A NEW MANY-PURPOSE CULTURE MEDIUM FOR CHEMOTHERAPEUTIC AND MICROBIOLOGIC STUDIES

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A new semisynthetic culture medium was prepared and found to serve many useful purposes in chemotherapeutical research and also in diagnostic microbiology. It contains 0.5% peptone, 0.1% glucose and is supplemented with 10% MCILVAINE's buffer. The medium is pH-stable and, being almost colorless, it is suitable for turbidimetric studies and enzymatic experiments which involve color-changes. For *in vitro* chemotherapeutic experimental studies it is a useful medium and, in certain cases, it behaves like an almost antagonist-free medium. Used as broth or in agar-base, it supports the growth of all of the 280 clinically important strains studied, including aerobic bacteria, *Candida* and *Trichophyton*. It promotes the germ tube formation of *C. albicans* and *C. stellatoidea*.

The culture media used in aerobic bacteriology and mycology are generally categorized into three major groups: 1. completely synthetic, 2. semisynthetic, and 3. complex organic media. Media within the last two groups may be good supporters of the growth of almost all aerobic microbes. Synthetic media composed of an inorganic nitrogen source, salts and glucose cannot be used universally because several bacterial genera including staphylococci do not grow in or on them. On the other hand, the composition of the complex organic media is not readily reproducible; they are usually colored and not regularly buffered. We were looking for a colorless and pH-stable medium which supports the growth of at least nonfastidious bacteria and fungi. The description of such a medium with a relatively simple composition and easy preparation and its use in studies on antibacterial agents, in microbial biochemistry and in diagnostic microbiology is the subject of this paper.

Materials and Methods

Chemicals and Antibiotics

The chemicals used (glucose, citric acid, dibasic sodium phosphate and bacto-peptone-Difco) were commercial preparations and, whenever applicable, of analytical grade. Nitrocefin (Glaxo chromogenic cephalosporin compound 87/312), primycin (Medimpex), clavulanic acid (Beecham), cefoxitin and cefmetazole (CS-1170) (Merck Sharp & Dohme), cefotaxime (Hoechst-Roussel), alaphosphin (Roche) were kindly provided as research samples; SK&F 73678 (a cephamycin) was synthesized at Smith Kline and French Laboratories.

Microbial Strains

All the microbial strains used throughout these studies were from the culture collection of Smith Kline and French Laboratories. They are maintained on appropriate microbiologic media under standard conditions.

Growth Support Studies

Our attempt to find a medium with the characteristics outlined in the introduction started with a 0.5% peptone water medium.[10] Since it did not support the growth of several microbes adequately, it was enriched with various amounts (0.1, 0.25, 0.5 and 1.0%) of glucose (dextrose). In all cases,

the pH was adjusted to 7.3 and the buffer capacity was reinforced by the addition of 10% McIlvaine's citric acid-phosphate buffer (pH 7.3).[7] In these five media, the optical density of nine microbial strains, regularly used in our screen to determine antimicrobial activity, was measured after overnight growth (37°C). In addition, the growth of three *Staphylococcus aureus*, three *Escherichia coli* and one *Enterobacter cloacae* strain all well-characterized previously[14], was monitored turbidimetrically[3] using a Bausch & Lomb spectrophotometer (Spectronic 70) at 500 nm. The final pH values of the cultures were measured (Fisher Accument Model 220 pH Meter). The growth supporting capacity of the medium found to be optimal in the above studies was then examined using an additional 280 mainly bacterial strains.

Composition and Preparation of the Medium

The addition of 0.1% glucose was found to support growth adequately and not to change the pH. Thus, the medium used in our subsequent study consisted of 0.5% peptone, 0.1% glucose (dextrose) and 10% McIlvaine's citric acid-phosphate buffer (pH 7.3),[7] and is referred to as PGB (peptone-glucose-buffered) medium. The ingredients were prepared and autoclaved separately and combined aseptically. The glucose was autoclaved as a 40% stock solution with the addition of two drops phosphoric acid to prevent caramelization. The medium can be solidified by addition of 1.7% agar to the peptone water and when cooled to 50°C, supplemented with the calculated volumes of the glucose and buffer solutions.

Studies Using the PGB Medium

For MIC determinations using the serial tube dilution method, 3 ml of the liquid medium was employed regularly. This volume permits monitoring of growth with the naked eye and turbidimetrically. The test compound and the inoculum (overnight grown culture in the same medium) were added in 0.05 ml volumes and the tubes were incubated at 37°C in the case of bacteria and 30°C in the case of *Candida* spp. and dermatophytes.

