JOURNAL OF CLINICAL MICROBIOLOGY, July 1980, p. 35-38
0095-1137/80/07-0035/04$02.00/0

Vol. 12, No. 1

SJ-GC, a Modified Complete Medium for Growth of *Neisseria gonorrhoeae*

R. K. SHOCKLEY, E. E. COFFEE, AND K. H. JOHNSTON*

Department of Microbiology, University of Texas Health Science Center at Dallas, Dallas, Texas 75235

A broth medim that supports growth of *Neisseria gonorrhoeae* to high densities with minimal lysis is described. With most gonococcal strains, inocula of 2×10^6 colony-forming units per ml yielded greater than 10^9 colony-forming units per ml after 8 h of incubation. Scale-up cultures produced 5 to 12 g (wet weight) of cells per liter.

Growth of gonococcal cultures in liquid medium to high densities with minimal cell lysis would facilitate the study of antigenic characteristics. Many types of broth media have been designed for the growth of *Neisseria gonorrhoeae*, and most of these have been reviewed recently by Catlin (3). Gerhardt and Heden (6) described a biphasic medium consisting of a solid medium overlayed with a broth medium. This medium supported the growth of selected strains of *N. gonorrhoeae* and maintained colony types. The disadvantages of this type of medium are twofold. First, in shaking cultures, the broth seeps under the agar and makes recovery of the organisms very difficult. Second, scale-up experiments are difficult. Reports of the growth of *N. gonorrhoeae* in single-phase media have also appeared. Brookes and Sikyta (2) and Brookes and Heden (1) described the growth of gonococcal strains in a broth medium consisting of proteose peptone no. 3 (Difco), 30.0 g; K_2HPO_4, 3.0 g; KCl, 6.5 g; glucose, 10.0 g; and distilled water, 1 liter. They found maximum yields of 1.12 mg ml^{-1} in a continuous culture at pH 6.75 and a dilution of 0.26 ml h^{-1}. The disadvantage of this medium was that the preparation of the initial inoculum for large cultures required 12 to 18 h of incubation. Tauber and Garson (17) reported yields of 60 to 100 mg (dry weight) of bacteria from 25 liters after 6 days of incubation. In 1972, Jephcott (11) reported the growth of certain strains of *N. gonorrhoeae* in a complex single-phase medium consisting of nutrient broth and 10% ascitic fluid. Although yields of 10^8 to 10^9 colony-forming units (CFU) ml^{-1} and generation times of 50 min were obtained, relatively large inocula were required. Hart and Goldberg (9) reported a biphasic medium consisting of 30 ml of gonococcal base agar containing defined supplements (GCBA-DS) overlayed with 70 ml of broth containing defined supplements. Yields of 10^{10} CFU ml^{-1} in 7 h were obtained; however, an inoculum of 10^8 CFU ml^{-1} was necessary. Chan et al. (4) described an enriched single-phase medium (ESP) consisting of GC base (Difco) without agar, 5% defined supplements, and 1% IsoVitaleX (BBL Microbiology Systems). Growth in their medium was significantly decreased without the addition of gaseous CO_2. Jones and Talley (13) reported that the gaseous CO_2 requirement was circumvented by the addition of $NaHCO_3$ to their simple complete medium.

In this communication, we report a modified complete medium that supports high-density growth of gonococcal cultures in the absence of CO_2. We also compare growth of gonococcal isolates in five single-phase media.

MATERIALS AND METHODS

Bacterial strains. The strains used in this study were isolated from patients in New York City, Seattle, Dallas, The Netherlands, The Philippines, and England. Strain RW-1 was obtained from a skin lesion of a patient having a disseminated gonococcal infection. The cultures were stored at −79°C in tryptic soy broth (BBL Microbiology Systems) and 25% (vol/vol) glycerol. All cultures were auxotyped with WSJ medium (19) before growth on SJ-GC medium.

