# Isolation of Gordon Group 1B *Bacillus* with Ammonium Based Agar

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**Abstract**

The genus *Bacillus* represents a large group of rod shaped Gram positive bacteria with the ability to make dormant endospores during periods of starvation. *Bacillus subtilis* is a well-known bacterium with industrial value due to its widespread use in agriculture, antibiotic development, and antigen development for vaccines. A novel medium with ammonium as the sole nitrogen source was developed and investigated for its effectiveness in the preferential isolation of Group 1B *Bacillus spp.* Through the observations of mannitol fermentation and spore characteristics, it was determined that Group 1B *Bacillus spp.* can be selectively isolated on an ammonium based agar.

## Introduction

The genus *Bacillus* represents a large group of rod shaped Gram positive bacteria with the ability to make dormant endospores during periods of starvation. A method was developed in the 1970's to classify the different species of the genus *Bacillus* into three groups based on the appearance of sporangia and mature spores, on the results from biochemical tests, and growth properties on various types of media (Gordon, 1973). Group 1 *Bacilli* form sporangia that are not swollen, and with spores that are ellipsoidal or cylindrical in shape while positioned central to terminal in the sporangium. Group 1 can further be divided into groups 1A and 1B, based on group 1B having the ability to grow in 7% NaCl agar, and ferment mannitol. Group 2 *Bacilli* exhibit swollen sporangia with ellipsoidal spores located central to terminal. Group 3 *Bacilli* develop swollen sporangia, spherical spores, and terminal spore position (Gordon, 1973).

*Bacillus subtilis* is a well-known bacterium with industrial value due to its widespread use in agriculture, in developing antibiotics (specifically Subtilin), and in the development of antigens for various vaccines (Zeigler, 2008). It is a part of group Group 1B, and is capable of growth in 7% NaCl, and fermenting mannitol salt agar. These qualities suggest it might be possible to create a selective medium for the isolation of Group 1B *Bacilli* for study.

The purpose of this investigation is to investigate the effectiveness of a novel media (MG5) for the preferential isolation of Group 1B *Bacillus spp.* from soils based on their salt tolerance and the ability to use ammonium as the sole nitrogen source. The agar should select specifically for Group 1B *Bacillus*, preventing the growth of other strains of the genus.

## Materials and Methods

### Media

For this investigation, the following media were used:

- Nutrient Agar - NA (Difco).
- Mannitol Salt Agar – MSA7 (Criterion), adjusted to 7% NaCl from 7.5% NaCl.
- MG5 – Novel medium consisting of 3.5g/L dipotassium phosphate, 1.5g/L monopotassium phosphate, 0.5g/L ammonium sulfate, 0.05g/L magnesium sulfate, 0.005g/L manganese chloride, 0.005g/L calcium chloride, ~5ul of ferric chloride, 1.25g/L MG + glucose, and 3.75g/L Agar Type A

### Isolation of Spore Formers from Soil

Five soil samples were retrieved from different locations within Taylor County Texas, with the GPS coordinates retrieved along with each sample. Soil was gathered in 50ml conical polypropylene centrifuge tubes up to the 10ml mark on each tube, and filled up to the 30ml mark with sterile deionized water. Each tube was placed in a shaker for 30 minutes at ambient temperature. After allowing shaken suspensions to settle for 15 minutes, 1ml of each suspension was transferred into sterile Eppendorf tubes and placed in a water bath set to 70°C for 30 minutes to heat shock spores and kill off non-sporeformers.

### Growth Studies

Heat shocked soil suspensions had 0.1 ml aliquots removed aseptically and pipetted onto both NA and MG5 plates, to be spread over the entirety of the agar with a sterile glass spreader rod. The plates for each site were placed in the incubator at 37°C, with NA plates incubated for one day, and MG5 plates incubated for four days. After incubation, colonies were selected based on observed differences and transferred to MSA7 plates via sterilized toothpicks for incubation overnight at 37°C to watch for fermentation. The results of the fermentation were documented, and all colonies, whether or not they fermented the mannitol, were transferred to NA plates and incubated for an additional two days at 37°C so that spore and sporangial morphology could be determined.

**Group 1B *Bacillus spp.* can be selectively isolated on an ammonium based agar.**


Figure 1. Soil sampling sites. Site 1: 32.3472001, -99.7820002 Site 2: 32.3512997, -99.7771997 Site 3: 32.3500002, -99.7784 Site 4: 32.3512, -99.7769 Site 5: 32.3502999, -99.7790001

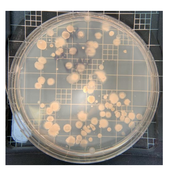

Fig. 2: Site 3 NA isolates

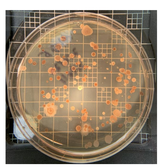

Fig 3: Site 3 MG5 isolates

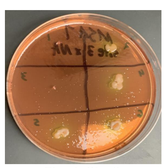

Fig. 4: Site 3 NA> MSA7 results

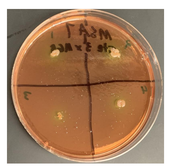

Fig. 5: Site 3 MG5>MSA7 results

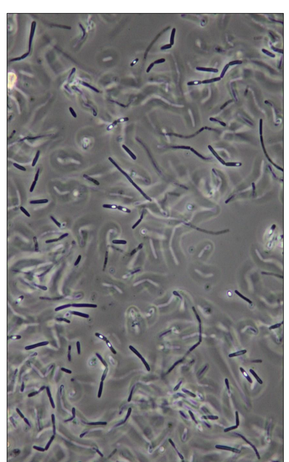

Fig. 6. NA-3-3: Ellipsoidal spores, C > T, grew on 7% agar, failed to ferment mannitol, Group 1A designation.

