 in the Thomas Jefferson University Hospital, Philadelphia, USA, and Gardner et al. (1970) observed that this group of microorganisms represented, in a 30 day period, 2.7% ot all the Gram-negative bacilli obtained from clinical materials at General Massachusetts Hospital, Massachusetts, USA.

When the microorganisms from the genus *Acinetobacter* are the only ones present in the infection, their isolation and identification cause no problem, but when they are mixed with other bacteria, their isolation becomes difficult because their colonial characteristics are not distinguishable from the ones of other microorganisms.

It is known that isolations the genus *Acinetobacter* have been made from urine, secretion from genito-urinary system, including cases of pseudo-gonorrhea, secretion from

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respiratory system and wounds (Irving & Herrich, 1967; Gilardi, 1968; Gardner et al. 1970, Randall & Lenegar, 1970; Zebral & Hofer, 1971) in which Gram-positive cocci and bacilli and Gram-negative bacilli, usually from the *Enterobacteriaceae* family are also present. To make easy the isolation of the genus *Acinetobacter* from the mixtures of microorganisms obtained from clinical material, we proposed here the preparation of a new selective culture medium based on the tolerance of *Acinetobacter* bacteria to pure chemical products and a dye (Zebral & Povoa, 1974), allowing a differentiation for the bacteria of the *Enterobacteriaceae* family and inhibition of the growth of cocci and Gram-positive bacilli.

MATERIAL AND METHODS

Origin of the used strains: For the present work the used strains were obtained from:

I.O.C. – Oswaldo Cruz Institute – Rio de Janeiro; I.M.U.F.R.J., Microbiology, Federal University of Rio de Janeiro; Dr. G.L. Gilardi, Microbiology Division, Department of Laboratories Hospital for Joint Diseases and Medical Center, New York, 10035, USA; Dr. E.J. Ordal, Microbiology Department, Washington University, Seatle, Washington, 98105, USA; Dr. E. Juni, Microbiology Department, Michigan University, Ann Arbor, Michigan, 48104, USA.

Strains that produce detectable acidity in a complex nitrogenous medium (22 strains)*

Order number	*Name*	*Source*	*Other date*
1	*Herellea vaginicola*	I.M.U.F.R.J.	CDC-7623
2	*Achromobacter conjuntivae*	Dr. G.L. Gilardi	ATCC-17905
3	*Achromobacter haemolyticus subs. haemolyticus*	Dr. G.L. Gilardi	ATCC-17906
4	*Herellea vaginicola*	Dr. E.J. Ordal	ATCC-9955
5	*Neisseria winogradsky*	Dr. E.J. Ordal	ATCC-17902
6	*Herellea caseolytica*	Dr. E.J. Ordal	ATCC-19002
7	*Cytophaga anitrata (A1)*	Dr. E.J. Ordal	Dr. Lautrop, 112.465
8	*Neisseria winogradsky*	Dr. E. Juni	ATCC-17902
9	*Achromobacter conjuntivae*	Dr. E. Juni	ATCC-17905
10	*Achromobacter winogradsky*	Dr. E. Juni	ATCC-17922
11	*Acinetobacter calcoa ceticus*	Dr. E. Juni	Dr. E. Juni IVL-10
12	*Acinetobacter calcoa ceticus*	Dr. E. Juni	Dr. E. Juni TREP-p-27
13 a 16	*Herellea vaginicola*	Zebral-Hofer	Human origin
17 a 22	*Herellea vaginicola*	Zebral-Povoa	Soil

*The original names were maintained.

Strains that do not produce detectable acidity in a complex nitrogenous medium (13 strains)*

Order number	*Name*	*Source*	*Other date*
23	*Mima polymorpha*	I.M.U.F.R.J.	CDC-7546
24	*Mima polymorpha*	I.M.U.F.R.J.	ATCC-9957
25	*Achromobacter metalcaligenes*	Dr. E.J. Ordal	ATCC-17909
26	*Moraxella Lwoffi*	Dr. E.J. Ordal	ATCC-17984
27	*Cytophaga Lwoffi (A3)*	Dr. E.J. Ordal	Dr. Lautrop 112.465
28	*Mima polymorpha*	Zebral-Hofer	Human origin
29	*Mima polymorpha*	Dr. E. Juni	CDC-7833
30	*Mima polymorpha*	Dr. E. Juni	ATCC-14291
31	*Vibrio 01*	Dr. E. Juni	ATCC-11171
32	*Achromobacter citro alcaligenes*	Dr. E. Juni	ATCC-17908
33	*Achromobacter metalcaligenes*	Dr. E. Juni	ATCC-17909
34	*Alcaligenes haemolysans*	Dr. E. Juni	ATCC-17988
35	*Mima polymorpha*	Dr. E. Juni	ATCC-17959

* The original names were maintained.