The bacterial growth-kinetics and responses to antibacterial agents were studied in PGB medium in stationary cultures (37°C) by recording changes in turbidity. Such a system allows measurement of drug induced or spontaneous bacteriolysis. Antibacterial agents were added to exponential phase cultures with an optical density (O.D.; absorbance) of about 0.4. Readings were taken at the start, then hourly for 8 hours and at 24 hours. The dynamics of the lytic potential of several β-lactam antibiotics and other cell-wall active agents have been studied in this medium.[14]

The PGB medium was evaluated for the detection of β-lactamase activity by following color production with nitrocefin[9]. This system allowed demonstration of the presence of β-lactamase inhibitors[18]. The inhibitor and nitrocefin were added in 0.05 ml volume each to 0.5 ml of PGB medium in which the bacteria were grown or suspended. The change from colorless to pink or purple could be read visually and spectrophotometrically.

Germ tube formation of *Candida* strains was studied in 2 ml of the PGB medium inoculated with 0.1 ml of an overnight culture (30°C) of *C. albicans* #759 or *C. stellatoidea* #2245 in the same medium. After two hours at 37°C, the germ tube formation was observed by light or phase-contrast microscopy.

Results

The results obtained by adding glucose to the buffered peptone water are presented in Table 1. With few exceptions (*Providencia* sp., *Enterobacter cloacae* and *Serratia marcescens*), the bacterial strains and *C. albicans* grew better on the glucose enriched medium. Most strains grew well at any of the glucose concentrations. At 0.1% glucose, the pH of the overnight cultures remained fairly constant (7.0~7.3). At higher glucose concentrations (0.25~1.0%), the medium tended to become acidic, which is undesirable for many experiments.

The growth of selected seven strains in Table 1 was followed kinetically. Again, the data clearly demonstrated that the addition of 0.1% glucose was optimal, both for growth promotion and for

Table 1. The influence of glucose concentrations on the growth and pH of selected microorganisms in peptone water. Incubation for 24 hours at 37°C.

Strains	Optical density (absorbance) in medium				
	Peptone− water	Peptone+ 0.1% glucose	Peptone+ 0.25% glucose	Peptone+ 0.5% glucose	Peptone+ 1.0% glucose
Staph. aureus ATCC 25923	0.56	1.2	1.1	1.1	1.1
Staph. aureus #910	0.40	1.3	1.2	1.3	1.2
Staph. aureus #127	0.68	1.4	1.3	1.3	1.3
Staph. aureus #671	0.69	1.3	1.2	1.3	1.3
Strep. faecalis #34358	0.29	0.94	0.66	0.55	0.86
E. coli #12140	0.75	1.6	1.6	1.3	1.2
E. coli #804	0.82	1.3	1.2	1.2	1.4
E. coli #211	0.84	1.3	1.1	1.1	1.3
Kleb. pneumoniae #4200	0.57	0.70	0.75	0.96	0.96
Enter. cloacae #31254	1.1	1.3	1.3	1.1	1.1
Proteus mirabilis #444	0.57	1.1	1.1	1.1	1.2
Proteus morganii #179	0.33	0.78	0.90	0.86	0.86
Providencia sp. #276	1.0	1.0	1.0	0.96	0.98
Salmonella gallinarum #595	0.28	0.86	0.94	0.93	1.0
Serratia marcescens #13880	1.0	1.1	1.1	1.1	1.1
C. albicans #759*	0.44	0.88	1.4	1.4	1.4
Final pH	7.3	7.3~7.0	7~5.5	6.5~5.5	5.5~4.5

* Incubated at 30°C.

Table 2. Microbial strains examined for growth in the peptone-glucose-buffered broth and agar.

Microbes	Number of strains studied	Microbes	Number of strains studied
Staphylococcus aureus and *epidermidis*	31	*Salmonella paratyphi* and *gallinarum*	2
Streptococcus faecalis	15	*Serratia marcescens*	26
Sarcina lutea	1	*Pseudomonas aeruginosa*	10
Bacillus subtilis ATCC 6633	1	*Mycobacterium phlei*	1
Escherichia coli	37	*Candida albicans*	10
Klebsiella pneumoniae	34	*Candida* spp. (other than *albicans*)	5
Enterobacter cloacae and *aerogenes*	27	*Trichophyton mentagrophytes*	4
Proteus mirabilis	25	*Microsporum versicolor*	1
Proteus, indole-producing	25		
Citrobacter, *Providencia*, *Arizona*, *Acinetobacter*	25	Total	280

stabilization of the pH.

The growth supporting quality of PGB medium using 280 microbial (bacterial and fungal) strains is summarized in Table 2. All strains grew well in or on this medium at 37° or 30°C overnight (longer in the case of some fungi). Since the medium is colorless, it is also convenient for the study of the mechanisms of pigment formation of *Pseudomonas aeruginosa*, *Serratia marcescens* and *Trichophyton* strains.