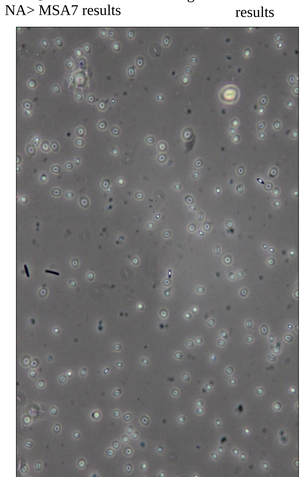

Fig. 7. MG5-3-1: Ellipsoidal spores, C > T, grew on 7% agar, fermented mannitol, Group 1B designation.

**Table 1. Results from growth and microscopic observations**

| Site | Isolation medium (NA *or* MG5) | Isolate designation | Growth on 7% NaCl (*+ or -*) | Ability to ferment mannitol (*+ or -*) | Spore shape (ellipsoidal *or* cylindrical *or* spherical) | Spore position (central to terminal *or* terminal to subterminal) | Sporangium (Not definitely swollen *or* Swollen) | Gordon Group (1A *or* 1B *or*2 *or* 3) |
|---|---|---|---|---|---|---|---|---|
| 1 | NA | NA-1-1 | + | + | Ellipsoidal | C > T | Not Swollen | 1B |
| | | NA-1-2 | + | + | Ellipsoidal | C > T | Not Swollen | 1B |
| | | NA-1-3 | + | + | Ellipsoidal | C > T | Not Swollen | 1B |
| | MG5 | MG5-1-1 | + | + | Ellipsoidal | C > T | Not Swollen | 1B |
| | | MG5-1-2 | + | + | Ellipsoidal | C > T | Not Swollen | 1B |
| | | MG5-1-3 | + | + | Ellipsoidal | C > T | Not Swollen | 1B |
| | | MG5-1-4 | + | + | Ellipsoidal | C > T | Not Swollen | 1B |
| | | MG5-1-5 | + | + | Ellipsoidal | C > T | Not Swollen | 1B |
| 2 | NA | NA-2-1 | - | - | Ellipsoidal | C > T | Not Swollen | 1A |
| | | NA-2-2 | - | - | Ellipsoidal | C > T | Not Swollen | 1A |
| | | NA-2-4 | - | - | Ellipsoidal | C > T | Not Swollen | 1A |
| | | NA-2-6 | - | - | Ellipsoidal | C > T | Not Swollen | 1A |
| | MG5 | MG5-2-1 | + | + | Ellipsoidal | C > T | Not Swollen | 1B |
| | | MG5-2-2 | + | + | Ellipsoidal | C > T | Not Swollen | 1B |
| | | MG5-2-3 | + | + | Ellipsoidal | C > T | Not Swollen | 1B |
| 3 | NA | NA-3-1 | - | - | Ellipsoidal | C > T | Not Swollen | 1A |
| | | NA-3-2 | + | + | Ellipsoidal | C > T | Not Swollen | 1B |
| | | NA-3-3 | - | - | Ellipsoidal | C > T | Not Swollen | 1A |
| | MG5 | MG5-3-1 | + | + | Ellipsoidal | C > T | Not Swollen | 1B |
| | | MG5-3-2 | + | + | Ellipsoidal | C > T | Not Swollen | 1B |
| 4 | NA | NA-4-1 | + | + | Ellipsoidal | C > T | Not Swollen | 1B |
| | | NA-4-2 | + | + | Ellipsoidal | C > T | Not Swollen | 1B |
| | MG5 | MG5-4-1 | + | + | Ellipsoidal | C > T | Not Swollen | 1B |
| | | MG5-4-2 | + | + | Ellipsoidal | C > T | Not Swollen | 1B |
| 5 | NA | NA-5-1 | + | + | Ellipsoidal | C > T | Not Swollen | 1B |
| | | NA-5-2 | + | + | Ellipsoidal | C > T | Not Swollen | 1B |
| | MG5 | MG5-5-1 | + | + | Ellipsoidal | C > T | Not Swollen | 1B |
| | | MG5-5-2 | + | + | Ellipsoidal | C > T | Not Swollen | 1B |

## Conclusions

The key factors in determining whether the results would indicate Group 1A or 1B was a combination between the morphological characteristics of the spores, and whether or not the selected colony was capable of growing on, and fermenting MSA7. Colonies on the NA plates had mixed results, with some colonies capable of fermentation (NA-3-2), while others were not (NA-3-1) (Table 1). The observation that every result observed was the same except for the MSA7 growth and fermentation, showed that all colonies incapable of fermentation were Group 1A, and all colonies that were capable of fermentation were designated Group 1B. Each MG5 plate and their respective colonies demonstrated the ability to ferment and grow on MSA7 agar, and had non-swollen spores, an indication of Group 1B *Bacillus spp.* These results show that Group 1B *Bacillus spp.* can be selectively isolated on an ammonium based agar, providing implications for a more streamlined approach to the isolation of Group 1B *Bacillus* at industrial levels.

## Literature Cited

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