Other strains used in this work: Staphylococcus aureus (Wood strain); *Staphylococcus aureus* (FDA 6538); *S. aureus, S. epidermides* (tree strains); *Sarcina lutea, Streptococcus alfa haemolyticus* (two strains); *Streptococcus faecalis, Bacillus subtilis, Corynebacterium sp., Listeria monocytogenes, Citrobacter sp., Salmonella para A, Salmonella para B, Salmonella typhi, Salmonella typhimurium, Salmonella aberdeen, Salmonella newport, Shigella flexneri, Shigella boydii, Escherichia coli* (tree strains), *E. coli* 026:B26, *Enterobacter sp,* (two strains), *Klebsiella pneumoniae* (two strains), *Serratia marcescens, Proteus vulgaris, P. morganii, P. mirabilis, P. rettgeri, Providencia sp.,* all strains from I.O.C.

Culture upkeep – The strains were maintained in meat infusion broth, prepared by the following procedure: 1) allow chopped beef (500 g to 1 litre of distilled water) to infuse in refrigerator overnight. 2) Boil and filter through gauze to remove meat particles and then through filter paper. 3) Restore the volume of water lost. 4) To this broth add: thiotone (BBL) 10 and NaC1, 5. 5) Heat and adjust reaction to pH 7.4. This medium was distributed in tubes 16mm x 160mm with 5ml in each and sterilized in autoclave (121°C-15 minutes). The cultures were inoculated and incubated at 37°C for 24 hours, after which they were maintained at room temperature and retransferred to new broth each two months.

Employed media – In accordance with the results obtained by Zebral & Povoa, 1974, the basic medium composition was in grams per litre: Thiotone (BBL), 15; yeast extract (BBL), 3; sodium cloride, 5; saccharose, 10; mannitol, 10; phenol red, 0.04; and agar-agar, 15. After dissolving the ingredients, the pH was adjusted to 7.4 and sterilized in autoclave at 121°C for 15 minutes. The blood-agar was prepared by adding 5ml of defibrinated sheep's blood to each 100ml of infusion agar. The basic medium added with selective substances *(Acinetobacter Agar)* was prepared as follows, in grams per litre of basic medium: 0.1 of sodium desoxycholate; 0.5 of sodium citrate and 0.001, 0.0005 and 0.00025 of crystal violet, respectively, were added.

To prepare cultures and inoculates – The 35 strains grew in infusion broth for 18-24 hours, at 37ºC. From those cultures a series of dilutions (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}) were made using test tubes with sterile saline. The test tubes containing the first dilution were homogenized by using a shaker (Cenco Meinzer Laboratory Shaker, Chicago, USA) at aproximately 400 shakings per minute during 5 minutes. From each dilution 0.1ml was pipetted to the central part of plates with agar base, blood-agar and agar base with the impedient substances. The inoculum was spread to all the area of the plates using Drigalski loops that were prepared at the time of the use by bending sterilized Pasteur pipettes.

Counting of colonies – The counting of colonies in the plates were made by using the Quebec Colony Counter, Optical Corporation, USA. The countings obtained from the blood-agar plates served for references basis by considering it as 100 per cent of recovery of the microorganisms.

RESULTS

It is shown in the Table I, that the 22 *Acinetobacter* strains producing detectable acidity from certain carbohydrates in complex nitrogenous medium, provided more similar results and most of them presented a very good degree of recovery, even in very high dilutions. The same findings were not observed with the other 13 strains (nºs 23 to 35) of *Acinetobacter* (Table II).

