Development of a Defined Minimal Medium for the Growth of
*Neisseria gonorrhoeae*

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This investigation describes the development of a solid and a liquid medium (Gonococcal Genetic Medium; GGM) which support the rapid growth of 41 gonococcal clinical isolates and laboratory strains with a minimum number of nutritional components. The complete medium contains minimal salts, eight amino acids, two nitrogen bases, vitamins, coenzymes, key metabolic intermediates, and some miscellaneous components. Results indicate that GGM can be modified and simplified even further than we described. In liquid GGM, several gonococcal strains grew logarithmically after a 2- to 3-h lag period with generation times ranging from 72 to 115 min, reaching optical densities of 175 to 320 Klett units in the presence of seven amino acids and in the absence of a CO₂ atmosphere. The development of a solid and a liquid defined minimal medium such as GGM should greatly broaden the avenues of experimentation for biochemical genetic studies with *N. gonorrhoeae*, especially gonococcal genetic transformation. *N. gonorrhoeae* can be classified into eight major and minor phenotypic groups, depending on its growth responses on GGM to just five amino acids: cysteine and cystine, arginine, proline, isoleucine, and serine. Such results demonstrate the feasibility of using GGM as a simple, sensitive, rapid probe for investigating the epidemiological patterns of gonorrhea.

Gonorrhea has become the most prevalent bacterial infectious disease today. However, in spite of this fact, knowledge of the genetic and biochemical mechanisms of the pathogenicity of *Neisseria gonorrhoeae* are strikingly limited. Therefore, it would be most desirable to establish a genetic system for this microorganism. The most essential initial requirement is the development of a defined minimal medium. Several chemically defined media have been described for the cultivation of the gonococci (3, 6, 10, 11). One was useful for the majority of gonococcal strains (3), whereas others appeared to be limited to just a selected number of strains (6, 10, 11). An extremely important contribution was the complete chemically defined medium developed by Carifo and Catlin for auxotyping (2, 3).

For genetics studies it is not sufficient that the medium be chemically defined; it also should have a minimum of nutritional components, especially amino acids, thereby allowing the isolation of a maximum number of auxotrophs. Furthermore, the medium with its minimal nutritional components should still permit the rapid growth of *N. gonorrhoeae* because certain mutants possess phenotypes that are distinguished from the wild type strain only by their rate of growth. For additional usefulness, the medium should be transparent to allow colonial typing of the gonococcus, as defined by Kellogg et al. (8, 9). Finally, it should be relatively simple to prepare.

This communication describes a solid and a liquid medium (Gonococcal Genetic Medium; GGM) that have a minimal number of nutritional components and yet support rapid growth of various strains of *N. gonorrhoeae*. Of 43 clinical isolates and laboratory strains tested, 41 grew rapidly on GGM. The complete medium contains minimal salts, eight amino acids, two nitrogen bases, vitamins, coenzymes, key metabolic intermediates, and some miscellaneous components. Essential and nonessential amino acids were determined for all strains that grew on GGM. As first shown by Carifo and Catlin (2), such chemically defined media can be used for rapidly determining the phenotypes of *N. gonorrhoeae* in clinical infections.

**MATERIALS AND METHODS**

**Nomenclature.** The *N. gonorrhoeae* clinical isolates used were assigned RUG (Rochester University gonococcus) numbers.
Bacterial strains. Thirty-three clinical isolates of *N. gonorrhoeae* were obtained from the Diagnostic Microbiology Laboratories of Strong Memorial Hospital and the Monroe County Venereal Disease Clinic. Eight laboratory strains were kindly provided by B. W. Catlin (Medical College of Wisconsin, Milwaukee, Wis.), D. S. Kellogg (Center for Disease Control, Atlanta, Ga.), and F. J. Tyreyar (Naval Medical Research Institute, Bethesda, Md.). All strains were isolated by three successive single-colony selections and were confirmed as *N. gonorrhoeae* by colonial morphology (8, 9), Gram stain, oxidase reaction, and fermentation of glucose but not maltose, lactose, or sucrose. The laboratory strains and clinical isolates used in this investigation, with their genotype designation, phenotype, and origin, are listed in Table 1. The genotype designations are according to the system of Demerec et al. (4).

