Mechanical Integrity of Agars with Time

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Introduction

Agar is the most widely used gelling agent in plant tissue culture media for micropropagation and research. Its broad use is due to mainly two factors: (1) being chemically inert to both media components and biochemicals released from plant tissue and, (2) physical stability during the preparation of media (i.e. melts during autoclaving, and gels upon cooling). Currently the global shortage of agars from *Gelidium* sp., has forced many companies and researchers to look for alternative sources to mitigate rising costs. One alternative is gellan gum. While less expensive on a per liter basis, and more consistent in quality and purity, gellan gums have traditionally been linked with hyperhydricity of plant tissue (Ivanova *et al.* 2011, Martinez-Rojas *et al.* 2010). Hyperhydricity (originally known as vitrification) is a physiological disorder in which the tissue takes up more water and leads to a glassy appearance in cultured explants (Martinez-Rojas *et al*. 2010). This hyperhydric effect has also been seen in tissue on agar used below the recommended 0.6-0.8% concentration (Brand, 1993).

Researchers have typically battled this unwanted side-effect in tissue through higher gelling agent concentrations (e.g., higher apparent gelling strength) (George *et al.* 2008, Ivanova *et al.* 2011, Klimaszewska *et al.* 2000, Martinez-Rojas *et al.* 2010). We recently found evidence that older agars can lose gelling strength. A drop in gelling strength could presumably have a similar effect to using a lower concentration of agar and could impart hyperhydridicity to plant tissue. To this end, we evaluated the gel strengths of agars from both *Gelidium* sp. (Product No. A296) and *Gracilaria* sp. (Product No. A111) manufactured over the last 15 years. Lots from both products stored dry for 15 years lost approximately half of the original gel strength. To our knowledge, this work is the first to demonstrate that the gelling strength of agar may be reduced when stored for long periods of time. This work illustrates the necessity of plant tissue culture researchers to use newer lots of agar, particularly where hyperhydricity may be an issue.

Materials and Methods

In order to test this hypothesis, past lots of *Phyto*technology Laboratories' A111 and A296 stored at room temperature in sealed containers were collected (A111: 2000-2015, A296: 1999-2015). Solutions were prepared containing just deionized water (1-2 μS/cm) and agar at 0.6% at pH 5.6-5.8. Each lot was prepared in duplicate with a total of 8 replicates. Solutions were then autoclaved for 25 minutes at 121°C and 1.1 bar and allowed to cool for 30 minutes while mixing. They were then dispensed into 25 mm x 150 mm round-bottom glass test tubes at a volume of 25 mL per replicate. The tubes were then capped and sealed with parafilm, and allowed to gel for 16 to 20 hours.

The mechanical integrity of gelled samples were tested with a digital force gauge (Model 475040 Extech Instruments, Nashua, NH, USA). The peak force required to break the surface tension of the gel was measured in g/cm^2 . Gel strengths from manufacturers are typically reported in a tensile mode with values on the order 700-1200 g/cm^2, whereas the mode of interest for plant tissue culture researchers is compression (e.g., forcing tissue down into a gelled medium) with values on the order of 140-200 g/cm^2. Though it is a different mode of gel strength, it still provides a baseline gel strength throughout the dataset.

Results

Average peak break forces are shown in Figure 1. (*Gracilaria* sp) and Figure 2. (*Gelidium* sp.) plotted as a function of year released.