TABLE I

Recovery of 22 strains of *Acinetobacter* in Blood-Agar, *Acinetobacter Agar (A.A.)* without inhibitory substances, A.A. (1), A.A. (2) and A.A. (3)

Organisms Nº	*Strains*	*Names*	*MEDIA* **					
			Blood-Agar		*A.A. without inhibitory substances*		*A.A. (1)**	
			Count per 0.1 ml	*recovery %*	*Count per 0.1 ml*	*recovery %*	*Count per 0.1 ml*	*recovery %*
1	CDC-72623	H. vaginicola	235×10^7	100	300×10^7	92.3	88×10^7	27.0
2	ATCC-17905	A. conjuntivae	210×10^6	100	201×10^6	95.7	100×10^6	47.6
3	ATCC-17906	A. haem. subsp. haem	344×10^7	100	324×10^7	94.1	325×10^7	94.4
4	ATCC-9955	H. vaginicola	280×10^7	100	260×10^7	92.8	180×10^7	64.2
5	ATCC-17902	N. winogradsky	56×10^7	100	66×10^7	117.0	3×10^7	5.3
6	ATCC-19002	H. caseolytica	360×10^7	100	344×10^7	95.5	99×10^7	27.5
7	Dr. Lautrop 112465(A1)	C. anitratum	220×10^7	100	222×10^7	100.0	50×10^7	22.7
8	ATCC-17902	N. winogradsky	200×10^7	100	180×10^7	90.0	110×10^7	55.0
9	ATCC-17905	A. conjuntivae	28×10^7	100	36×10^7	127.1	16×10^7	57.0
10	ATCC-17922	A. winogradsky	200×10^6	100	189×10^7	94.5	***	–
11	ATCC-IVL-10	A. calcoaceticus	34×10^7	100	30×10^7	88.2	25×10^7	73.5
12	ATCC-TBE-P-27	A. calcoaceticus	60×10^7	100	62×10^7	103.3	40×10^7	66.6
13	Humana-253	H. vaginicola	200×10^6	100	202×10^6	101.0	110×10^6	55.0
14	Humana-274	H. vaginicola	96×10^7	100	93×10^7	96.8	15×10^7	15.6
15	Humana-330	H. vaginicola	31×10^7	100	30×10^7	96.7	14×10^7	45.1
16	Humana-S.J.	H. vaginicola	30×10^7	100	29×10^7	96.6	***	–
17	Humana	H. vaginicola	88×10^7	100	90×10^7	102.2	40×10^7	45.4
18	Soil	H. vaginicola	263×10^7	100	250×10^7	95.1	190×10^7	72.2
19	Soil	H. vaginicola	240×10^7	100	222×10^7	92.5	180×10^7	75.0
20	Soil	H. vaginicola	230×10^7	100	301×10^7	94.0	252×10^7	78.7
21	Soil	H. vaginicola	190×10^7	100	185×10^7	97.3	105×10^7	55.2
22	Soil	H. vaginicola	170×10^7	100	165×10^7	97.0	100×10^7	58.8

*Sodium-desoxycholate, 0.1%: sodium citrate, 0.5%; crystal violet, 0.001%
**Overall average recovery; Blood-agar, 100%; A.A. without inhubitory substances 98.2%; A.A. (1), 47.3%
***No growth.

TABLE I

(continuation)

Organisms Nº	*MEDIA* ***			
	*A. A. (2)**		*A. A. (3)***	
	Count per 0.1 ml	*recovery (%)*	*Count per 0.1 ml*	*recovery (%)*
1	200 x 10^7	61.5	310 x 10^7	95.4
2	180 x 10^7	85.7	200 x 10^7	95.2
3	315 x 10^7	91.5	320 x 10^7	93.0
4	220 x 10^7	78.5	200 x 10^7	92.8
5	43 x 10^7	76.7	58 x 10^7	103.5
6	130 x 10^7	36.1	324 x 10^7	90.0
7	148 x 10^7	67.2	224 x 10^7	101.8
8	130 x 10^7	65.0	200 x 10^7	100.8
9	18 x 10^7	64.2	30 x 10^7	107.1
10	48 x 10^7	24.2	205 x 10^6	102.5
11	28 x 10^7	82.3	30 x 10^7	88.2
12	52 x 10^7	86.6	70 x 10^7	116.6
13	120 x 10^6	60.0	190 x 10^6	95.0
14	49 x 10^7	51.0	98 x 10^7	102.0
15	20 x 10^7	64.5	38 x 10^7	122.0
16	20 x 10^7	66.6	39 x 10^7	130.0
17	60 x 10^7	68.1	82 x 10^7	93.1
18	202 x 10^7	76.7	240 x 10^7	91.2
19	190 x 10^7	79.1	215 x 10^7	89.6
20	291 x 10^7	90.9	310 x 10^7	86.8
21	130 x 10^7	68.4	180 x 10^7	94.7
22	150 x 10^7	88.2	160 x 10^7	93.1

*Sodium desoxycholate, 0.1%; sodium citrate, 0.5%; crystal violet, 0,000.5%.
**Sodium desoxycholate, 0.1%; sodium citrate, 0.5%; crystal violet, 0,000.25%.
***Overall average recovery: A.A. (2), 69.6%; A.A. (3), 99.7%.