Media. Shockley-Johnston-modified gonococcal medium (SJ-GC) consisted of proteose peptone no. 3 (Difco), 15 g; K_2HPO_4, 4 g; KH_2PO_4, 1 g; NaCl, 5 g; and soluble potato starch (Sigma), 1 g. One percent defined supplements as described by Kellogg et al.(14) were added. In addition, 5 ml of solution B containing 40% (wt/vol) dextrose, 10% (wt/vol) Casamino Acids (GIBCO), 0.4% (wt/vol) cysteine-hydrochloride, and 6% (wt/vol) magnesium sulfate were added per 100 ml of culture medium. Growth in SJ-GC medium was compared with that in other media: (i) gonococcal base broth (GCBB), which was similar to SJ-GC, except solution B was omitted; (ii) a chemically defined medium (WSJ medium) (19), which supports the growth of *Neisseria* spp., designed in this laboratory; (iii) tryptic soy broth obtained from Difco; and (iv) Frantz medium (5).

Preparation of inocula and growth of bacterial strains. Inocula for each medium were prepared for cultures grown on GC base agar for 16 h in an atmos-

phere containing 10% (vol/vol) CO_2. For comparison of each medium, colonies from a single plate were suspended in 5 ml of the appropriate test medium. Portions of the suspensions were transferred to 100 ml of the same test medium in a 300-ml nephelometer flask until a final absorbance at 600 nm of 0.03 to 0.035 was obtained. WSJ medium was inoculated to an absorbance of 0.045. The final absorbances corresponded to viable cell counts of 2×10^6 to 4×10^7 CFU ml^{-1}.

For comparison of total yields, batch cultures were grown. Cultures grown on GC base agar for 16 h were removed with cotton-tipped swabs and were suspended in 5 ml of the appropriate test medium. The suspensions, containing approximately 2×10^9 CFU ml^{-1}, were added to 200 ml of the same medium. The 200-ml cultures were incubated in the absence of CO_2 at 37°C and shaken at 125 rpm until early stationary phase of growth. The 200-ml cultures were diluted into 800 ml of prewarmed medium of appropriate composition. One-liter cultures were incubated in the absence of CO_2 at 37°C and shaken at 100 rpm. After 16 h, the cells were harvested by centrifugation at 12,000 × *g* for 20 min at 10°C. The wet weight yield of cells obtained from each medium was determined by direct measurement of the cell pellet.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The outer membranes of gonococcal strains grown in different media were prepared by the procedure described by Johnston (12), which uses the detergent, sodium *N*-lauroyl sarcosinate, to isolate the outer membranes. One hundred micrograms of outer membrane protein was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis by the procedure of Weber and Osborn (18).

RESULTS

Growth responses. Growth without a lag period was observed with strain K_2 (Pro^- Met^-) in SJ-GC, GCBB, and Frantz media (Fig. 1). Generation times for strain K_2 were 48 min in SJ-GC, 54 min in GCBB, and 68 min in Frantz. After a lag period of 1 h, a generation time of 77 min was observed in WSJ medium. No significant growth was observed in tryptic soy broth medium. Maximum absorbances were observed in each medium after 8 h of incubation at 37°C in the absence of CO_2. Observed yields ranged from 4×10^7 CFU ml^{-1} for tryptic soy broth to 7×10^9 CFU ml^{-1} for SJ-GC. The yield from SJ-GC was approximately twofold greater than that from GCBB. Extended incubation of cultures in SJ-GC and GCBB for 36 h yielded 6×10^8 and 4×10^7 CFU ml^{-1}, respectively. After 36 h, cells grown in SJ-GC and GCBB were examined microscopically for changes in their staining properties and cellular morphology. Cells obtained from SJ-GC medium appeared more uniform with respect to staining and cellular morphology than cells grown in GCBB, which appeared enlarged and stained poorly. For 30 gonococcal strains tested, variations in generation times and