### Table 1. Characterization of *N. gonorrhoeae* strains

| Strain | Genotype designation | Phenotype | Origin |
|--------|----------------------|-----------|--------|
| F62    | pro-1 Sm<sup>a</sup>  | Pro<sup>-</sup> Sm<sup>-</sup> | F joint fluid |
| 2666   | pro-2                | Pro<sup>-</sup> Ile<sup>-</sup> | F rectal |
| MHD 340 | ile-1          | Ile<sup>-</sup> | F cervix |
| MHD 446 | arg-1            | Arg<sup>-</sup> | F cervix |
| RD<sub>s</sub> | ile-2         | Ile<sup>-</sup> | F cervix |
| H<sub>s</sub>          | pro-3          | Pro<sup>-</sup> | F cervix |
| ATCC 19424 | met-1       | Met<sup>-</sup> | F cervix |
| #9     | wt<sup>b</sup>     | Wt        | F cervix |
| RUG 4  | pro-4             | Pro<sup>-</sup> | F cervix |
| RUG 5  | pro-5             | Pro<sup>-</sup> | F cervix |
| RUG 6  | wt                 | Wt        | F cervix |
| RUG 7  | wt                 | Wt        | F cervix |
| RUG 8  | pro-6, ser-1, ile-3 | Pro<sup>-</sup> Ser<sup>-</sup> Ile<sup>-</sup> | F cervix |
| RUG 9  | arg-2, ile-4       | Arg<sup>-</sup> Ile<sup>-</sup> | F cervix |
| RUG 10 | wt                 | Wt Ile<sup>-</sup> | F cervix |
| RUG 11 | arg-3             | Arg<sup>-</sup> Ile<sup>-</sup> | F cervix |
| RUG 12 | arg-4             | Arg<sup>-</sup> | F cervix |
| RUG 13 | arg-5, pro-7, ile-5 | Arg<sup>-</sup> Pro<sup>-</sup> Ile<sup>-</sup> | F cervix |
| RUG 14 | arg-6, ile-6       | Arg<sup>-</sup> Ile<sup>-</sup> | F cervix |
| RUG 15 | wt                 | Wt        | F cervix |
| RUG 16 | pro-8, ile-7       | Pro<sup>-</sup> Ile<sup>-</sup> | F cervix |
| RUG 17 | pro-9, ser-2       | Pro<sup>-</sup> Ser<sup>-</sup> Ile<sup>-</sup> | F cervix |
| RUG 18 | arg-7, ile-8       | Arg<sup>-</sup> Ile<sup>-</sup> | F cervix |
| RUG 19 | wt                 | Wt Arg<sup>-</sup> Isoleu<sup>-</sup> | F cervix |
| RUG 20 | arg-8, ile-9       | Arg<sup>-</sup> Ile<sup>-</sup> | F cervix |
| RUG 21 | arg-9, ile-10      | Arg<sup>-</sup> Ile<sup>-</sup> | F cervix |
| RUG 22 | pro-10             | Pro<sup>-</sup> Ser<sup>-</sup> Ile<sup>-</sup> | F cervix |
| RUG 23 | pro-11             | Pro<sup>-</sup> Ser<sup>-</sup> Ile<sup>-</sup> | F cervix |
| RUG 24 | pro-12             | Pro<sup>-</sup> | F cervix |
| RUG 25 | pro-13, ile-11     | Pro<sup>-</sup> Ile<sup>-</sup> Ser<sup>-</sup> | F cervix |
| RUG 26 | wt                 | Wt        | F cervix |
| RUG 27 | pro-14, ser-3      | Pro<sup>-</sup> Ser<sup>-</sup> Ile<sup>-</sup> | F cervix |
| RUG 28 | ser-4              | Ser<sup>-</sup> Pro<sup>-</sup> | F cervix |
| RUG 29 | pro-15, ser-5      | Pro<sup>-</sup> Ser<sup>-</sup> Asp. Acid<sup>-</sup> | F cervix |
| RUG 30 | wt                 | Wt        | F cervix |
| RUG 31 | arg-10, pro-16, ser-6, ile-12 | Arg<sup>-</sup> Pro<sup>-</sup> Ser<sup>-</sup> Ile<sup>-</sup> | F cervix |
| RUG 32 | pro-17             | Pro<sup>-</sup> | F cervix |
| RUG 33 | wt                 | Wt        | F cervix |
| RUG 34 | wt                 | Wt Ile<sup>-</sup> | F cervix |
| RUG 35 | wt                 | Wt        | F cervix |
| RUG 36 | pro-18             | Pro<sup>-</sup> Ile<sup>-</sup> | F cervix |

<sup>a</sup>The first eight strains are laboratory strains; the remaining thirty-three are clinical isolates. Abbreviations: F, female; exponent s, stimulatory for growth; Sm<sup>-</sup>, resistant to 1 mg of streptomycin sulfate per ml.