TABLE II

Recovery of 13 strains of *Acinetobacter* in Blood-Agar, *Acinetobacter Agar* (A.A.) without inhibitory substances, A.A. (1), A.A. (2) and A.A. (3)

Organisms Nº	*Strains*	*Names*	*MEDIA* **					
			Blood-Agar		*A.A. without inhibitory substances*		*A.A. (1)**	
			Count per 0.1 ml	*recovery (%)*	*Count per 0.1 ml*	*recovery (%)*	*Count per 0.1 ml*	*recovery (%)*
23	CDC-7546	M. polymorpha	60 x 10^4	100	58 x 10^4	96.4	20 x 10^4	33.3
24	CDC-9957	M. polymorpha	86 x 10^6	100	89 x 10^6	103.4	40 x 10^6	46.5
25	ATCC-17909	A. metalcaligenes	50 x 10^4	100	46 x 10^4	92.0	20 x 10^4	40.0
26	ATCC-17984	M. lwoffi	170 x 10^7	100	152 x 10^7	89.1	***	–
27	Dr. Lautrop 112465	C. lwoffi	90 x 10^4	100	85 x 10^4	94.4	40 x 104	44.4
28	236 humana	M. polymorpha	300 x 10^7	100	306 x 10^7	102.0	240 x 10^7	102.5
29	CDC-7833	M. polymorpha	156 x 10^7	100	150 x 10^7	96.1	160 x 10^7	102.5
30	ATCC-14291	M. polymorpha	46 x 10^4	100	40 x 10^4	86.4	***	–
31	ATCC-11171	Vibrio 01	60 x 10^4	100	58 x 10^4	96.6	***	–
32	ATCC-17908	A. citroalcaligenes	264 x 10^7	100	244 x 10^7	95.0	***	–
33	ATCC-17909	A. metalcaligenes	160 x 10^4	100	152 x 10^4	95.0	6 x 10^4	3.7
34	ATCC-17989	A. haemolysans	160 x 10^7	100	145 x 10^7	90.6	***	–
35	ATCC-17959	M. polymorpha	160 x 10^7	100	155 x 10^7	96.8	100 x 10^7	62.5

*Sodium desoxycholate, 0.1%; sodium citrate, 0.5%; crystal violet 0.001%.
**Overall average recovery: Blood-agar, 100%; A.A. without inhibitory substances 94.8%; A.A. (1), 31,7%.
***No growth.

TABLE II
(continuation)

Organisms Nº	*MEDIA* ***			
	*A. A. (2)**		*A. A. (3)***	
	Count per 0.1 ml	*recovery (%)*	*Count per 0.1 ml*	*recovery (%)*
23	22 x 10^4	36.6	30 x 10^4	83.3
24	64 x 10^6	74.6	80 x 10^6	93.0
25	27 x 10^4	54.0	40 x 10^4	80.0
26	–	–	145 x 10^7	85.2
27	61 x 10^4	67.7	80 x 10^4	88.8
28	300 x 10^4	100.0	288 x 10^7	96.0
29	122 x 10^7	78.2	200 x 10^7	128.2
30	****	–	****	–
31	****	–	****	–
32	120 x 10^7	45.1	252 x 10^7	95.4
33	92 x 10^7	57.5	140 x 10^4	87.5
34	80 x 10^7	50.0	160 x 10^7	100.0
35	110 x 10^7	68.7	150 x 10^7	93.7

* Sodium desoxycholate, 0.1%; sodium citrate, 0.5%; crystal violet, 0.000.5%.
** Sodium desoxycholate, 0.1%; sodium citrate, 0.5%; crystal violet, 0.000.25%.
*** Overall average recovery: A.A. (2), 48.6%; A.A. (3), 79.3%.
**** No growth.

The 13 strains of *Acinetobacter* that were non-producers of a detectable acidity from carbohydrates in complex nitrogenous medium seem to be more sensitive to little variations of the media and generally, under normal culture conditions, they present less growth density, in comparison with the other 22 strains.