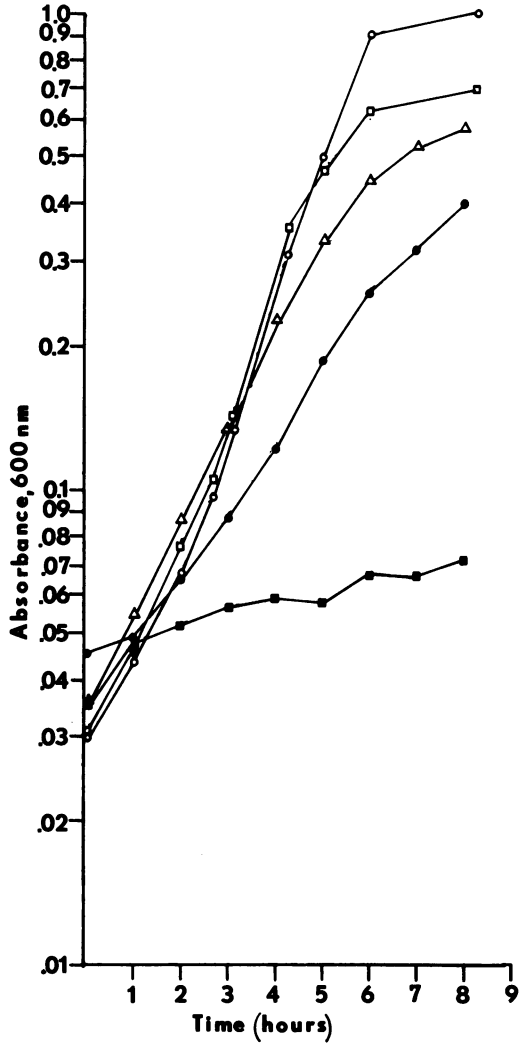

FIG. 1. *Growth responses of N. gonorrhoeae* K_2 *Pro⁻ Met⁻ in SJ-GC* (○), *GCBB* (□), *Frantz* (△), *WSJ* (●), *and tryptic soy broth* (■) *media.*

maximum yields were observed. For instance, the generation times ranged from 48 min to 60 min in SJ-GC. However, the relative differences in growth responses in the different test media were similar to those in strain K_2 (Pro^- Met^-).

Wet weight yields. Scale-up experiments for the growth of liter quantities of *N. gonorrhoeae* were performed to determine bacterial yields in each medium. Bacterial yields, expressed as grams (wet weight) per liter from SJ-GC, WSJ, and Frantz, are compared in Table 1. Yields from SJ-GC ranged from 5 to 13 g (wet weight) $liter^{-1}$, whereas, in WSJ yields were slightly less, ranging from 4 to 9 g (wet weight) $liter^{-1}$. Wet weight yields from tryptic soy broth and Frantz

TABLE 1. *Growth of N. gonorrhoeae in SJ-GC, WSJ,[a] and Frantz[b] media*

Strain	Phenotype	*N. gonorrhoeae* (g [wet wt] liter^{-1}) in:		
		SJ-GC	WSJ	Frantz
F62	Pro$^-$	7.5	6.4	1.1
N078	Pro$^-$ Met$^-$	8.0	6.1	1.2
N158	Hyx$^-$	9.8	8.4	0.7
N007	Pro$^-$	6.5	5.5	1.4
N165	Hyx$^-$	11.0	7.6	1.1
SK-4-2	Pro$^-$	7.0	5.2	0.8
K_2	Pro$^-$ Met$^-$	12.0	7.2	1.6
N126	Arg$^-$ Ile$^-$	6.8	5.7	1.2
N149	Arg$^-$	6.8	5.0	1.6
5042	Arg$^-$	6.2	5.5	0.9
RW-1	Arg$^-$ Hyx$^-$ Ura$^-$	9.0	7.4	0.3
5019	WT[c]	7.8	6.4	0.8
N082	Arg$^-$ Ser$^-$	6.6	4.8	0.9
N033	WT[c]	9.3	7.1	1.2
N073	Pro$^-$	12.7	8.6	0.7
SK 16-4	Pro$^-$	6.1	4.5	1.3
D 75	WT[c]	7.5	6.7	1.4

[a] Wong et al. (19).
[b] Frantz (5).
[c] WT, Wild-type prototroph.

were poor. Although not listed in Table 1, yields from GCBB were 10 to 20% less than those from SJ-GC.

Qualitative differences in membrane proteins. To determine whether any changes (qualitative) had taken place with respect to the membrane proteins produced by certain strains of gonococci, we examined the protein profiles of outer membranes of cells grown in each of the four media, isolated by the sodium *N*-lauroyl sarcosinate procedure described by Johnston (12). The outer membrane protein profiles of cells grown in SJ-GC, GCBB, WSJ, and Frantz were identical. The outer membrane samples contained proteins with molecular weights ranging from 23,000 to 105,000.

DISCUSSION

A modification of GC medium base (minus agar) that supports high density growth of gonococcal strains has been described. A comparison of the cell yields in the five media indicated that yields from SJ-GC were greater. Microscopically, cells grown in SJ-GC were more uniform morphologically than cells grown in GCBB. Although this does not directly measure the amount of cell lysis, it indicates that the cells grown in GCBB appear to be stressed more than cells grown in SJ-GC medium.