<sup>b</sup>All gonococcal strains tested to date require cysteine and/or cystine. Strains that required just cysteine and its oxidized form, cystine, were designated wild-type (wt or Wt) strains.

### Maintenance of bacterial strains. The gonococcal strains were stored at −76°C in Trypticase soy broth containing 15% glycerol. For use, the cultures were thawed at room temperature and inoculated onto the appropriate media. The organisms were stable upon repeated freezing and thawing in this medium. Stocks were also kept for short periods (3 to 4 weeks) on dextrose starch agar slants with some minor modifications of the procedure described by Hunter and McVeigh (6).

**Gonococcal Genetic Medium.** The composition of the GGM for growth of *N. gonorrhoeae* is given in

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**DEFINED MINIMAL MEDIUM FOR *N. GONORRHOEAE***

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Table 2. Minimal salts solution I contained (grams per liter of distilled, demineralized water): KCl, 4; NaCl, 70; Na$_2$C$_5$H$_7$O$_2$, 2H$_2$O, 15; MgCl$_2$, 6H$_2$O, 6; NH$_4$Cl, 4; Na$_2$SO$_4$, 10; K$_2$SO$_4$, 12; and Na$_2$HPO$_4$·3H$_2$O, 20. Minimal salts solution II contained (grams per liter of distilled, demineralized water): K$_2$HPO$_4$, 140; KH$_2$PO$_4$, 60. The stock solutions for solution III contained the following amino acids (in milligrams per milliliter): L-glutamic acid, 7.5; L-arginine and L-proline, 20; L-methionine, 15; L-aspartic acid, L-serine, and L-isoleucine, 10. Their final concentrations per liter of GGM (in micrograms per milliliter) were: L-glutamic acid, 188; L-arginine, 250; L-proline, 250; L-methionine, 190; L-aspartic acid, 125; L-serine, 125; and L-isoleucine, 125. L-Cysteine was dissolved in the required volume of hot, distilled, demineralized water by the addition of a few drops of 1 N HCl. L-Cysteine and L-cystine were prepared immediately before use. Stock solutions of uracil (2 mg/ml) and hypoxanthine (1 mg/ml) were mixed in a 1:1 ratio, and 5 ml was added per liter to yield final concentrations of 5 µg/ml for uracil and 2.5 µg/ml for hypoxanthine. The vitamin and coenzyme solution contained the following (in milligrams per milliliter): nicotinamide adenine dinucleotide, 5; thiamine-hydrochloride, 10; thiamine-pyrophosphate chloride, 10; and calcium pantothenate, 10. A 2-ml amount of a solution containing the vitamins and coenzymes at the above concentrations was added per liter to yield final concentrations of 10 µg/ml for nicotinamide adenine dinucleotide and 20 µg/ml for the last three components. Portions (2 ml) of the vitamin and coenzyme solution were added to plastic vials and stored at −20 C. Heat or alkali was used to dissolve the biotin in the required volume of distilled, demineralized water. The solution of metabolic intermediates contained oxalacetic acid at 10 mg/ml and pyruvic acid and glycerol each at 5% (vol/vol). After the addition of 25 ml of this solution per liter, the final concentrations were 250 mg/ml for oxalacetic acid and 0.125% (vol/vol) for pyruvic acid and glycerol. The pH of this solution was adjusted to 7.3 by first adding 6 N NaOH to pH 7.0 and then adding 1 N NaOH to pH 7.3.

All stock solutions were filter sterilized with either a 0.2- or 0.45-µm membrane filter. The solutions that were refrigerated were the metabolic intermediates, ferric nitrate (wrapped in aluminum foil), sodium thioglycolate, and CaCl$_2$. The remaining solutions were kept at room temperature with the exception of the vitamins and coenzymes, which were stored at −20 C. The procedure for the liquid GGM was exactly the same as for the solid medium, except that the Ionagar was omitted. The liquid and solid GGM were stored at 4 C. Glass-distilled, demineralized water was used for the preparation of all solutions. The glassware used in experiments was cleaned with nitric acid. The chemicals used were of the best available grade from the following companies: Fisher Scientific Co., GIBCO Diagnostics, and Sigma Chemical Co. For GGM it was not necessary to prewash the Ionagar.