The largest recuperation degree in all the strains of *Acinetobacter* (Tables I and II), was found in the *Acinetobacter Agar Medium* number 3, where the crystal violet was used in 0.00025 grams per litre, in relation to the media number 1 and 2, and comparable with the recovery obtained in blood-agar. This finding was confirmed by the average of all the recoveries, as shown in Table III, where the *Acinetobacter Agar Medium* number 3, presented 99.7% average recuperation for the strains that produced acidity in the complex nitrogenous medium, and only 79.3% for the other strains.

TABLE III

Comparison of recovery ratios of 35 strains of *Acinetobacter* on Blood-Agar, *Acinetobacter Agar* (A.A.) without inhibitory substances, A.A. (1), A.A. (2), and A.A. (3)

Media Organisms Nº	*Blood-Agar*	Acinetobacter-Agar *(A.A.) withouth inhibitory substances*	*A.A. (1)*	*A.A. (2)*	*A.A. (3)*
1 a 22	100%	98.2%	47.3%	69.6%	99.7%
23 a 35	100%	94.8%	26.9%	48.6%	79.3%

A.A. (1): Sodium desoxycholate, 0.1%; sodium citrate, 0.5%; crystal violet 0.001%.
A.A. (2): Sodium desoxycholate, 0.1%; sodium citrate, 0.5%; crystal violet 0.000.5%.
A.A. (3): Sodium desoxycholate, 0.1%; sodium citrate, 0.5%; crystal violet 0.000.25%.

Table IV shows: 1º) absence of growth for cocci and Gram-positive bacilli; 2º) the growth of the principal biochemical and serological groups from the *Enterobacteriaceae* are differentiated by the action on saccharose and/or mannitol, with the consequent turn of the pH indicator (phenol red), to the acid side (yellow color); 3º) the growth of the 35 strains of *Acinetobacter* that do not act on saccharose and mannitol under any condition tend to alkalize the medium (red color).

TABLE IV

Reactions and growth of various Gram-positive and negative microorganisms in *Acinetobacter* Agar (3).

Organisms	*Medium A.A. (3)*		
	24 hs	*48 hs*	*Color*
Sarcina lutea	–	–	–
Staphylococcus epidermidis	–	–	–
Staphylococcus epidermidis	–	–	–
Staphylococcus epidermidis	–	–	–
Staphylococcus aureus (F.D.A.)	–	–	–
Staphylococcus aureus (WOOD)	–	–	–
Staphylococcus aureus	–	–	–
Streptococcus group alfa	–	–	–
Streptococcus group beta	–	–	–
Streptococcus group gama	–	–	–
Bacillus subtilis	–	–	–
Corynebacterium sp	–	–	–
Listeria monocytogenes	–	–	–

– = No growth

TABLE IV

(continuation)

Organisms	*Medium A.A. (3)*		
	24 hs	*48 hs*	*Color*
Arizona	A	A	Yellow
Citrobacter	A	A	"
Salmonella para A	A	A	"
Salmonella para B	A	A	"
Salmonella typhimurium	A	A	"
Salmonella newport	A	A	"
Salmonella typhi	A	A	"
Salmonella anatum	A	A	"
Shigella dysenteriae	K	K	Red
Shigella sonnei 丶	A	A	Yellow
Shigella flexneri	A	A	"
Shigella boydii	A	A	"

TABLE IV (continuation)

Escherichia coli (3 strains)	A	A	"
Escherichia coli 026-B6	A	A	"
Klebsiella (2 strains)	A	A	"
Enterobacter (2 strains)	A	A	"
Serratia	A	A	"
Proteus vulgaris	A	A	"
Proteus morganii	K	K	Red
Proteus mirabilis	K	K	"
Proteus rettgeri	K	K	"
Providencia	K	K	"
Acinetobacter Nº 1 a 22	K	K	"
Acinetobacter 23 a 35	K	K	"

A = Acid reaction. Yellow zones around the colonies.
K = Alkali reaction. Red zones around the colonies.

DISCUSSION

The *Acinetobacter Agar Medium* presents the advantage to use pure chemical substances (sodium citrate and sodium desoxycholate) responsable for the inhibition of cocci and Gram-positive bacilli. The crystal violet, in the used concentration, increases the inhibitory action for the *Streptococcus* and *Staphylococcus,* without changing the sensitiveness of the medium for the *Acinetobacter* genus bacteria (Table I).