Increased cell yields and more rapid generation times of strains grown in SJ-GC medium could be associated with the high concentration of glucose in SJ-GC medium. Morse and Hebeler (16) have demonstrated that cells grown at pH 7.2 in the presence of glucose have faster generation times than cells grown at a lower pH. The initial pH of SJ-GC medium was 7.2, and the final pH was 6.9. They also reported that at pH 7.2 the tricarboxylic acid cycle activity was decreased and that energy was generated from glucose by increased activity of the Entner-Doudoroff pathway. In addition, they reported that at pH 7.2 a large portion of the glucose was used for energy and less of the glucose carbon was available for biosynthesis than at a lower pH. The addition of casein hydrolysate to SJ-GC medium supplied a utilizable amino acid pool that could free more glucose carbon for biosynthesis by conserving energy normally expended for the synthesis of amino acids.

Growth of *N. gonorrhoeae* in vitro usually requires an increased CO_2 tension. Jones and Talley (13) reported that sodium bicarbonate could be substituted for gaseous CO_2 in broth cultures. In the present study, addition of $NaHCO_3$ did not improve the growth of gonococci in SJ-GC medium. The reasons for this apparent discrepancy are unclear; however, two possible explanations may be presented. First, the strains used in the present study may have adapted to growth without CO_2. Although this is probable, it does not appear to be the case in this study since strains cultured on GC base in the absence of CO_2 did not grow. Second, the metabolism of the extra glucose and amino acids may eliminate the requirement for exogenous CO_2 by *N. gonorrhoeae*.

The growth of a variety of auxotrophs in SJ-GC was tested (Table 1). Strains having requirements for arginine, hypoxanthine, and uracil or arginine and uracil grew in SJ-GC. With the exception of RW-1, an isolate from a skin lesion, the disseminated gonococcal strains grew very well in SJ-GC after the third subculture. Growth occurred without the addition of hypoxanthine or uracil to the medium. Strain RW-1 did not grow very well on GC base agar in the presence of 10% CO_2, requiring approximately 24 h before confluent growth was observed. However, after subculturing and use of a large inoculum, high yields were obtained (Table 1). It is well known (15) that hypoxanthine requirement is unstable and reverts at a very high frequency; growth in the absence of hypoxanthine may have selected for revertants. However, RW-1 was tested for the requirement for hypoxanthine after growth on SG-GC; the subcultured bacterial population still expressed hypoxanthine auxotrophy.

Variations in growth parameters can cause changes in the expression of bacterial antigens. Hebeler et al. (10) have reported that the amount of peptidoglycan-associated protein in-

creased in two gonococcal strains when grown at pH 6.0. In this study, changes in sodium *N*-lauroyl sarcosinate-insoluble outer membrane proteins were not observed at pH 7.2 after the addition of Casamino Acids and a high concentration of glucose. These data suggest that this modification of GC base did not cause gross changes in the outer membrane proteins of *N. gonorrhoeae*. The peptidoglycan, phospholipids, and lipopolysaccharide were not examined for changes in this study.

Throughout this study, it was observed that errors could be made in determining cell yields if the yields from small cultures were used to compute the yield from a liter of medium. The values derived from the small cultures were consistently greater than the values determined by direct measurement of the yields from a liter of medium. The values reported in this study were determined by direct measurements.

Although direct measurement of cell lysis was not done, microscopic examination of cells grown in SJ-GC medium indicated that they appeared to be more uniform morphologically than cells grown in the other test media. In conclusion, we have reported a modification of GC base medium that allows the high density growth of gonococcal strains.

ACKNOWLEDGMENTS

This research was generously supported by Public Health Service contract NO1-AI-52534 from the National Institute of Allergy and Infectious Diseases.

We thank R. L. McDade, Jr. and T.-P. Wong for excellent technical assistance. In addition, the expert typing skills of Daisi Marcoulides are greatly appreciated.