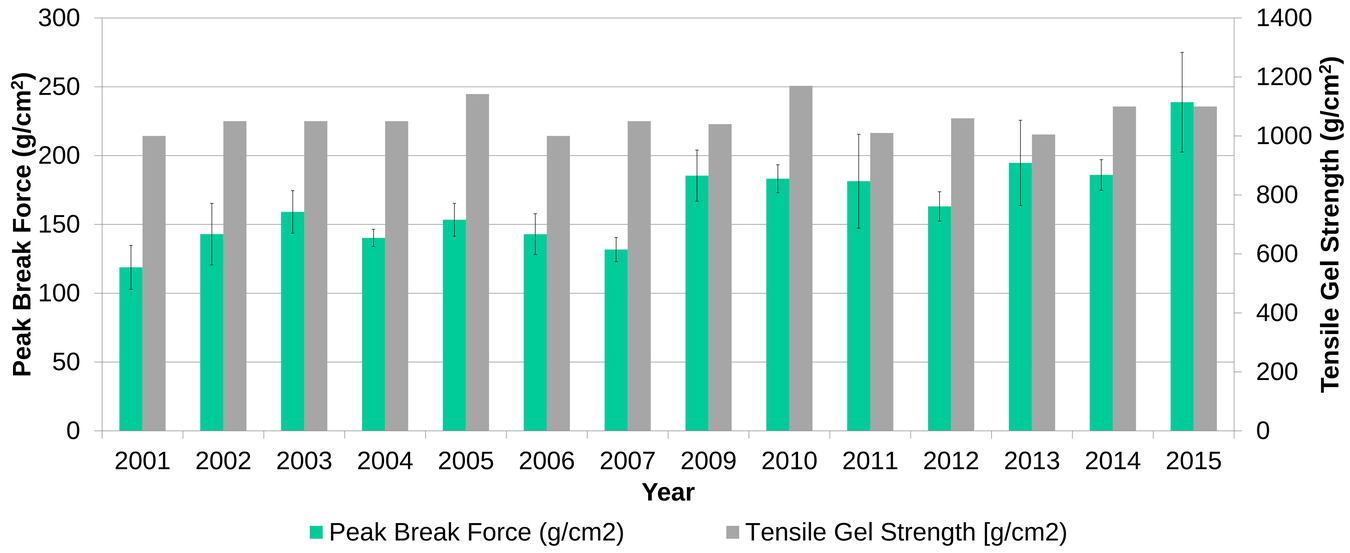

Figure 1. Comparison of average peak break force (g/cm^2) and tensile gel strength (g/cm^2) for 0.6% Agar from *Gracilaria* sp (Product No. A111). Error bars are +/- one standard deviation.

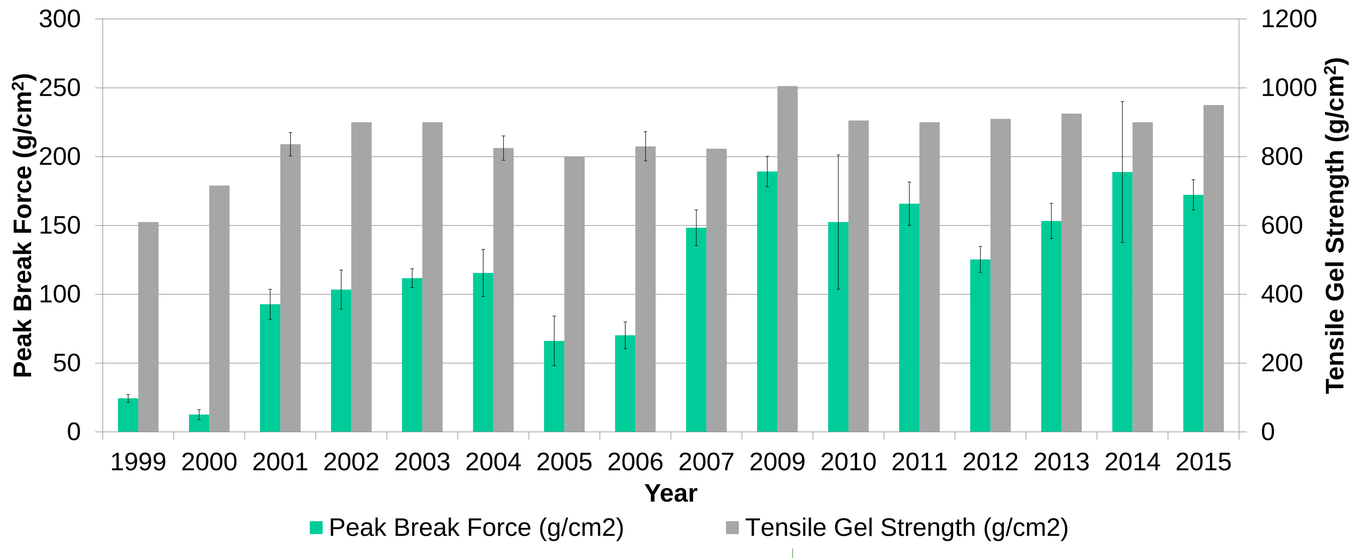

Figure 2. Comparison of average peak break force (g/cm^2) and tensile gel strength (g/cm^2) for 0.6% Agar from *Gelidium* sp (Product No. A296). Error bars are +/- one standard deviation.

Discussion

As shown in Figures 1 and 2, the average peak break force measured decreases as the age of the agar increases. Yet the tensile gel strengths reported from the manufacturers are generally consistently high. In Figure 2, the tensile gel strength quality increased significantly from 1999-2001. Figure 1 shows A111 prior to 2009 has peak break forces that are approximately half of what lots 2009 and later are. Figure 2 also shows that A296 prior to 2007 has approximately half the peak break force compared to lots 2007 and newer.

These figures also depict year-to-year variability in terms of the average peak break force. This variability stems from the fact that the origin of agar is biologic (seaweed) and susceptible to seasonal growth and climate fluctuations. Since agar is a commodity, we have established criteria to evaluate each lot we receive to ensure that the agars we offer are of the highest quality for plant tissue culture.

Though tensile gelling strengths from manufacturers are not equivalent to the compression mode of gelling strength; peak break force measurements do provide a method to test the relative gel strength in a mode more fitting for plant tissue culture.

Conclusions

- The results indicate that agar can lose it peak break force (e.g. gelling strength) with time.
- In order to reduce potential issues with hyperhydricity, it is important to avoid using agar after its shelf life date.
- It is also important to know how your agar supplier ensures that the material has retained the tensile gelling strength measured at the time of manufacture.

Future Work

- Expand testing to other gelling agents such as gellan gums, carrageenans.

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