Other media. Strains of N. gonorrhoeae were routinely grown on chocolate agar and GC medium base (Difco) supplemented with either IsoVitaleX or yeast extract to final concentrations of 1 and 0.2%, respectively.

Growth experiments in liquid GGM. The inoculum for growth experiments was obtained from N. gonorrhoeae that was grown for 15 to 17 h on solid GGM at 37 C with 8 to 10% CO$_2$. A heavy suspension (approximately 10$^8$ cells per ml) was prepared in liquid GGM preincubated overnight at 37 C in 8 to 10% CO$_2$. A sample of this inoculum was transferred to an acid-washed, 125-ml nephelometer flask containing 15 ml of liquid GGM that had also been preincubated overnight at 37 C in 8 to 10% CO$_2$. Usually the inoculum was diluted 1:50 to obtain an initial turbidity of 5 to 9 Klett units (Klett Sommerson spectrophotometer, filter no. 54). Cultures were incubated at 37 C in a New Brunswick G76 water bath at 120 rpm in air. With the preincubation of the medium in a CO$_2$ atmosphere, no further CO$_2$ was required for growth. The liquid GGM used in these studies had a total of seven amino acids as a result of

| Component* | Amount per liter | Final concentration (µg/ml) |
|------------|-----------------|-----------------------------|
| Part 1     |                 |                             |
| Minimal salts I | 75 ml | NA*                         |
| Minimal salts II | 75 ml | NA                          |
| Solution III | 100 ml | NA                          |
| L-Cysteine (40 mg/ml) | 30 ml | 1,200                        |
| L-Cystine (10 mg/ml) | 5 ml  | 50                           |
| Nitrogen bases | 5 ml  | NA                          |
| Vitamins and coenzymes | 2 ml  | NA                          |
| Separate solution of biotin (0.4 mg/ml) | 10 ml | 4                             |
| Metabolic intermediates | 25 ml | NA                          |
| Glucose (50%)* | 15 ml | 0.75%                       |
| NaHCO$_3$ (5%)* | 20 ml | 0.1%                       |
| Fe(NO$_3$)$_3$ (1 mg/ml) | 5 ml  | 5                           |
| CaCl$_2$ (1 mg/ml) | 5 ml  | 5                           |
| Sodium thioglycolate (5 mg/ml) | 5 ml  | 25                          |
| Water | 123 ml | NA                          |
| Part 2     |                 |                             |
| Soluble starch | 1 g  | 0.1%                         |
| Ionagar | 10 g  | 1%                          |
| Water | 500 ml | NA                          |

* Part II was autoclaved (15 lb. [about 7.8 kg] for 15 min), cooled, and held at 50 C in a water bath. The pH of part I was adjusted to 7.20 to 7.25 with 6 N NaOH (above pH 7.3 an irreversible precipitate will form). Part I was then equilibrated for 45 min at 50 C, filtered through a 0.20-µm filter, and added to part II. The final medium was mixed thoroughly and dispensed into petri dishes. Ionagar was omitted from liquid GGM.

* The composition of these particular solutions is given in the text.

* All solutions adjusted grams per volume percentage.
the omission of L-aspartic acid (Table 2). These growth experiments were repeated at least two times with the monitoring of cell morphology by phase microscopy.

Phenotypic determination on solid GGM. The phenotypes of various gonococcal strains, incubated for 24 h at 37° C in 8 to 10% CO₂ on GC medium base plates supplemented with yeast extract, were determined by streaking on agar composed of the complete GGM minus the individual amino acids. Whenever cysteine or cystine were deleted, they were omitted together. In all studies, the medium was prewarmed to room temperature. The cultures were then incubated at 37° C in 8 to 10% CO₂ and were examined at 24 and 48 h. The phenotypic determinations were repeated two to four times. If a particular amino acid was essential for growth on solid GGM, no growth was obtained after 48 h at 37° C in 8 to 10% CO₂. However, if a particular amino acid stimulated growth on solid GGM, sparse growth was obtained under these conditions.

