



Bacterial Production of Gellan Gum as a Do-It-Yourself Alternative to Agar

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INTRODUCTION

Cost can be a steep barrier to participation in science. As a result, most research takes place in universities, government, and industry. Recently, a do-it-yourself (DIY) biology community has emerged outside of this system (1). DIY scientists often conduct their experiments in non-traditional settings (e.g., kitchens, garages, maker spaces) and fund their own work. To make microbiology more affordable in this and other educational environments, we became interested in a DIY replacement for agar.

Agar is one of the most expensive and routinely utilized reagents in microbiology labs. It is used as a gelling agent in solid media for growing colonies. Agar is a mixture of polysaccharides that is processed from the cell walls of seaweed (red algae). These algae are currently harvested from the wild, which has contributed to fluctuations and shortages in the agar supply, most recently in 2015 (2). Microbes are already the source of many nutrients (e.g., yeast extract) and supplements (e.g., amino acids and antibiotics) used in common culture media. What if microbes could also cheaply produce a gelling agent?

They can! Gellan gum is a capsular exopolysaccharide (EPS) that is naturally synthesized by the bacterium *Sphingomonas paucimobilis* ATCC 31461 (3, 4). Gellan gum is widely used as a solidifying agent in the food industry, and it is sold as a chemically purified product (e.g., GELRITE) for use in microbial growth media (3). Purification of gellan gum normally involves cultivating *S. paucimobilis*, several precipitation steps to separate the polysaccharide from cells, and a final drying phase (5). Here, we present a streamlined procedure for affordably producing gellan gum plates that requires only DIY equipment and ingredients.

PROCEDURE

Prepare DIY media for gellan gum production

Culturing *S. paucimobilis* for gellan gum production is a two-step process in which a starter culture is propagated in a rich medium and then transferred to a minimal medium. The initial culture phase in rich medium allows cells to rapidly achieve high densities before they are used to inoculate the gellan production medium, where little further growth occurs (6). Gellan gum production is induced under nitrogen starvation in the presence of excess carbon, which serves as the substrate for EPS synthesis. Therefore, the gellan production minimal medium contains a high C:N ratio to favor maximal EPS accumulation (3).

When carrying out this procedure, you should wear appropriate personal protective equipment, such as safety goggles, gloves, and a lab coat, and use heat-resistant glassware (e.g., Pyrex). For the two-step culturing method, you will need to first make a DIY rich medium, General Kitchen Broth (GKB) (7), which substitutes for a conventional *S. paucimobilis* rich medium (e.g., YPG) used in the first step. To make GKB, mix the following ingredients in 100 mL of water: 0.5 g dried skim milk, 0.25 g marmite, and 0.1 g honey. For the second step, we developed a minimal medium recipe (DIY-GPM) by identifying DIY equivalents for components of standard gellan production medium (6). To make DIY-GPM, mix the following in 1 L of water: 0.03 g dried skim milk, 0.5 g marmite, 24.35 g honey, 1 g table salt, 1.2 g Epsom salt, 10 g trisodium phosphate, 3.75 mL clear ammonia (e.g., Austin's brand, ~2.5% w/v ammonium hydroxide), and 3.1 g citric acid. Ensure that the GKB and DIY-GPM components are well mixed and sterilize them, using an autoclave or pressure cooker (7).

Culture *S. paucimobilis* for gellan gum production

Once the media have cooled to room temperature, start a 100 mL culture of *Sphingomonas paucimobilis* ATCC 31461 in GKB. Allow this to grow to saturation by incubating for two days, with shaking and at 30°C if possible (Fig.

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Received: 17 November 2017, Accepted: 10 April 2018, Published: 29 June 2018.

1a). Next, add this entire culture to 1 L of sterile DIY-GPM and incubate under the same conditions until the culture becomes gelatinous and homogenous. We found that incubating for eight days at 30°C with shaking produced a sufficient yield of gellan gum. You should inspect these cultures every day. A culture that is ready for pouring plates should be highly viscous and adhere to the side of the flask when it is tilted (Fig. 1b).

Pour gellan gum plates

Next, add 0.4 g/L of Epsom salt to the *S. paucimobilis* culture. This provides magnesium to strengthen the gellan polymer matrix (3). Also, add any other nutrients needed by your microbe of interest (7). For example, we found that adding 10 g/L of marmite improved growth of *E. coli* and *S. cerevisiae*. Heat the entire culture in a microwave using a medium setting until it becomes fluid and homogenous, watching carefully to avoid overboiling. At this point, the mixture is sterile and can be poured into petri dishes to make ~40 standard plates. We observed normal colony growth rates and morphology on these DIY gellan gum plates (Fig. 1c).