The PGB broth was found to be convenient for MIC determination of various antimicrobial agents. The results of a typical *in vitro* assay are presented in Table 3, although many experiments were per-

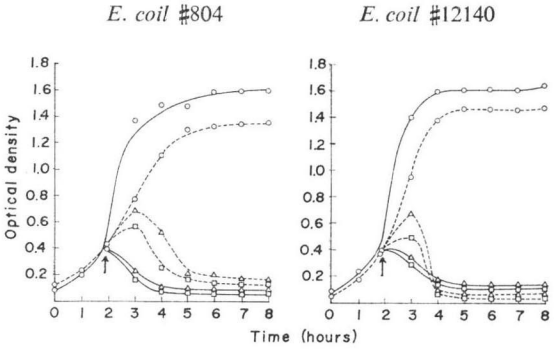
Table 3. Representative MIC values of cefotaxime and turbidimetric results in peptone-glucose-buffered (PGB) broth.

Strains	Minimal inhibitory concentration μg/ml	Optical density	
		at MIC	Control
Staphylococcus aureus #674	1.5	0.10	1.15
Staphylococcus aureus #671*	1.5	0.11	1.3
Escherichia coli #12140	0.1～0.05	0.12	1.4
Escherichia coli #804*	0.1	0.61	1.6

* Strong β-lactamase producing strains.

Fig. 1. Lytic action of alaphosphin on the non-β-lactamase producing *E. coli* #12140 and the strong β-lactamase producing *E. coli* #804 in peptone-glucose-buffered broth and in supplemented antagonist-free medium.

Symbols: ——, antagonist-free medium; ------, peptone-glucose-buffered medium; ○, control; △, 1.5 μg/ml and □, 3 μg/ml alaphosphin; ↑, time of addition of alaphosphin to culture.



formed. The MIC's for cefotaxime (HR-756) are in agreement with those obtained in conventional media[2,5]. An added advantage of PGB medium is that the MIC can be measured turbidimetrically, not only visually (Table 3).

PGB medium is particularly useful for the study of growth kinetics and the influence of antibiotics on growth. The bacteriolytic-effect of cephamycins (cefoxitin, CS-1170 and SK&F 73678) on various bacterial strains in this medium has been published[14]. Additional studies include other cephamycins, ampicillin, and cephalosporins (to be published). Although PGB medium was rich enough to support the growth of all bacteria and fungi studied, in certain experiments it behaved like an "antagonist-free" medium. In turbidimetric experiments using a supplemented defined antagonist-free medium[1] alaphosphin was shown to produce a rapid fall of optical density with complete lysis of the cells. A similar effect was found with *Escherichia coli* strains grown in PGB medium (Fig. 1). In the defined medium, the *E. coli* cells start to lyse at the time of addition of 1.5 or 3.0 μg/ml of alaphosphin, whereas in PGB broth there is some further growth before the lysis begins. Complete lysis occurs in both media after 4～5 hours of incubation. Lysis is not observed in complex organic media because of the presence of antagonistic factor(s) that counteract the antibacterial effect of alaphosphin.

Candida albicans #759 forms germ tubes in great frequency in PGB broth[15] as it does in human serum[12] or in peptone water[6]. *C. stellatoidea* was also found to produce germ tubes but other *Candida* spp. did not. PGB medium may be useful for the differentiation of *C. albicans* and *C. stellatoidea*[10], and for the study of the mechanism of germ tube or bud formation[4,8]. The concentration-dependent inhibition of germ tube formation of *C. albicans* #759 by primycin correlated well with its static and candicidal activity in this medium.[15]

Discussion

The results obtained with the new PGB medium demonstrate its versatility in the microbiology laboratory. It is not only useful for the diagnostic propagation of various aerobic bacteria and fungi but because it is colorless and maintains pH during growth, it has wide applicability in various microbial biochemical and chemotherapeutic studies.

The PGB colorless medium can be advantageously used for the study of microbial enzymatic

reactions which are accompanied by color change. It served as a basis for the development of a rapid and simple method for the detection of β-lactamase(s) inhibitors. The results obtained with clavulanic acid using 14 β-lactamase producing bacterial strains have recently been published[13].

Its behavior in experiments as an antagonist-free medium with alaphosphin may extend to the study of other agents such as sulfonamides, D-cycloserine, bacilysin, vancomycin, fosfomycin, and the like, the action of which is known to be influenced by medium ingredients. The germ tube formation of *C. albicans* for diagnostic and research purposes can easily be studied in this simple, colorless, pH-balanced and easily preparable medium, replacing serum, albumin and complex amino-acid containing media.

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