RESULTS

Growth of N. gonorrhoeae in GGM. Because biochemical and genetic studies of N. gonorrhoeae have been hampered by the lack of a simple, defined, minimal medium, the first aim of this investigation was to develop a defined, minimal medium that could serve as a solid and a liquid medium for the growth of a wide variety of N. gonorrhoeae clinical isolates and laboratory strains. GGM was developed by the modification and reduction of several of the components of previously described media (3, 6, 10, 11). Complete GGM consists primarily of minimal salts, eight amino acids, two nitrogen bases, vitamins, coenzymes, key metabolic intermediates, and some miscellaneous components (Table 2). It was decided to incorporate soluble starch, since it was reported that this component could neutralize fatty acids having an antibacterial effect that are present in the agar (5). Furthermore, the toxic effect of ordinary agar on gonococci has been noted in a number of investigations (1, 3, 6, 10, 11), and so a purified agar (lonagar) was used for GGM.

In a study of 43 clinical isolates and laboratory strains, only two failed to grow on the complete GGM. The growth patterns of some representative clinical isolates and laboratory strains are shown in Fig. 1. Complete GGM contained the maximal number of components. For some experiments the medium can be simplified. This is best exemplified by the phenotypic patterns that were obtained by streaking out 41 gonococcal strains on GGM minus individual amino acids (Table 1). These phenotypic determinations demonstrate that for almost all gonococcal strains the amino acids L-glutamic acid, L-methionine, and L-aspartic acid are individually not essential for growth on GGM.

Since a liquid medium is essential for various genetic and biochemical studies, it was important to determine whether the liquid GGM could support the growth of the gonococcus. Nine representative gonococcal strains were examined for growth in liquid GGM (Fig. 2). In this medium the laboratory strains H₄, F62, RD₄, and ATCC 19424 (Fig. 2A and B) and clinical isolates RUG 5, 6, 7, 12, and 15 (Fig. 2C and D) grew logarithmically after a 2- to 3-h lag period, with generation times ranging from 72 min for F62 to 115 min for RUG 12 (Table 3), and reaching final optical densities of 175 to 320 Klett units. These growth experiments demonstrate that liquid GGM is able to support rapid growth of various gonococcal strains. Secondly, the omission of L-aspartic acid in these growth experiments indicates that GGM can be further simplified. Thirdly, these gonococcal strains grew at 37° C in air (Materials and Methods); therefore, the usual 8 to 10% atmosphere of CO₂ is not necessary for growth. It should be noted, however, that a few strains had a very long lag period before entering log phase, which could simply be a direct result of requiring an increased CO₂ tension for rapid growth.

Use of GGM for epidemiological studies. Epidemiological investigations of various bacterial diseases require a method for classifying the isolates of a given species. Carifo and Catlin (2) described the development of a completely defined medium which could be used to type N. gonorrhoeae strains according to a wide diversity of nutritional requirements (auxotypes), such as a number of vitamins, nitrogen bases, amino acids, and metabolic intermediates. GGM classified N. gonorrhoeae strains into eight major and minor phenotypic groups based
Fig. 2. Growth of *N. gonorrhoeae* in liquid GGM. The laboratory strains (F62, H4, RD8, and ATCC 19424; A and B) and the clinical isolates (RUG 5 through 7, 12, and 15; C and D) were incubated in liquid GGM, with the exception that L-aspartic acid was omitted from this medium.

Table 3. Generation times of *N. gonorrhoeae* laboratory and clinical isolates in liquid GGM

| Strain     | Generation time (min) |
|------------|-----------------------|
| F62        | 72                    |
| H4         | 87                    |
| RD8        | 83                    |
| ATCC 19424 | 84                    |
| RUG 5      | 87                    |
| RUG 6      | 90                    |
| RUG 7      | 88                    |
| RUG 12     | 115                   |
| RUG 15     | 85                    |

*The medium used was L-aspartic acid-free liquid GGM (Table 2). The generation times represent the average value from at least two experiments.*

on their growth patterns to the amino acids cysteine and cystine, arginine, proline, isoleucine, and serine (Table 4). These five amino acids appear to be the most important ones for gonococcal growth on GGM. In agreement with Catlin's auxotyping system (2), there are two major gonococcal phenotypic classes, the cysteine and cystine requireurs and the proline requireurs. The group requiring arginine appears not to be a major class on GGM; however, a new major phenotype group seems to be the gonococci requiring both arginine and isoleucine. These phenotypic patterns, in addition to others, are being investigated at the present time. Additional *N. gonorrhoeae* phenotype groups can be created by analyzing the requirements for more than two amino acids (Table 1). There appears to be some variance between the phenotypic patterns on GGM and Catlin's chemically defined media (2, 3); however, it should be remembered that growth patterns are