Safety issues

S. paucimobilis ATCC 31461 is classified as a biosafety level 1 (BSL1) organism. Students and DIY scientists should follow the American Society for Microbiology's guidelines for teaching laboratories, which describe appropriate personal

protective equipment and sterile procedures for working safely with BSL1 microbes (8).

CONCLUSION

This protocol can be used to sustainably and affordably source a homegrown agar replacement. We estimate that the cost of the culture components needed to produce DIY gellan gum is roughly one tenth the cost of agar (\$0.58 versus \$5.60 per liter of medium). Cultures of *S. paucimobilis* become extremely gelatinous, which also makes it an interesting microbe for classroom demonstrations related to the material properties of extracellular polysaccharides. *S. paucimobilis* strains used for commercial production have mutations to make them unpigmented or to produce deacetylated variants of gellan gum with improved gelling properties (9, 10). It is possible that DIY researchers or students could also alter these properties or even engineer *S. paucimobilis* variants that incorporate metabolic pathways for manufacturing other expensive media components (e.g., cofactors or antibiotics) in the future.

ACKNOWLEDGMENTS

We thank the 2016 UT Austin iGEM team and Microbe Hackers Freshman Research Initiative stream for helpful discussions. We acknowledge support from the National Science Foundation (NSF) (CBET-1554179) and the NSF BEACON Center for the Study of Evolution in Action

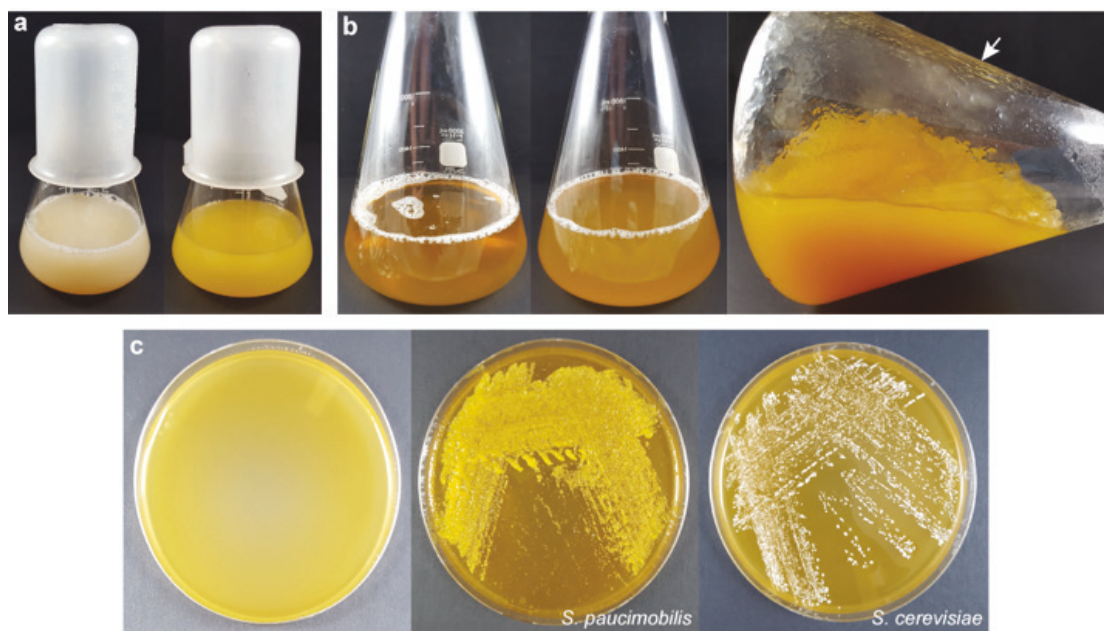


FIGURE 1. DIY gellan gum plates. (a) Initial phase of culturing *S. paucimobilis* in rich media. GKB medium before inoculation with *S. paucimobilis* (left) and after 2 days of growth (right). (b) Second stage of culturing for gellan gum production. DIY-GPM medium before inoculation (left), immediately after inoculation (middle), and after 8 days of growth (right). The arrow points to gellan gum adhering to the side of the flask. (c) DIY gellan gum plates supplemented with marmite support the growth of colonies of *S. paucimobilis* (middle), *S. cerevisiae* (right), and *E. coli* (not shown).

(DBI-0939454) for work on this protocol. JCM acknowledges a UT Austin TIDES fellowship. DL acknowledges an ASM Watkins Graduate Research Fellowship. The authors declare that there are no conflicts of interest.

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