LITERATURE CITED

1. **Brookes, R., and C.-G. Heden.** 1967. Dense cultures of *Neisseria gonorrhoeae* in liquid medium. Appl. Microbiol. **15**:219-223.
2. **Brookes, R., and B. Sikyta.** 1967. Influence of pH on the growth characteristics of *Neisseria gonorrhoeae* in continuous culture. Appl. Microbiol. **15**:224-227.
3. **Catlin, B. W.** 1977. Nutritional requirements and auxotyping, p. 91-109. *In* R. R. Roberts (ed.), The gonococcus. John Wiley and Sons, Inc., New York.
4. **Chan, K., G. M. Wiseman, and J. D. Caird.** 1975. Cultivation of type 1 *N. gonorrhoeae* in liquid medium. Br. J. Vener. Dis. **51**:382-386.
5. **Frantz, I. D.** 1941. Growth requirements of the meningococcus. J. Bacteriol. **43**:757-761.
6. **Gerhardt, P., and C. G. Heden.** 1960. Concentrated culture of gonococci in clear liquid medium. Proc. Soc. Exp. Biol. Med. **105**:49-51.
7. **Hafiz, S., and M. G. McEntegart.** 1976. Prolonged survival of *Neisseria gonorrhoeae* in a new liquid medium. Br. J. Vener. Dis. **52**:381-383.
8. **Hafiz, S., M. G. McEntegart, and A. E. Jephcott.** 1976. Reversion of Kellogg's colonial types of *Neisseria gonorrhoeae* in liquid medium. J. Med. Microbiol. **10**:377-380.
9. **Hart. E. J., and I. D. Goldberg.** 1975. Conditions required for attainment of colony-type stability of *Neisseria gonorrhoeae* in liquid culture. J. Clin. Microbiol. **2**:387-390.
10. **Hebeler, B. H., S. A. Morse, W. Wong, and F. E. Young.** 1978. Effect of pH on the chemical and biological properties of the cell envelope of *Neisseria gonorrhoeae:* isolation of a protein-peptidoglycan complex; p. 18-25. *In* G. F. Brooks, E. C. Gotschlich, K. K. Holmes, W. D. Sawyer, and F. E. Young (ed.), Immunobiology of *Neisseria gonorrhoeae*. American Society for Microbiology, Washington, D. C.
11. **Jephcott, A. E.** 1972. Preliminary study of colony type stability of *Neisseria gonorrhoeae* in liquid culture. Br. J. Vener. Dis. **48**:396-375.
12. **Johnston, K. H.** 1978. Antigenic profile of an outer membrane complex of *Neisseria gonorrhoeae* responsible for serotypic specificity, p. 121-129. *In* G. F. Brooks, E. C. Gotschlich, K. K. Holmes, W. D. Sawyer, and F. J. Young (ed.), Immunobiology of *Neisseria gonorrhoeae*. American Society for Microbiology, Washington, D. C.
13. **Jones, R. T., and R. S. Talley.** 1977. Simplified complete medium for the growth of *Neisseria gonorrhoeae*. J. Clin. Microbiol. **5**:9-14.
14. **Kellogg, D. S., W. L. Peacock, Jr., W. E. Deacon, L. Browh, and C. I. Perkle.** 1963. *Neisseria gonorrhoeae*. I. Virulence genetically linked to colonial variation. J. Bacteriol. **85**:1274-1279.
15. **Mayer, L. W., G. K. Schoolnik, and S. Flakow.** 1977. Genetic studies on *Neisseria gonorrhoeae* from disseminated gonococcal infections. Infect. Immun. **18**:165-172.
16. **Morse, S. A., and B. H. Hebeler.** 1978. Effect of pH on growth and glucose metabolism of *Neisseria gonorrhoeae*, p. 9-17. *In* G. F. Brooks, E. C. Gotschlich, K. K. Holmes, W. D. Sawyer, and F. E. Young, (ed.), Immunobiology of *Neisseria gonorrhoeae*. American Society for Microbiology, Washington, D. C.
17. **Tauber, H., and W. Garson.** 1957. Preparation and some properties of *Neisseria gonorrhoeae* endotoxin. Proc. Soc. Expl. Biol. Med. **95**:669-672.
18. **Weber, K., and M. Osborn.** 1969. The reliability of molecular weight determination by dodecyl sulfate-polyacrylamide electrophoresis. J. Biol. Chem. **244**: 4406-4412.
19. **Wong, T. P., R. K. Shockley, and K. H. Johnston.** 1980. WSJM, a simple chemically defined medium for growth of *Neisseria gonorrhoeae*. J. Clin. Microbiol. **11**:363-369.