The use of saccharose and the poly-alcohol mannitol, is based on the findings of 19 basic works published in world literature. Those works show that under any condition, none of those strains of *Acinetobacter* metabolized the two substances, while the others sugars could be utilized, even in complex nitrogenous medium (Samuels, Pitman & Cherry, 1969; Gilardi, 1971; Gilardi, 1973).

TABLE V

Strains of *Acinetobacter* with no action upon the sucrose and or manitol.

Authors	*Year*	*Source*	*Nº of Strains*
Schaub & Hauber	1948	Human	15
Pièchaud, Pièchaud & Second	1951	Human	26
Pièchaud, Pièchaud & Second	1956	Human	10
Travassos	1960	Human	5
Curtieu, Chassignal & Longerey	1961	Human	214
Henderson	1965	Human	60
Snodgrasse & Koburger	1967	Food	33
Irwing & Herrich	1967	Human	175
Solé-Vernin & Ciconelli	1968	Human	4
Casellas	1968	Soil	24

TABLE V (continuation)

Baumann, Doudoroff & Stanier	1968b	Soil, human, water, etc.	104
Gilardi	1968	Human	157
Samuel, Pittman & Cherry	1969	Human	55
Randall & Linegar	1970	Human	121
Gilardi	1971	Human	166
Ledermann	1971	Human	201
Zebral & Hofer	1971	Human	17
Gilardi	1973	Human	307
Zebral	1974	Soil	5
Total			1 699

On the other hand, with rare exceptions *(Shigella dysenteriae,* some *Proteus,* and some strains of *Providencia),* as shown in Table IV, all the Enterobacteria leaven the sugar or the poly-alcohol or both together, making the culture medium first rose-colored, to change to a yellow color around the colony, which indicates the turn of the pH indicators (phenol red) to the acid side.

Others nonfermentatives, nonfastidious and of medical interest, Gram-negative bacilli may grow in this culture medium. The most important, *Pseudomonas,* can be easily differentiated by the colonial morphology that is completely different from the genus *Acinetobacter* as well as by the characteristic smell, and diffusible pigment that they present.

The *Acinetobacter Agar Medium* (pH 7.4) which final formula is in grams per litre: thiotone (BBL), 10; yeast extract (BBL), 3; NaCl (BBL), 5; saccharose, 10; mannitol, 10; sodium citrate, 0.5; sodium desoxycholate, 0.1; crystal violet, 0.00025; phenol red, 0.04; agar-agar, 15, presents good selectivity and high sensitiveness to the bacteria belonging to the genus *Acinetobacter.* It has the advantage of use pure chemical substances which inhibition degree can be exactly controled; it has also the advantage to show the fermentative activity of the Enterobacteria upon saccharose and/or mannitol and complete inactivity of the *Acinetobacter* genus bacteria on the same substances.

Due to this last condition *Acinetobacter* causes a change to the red color of the medium around the colonies, indicating alkalinization due to the metabolism of nitrogenus substances.

Consequently, the *Acinetobacter Agar* can be used for the isolation of *Acinetobacter* genus bacteria from his natural habitat (soil and water), as well as from clinical specimens with heavy contamination and therefore increasing the efficiency of isolation methods and decreasing the amount of material and consumed work time.

RESUMO

Um Meio Seletivo para o Isolamento de Bactérias do Gênero "Acinetobacter"

Um meio seletivo e diferenciador foi desenvolvido para facilitar o isolamento das bactérias do gênero *Acinetobacter.* Este meio *(Agar Acinetobacter)* contém em gramas

por litro: Tiotone (BBL), 10; extrato de levedura (BBL), 3; NaC1, 5; sacarose, 10; manitol, 10; citrato de sódio, 0,5; desoxicolato de sódio, 0,1; cristal violeta, 0,00025; vermelho de fenol, 0,04 e pH 7,4. Apresenta a vantagem de impedir o crescimento dos cocos e bacilos Gram positivos, pelo emprego do citrato de sódio e do desoxicolato de sódio, associados ao cristal violeta e de diferenciar os bacilos Gram negativos da família *Enterobacteriaceae*, pela sua atividade fermentativa sobre a sacarose e/ou manitol, em contraste com a completa inatividade das bactérias do gênero *Acinetobacter* sobre os mesmos substratos.

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