Table 4. Amino acid classification of *N. gonorrhoeae* strains on solid GGM

| Amino acid requirements | Strain       | % of total strains |
|-------------------------|--------------|--------------------|
| wt                      | #9, RUG 6, 7, 10, 15, 19, 26, 30, 33, 34, 35 | 27                 |
| Pro−                    | F62, 2686, H4, RUG 4, 5, 22, 23, 24, 32, 36 | 24                 |
| Arg−                    | MHD 446, RUG 11, 12 | 7                  |
| Ile−                    | MHD 340, RD8 | 5                  |
| Ser−                    | RUG 28      | 2                  |
| Pro− Ile−               | RUG 16, 25  | 5                  |
| Pro− Ser−               | RUG 17, 27, 29 | 7             |
| Ile− Arg−               | RUG 9, 14, 18, 20, 21 | 12            |

*On cystine- and cysteine-free GGM, all the gonococcal strains found these components essential for growth. Strains that just required cysteine and its oxidized form, cystine, were designated wild-type (wt) strains.*
a function of the composition of the chemically
defined media. Based on just these five specific
amino acids, GGM has the potential to develop
into a simple, sensitive, and rapid typing sys-
tem for studying the epidemiological patterns of
gonorrhea.

DISCUSSION

The results described in this investigation
demonstrate the development of a solid and a
liquid medium (GGM) that support the rapid
growth of a wide variety of gonococcal strains
with a minimum number of nutritional compo-
nents. In addition, GGM has the potential to be
modified and simplified even further. Also, it
appears that for several strains rapid growth
can be obtained in liquid GGM in the absence
of a CO₂ atmosphere. Finally, GGM is simple to
prepare and is transparent for studying the
colonial types of the gonococcus as defined by
Kellogg et al. (8, 9). Experiments are now in
progress to determine the colonial types main-
tained on solid and liquid GGM. Furthermore,
the development of a solid and a liquid GGM
should greatly broaden and enhance the aven-
ues of experimentation for biochemical genetic
studies with Neisseria gonorrhoeae. For instance,
virulent isolates and nonvirulent labora-
tory-adapted strains differ in colonial mor-
phology (8, 9), autoagglutinability (9), ability to
irreversibly bind naked deoxyribonucleic acid
by genetic transformation (12), possession of
pili (7, 13), resistance to phagocytosis (14), and
sensitivity to the bactericidal action of anti-
serum plus complement (15). These findings
suggest that there may be specific genetic and
nutritional differences between the virulent
gonococci and laboratory-adapted strains. Em-
phasis will be placed on gonococcal genetic
transformation, using GGM for the selection
and mapping of a wide variety of structural and
regulatory mutants from a common genetic
background. In addition, it is possible to incor-
porate radioactive diaminopimelic acid, N-
acetyl glucosamine, and glucosamine into the
peptidoglycan of Neisseria gonorrhoeae (B. Hebeler
and F. E. Young, unpublished observations). Finally, it is important to note that the ability
to grow large quantities of gonococcal cells with
liquid GGM will greatly facilitate bio-
chemical experiments.

GGM, as a probe for studying the metabolic
processes of Neisseria gonorrhoeae, could also be used
to examine the epidemiological patterns of
gonorrhea by using metabolic markers, as first
introduced by Carifo and Catlin (2). Our results
show that GGM classified Neisseria gonorrhoeae
strains into eight major and minor phenotypic
groups, depending on their growth responses to
just five amino acids: cysteine and cystine,
arginine, proline, isoleucine, and serine. By
analyzing the requirements for more than two
amino acids, additional phenotype groups could
be established. Therefore, GGM has the poten-
tial to develop into a simple, sensitive, rapid
typing system for investigating contacts and for
evaluating the success of therapy.

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Addendum in Proof

Upon continual maintenance of stocks, it was noted that the isoleucine genetic marker was unstable and reverted to prototrophy in some strains.

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