Homemade Media for DIY Inoculant

Introduction

The ability for farmers to produce inoculants using microbes native to their soil has the potential to open exciting opportunities for a distributed on-farm network of microbial research. Our goal with this experiment was to see if basic ingredients, bought from the grocery store, and without specialized lab equipment, could serve as a substitute for the traditional media that we make in the lab to grow rhizobia.

The experiment was conducted over multiple time courses, with 4 solutions being tested against a standard control (VR) media. We used 3 reps for each solution as well as 3 controls that were not inoculated. The solutions were mixed on a hot plate with a magnetic stir stick, autoclaved in a pressure cooker (InstantPot™), and transferred into 50 mL falcon tubes. The specified reps were inoculated with a 10 microliter drop of pre-made rhizobial inoculant. The solutions were tested immediately after inoculating, then every 24 hours using a spectrophotometer at a wavelength of 600 nm (OD600). OD600 is traditionally used as a proxy for microbially growth with higher values indicating more growth. Solutions were kept in a shaker at room temperature.

Results

Below are the solutions for week 1, with the VR media (Control) used as the 5th solution.

Solution 1 (S1) (250mL)

Starting PH : 5.27

0.3 g NaCl
0.4 g bakers yeast
0.2 epsom salt
1 tbsp molasses
Distilled H2O to 250 mL

Solution 2 (S2)

Starting PH : 4.95

0.3 g NaCl
0.4 g bakers yeast
0.2 epsom salt
2 g granulated sugar
Pedialyte to 250 mL

Solution 3 (S3)

Starting PH : 2.76

0.3 g NaCl
0.4 g bakers yeast
0.2 epsom salt
3 g granulated sugar
Propel (Gatorade) to 250 mL

Solution 4 (S4)

Starting PH :
6.7

0.3 g NaCl
0.4 g bakers yeast
0.2 epsom salt
3 g granulated sugar
coconut milk to 250 mL

Ending PH:

Solution 5 (VR) - Control

Starting PH :
5.23

0.025 NaCl
0.05 MgSO4
0.1 yeast extract
0.1625 K2HPO4
2.5 g mannitol
H2O to 250mL

Table 1. Absorbance time series of DIY Trial 1.

OD 600	S1	S1 control	S2	S2 control	S3	S3 control	S4	S4 control	VR	VR control	
Rep 1	0.371	0.376	0.109	0.09	0.137	0.142	2.053	2.111	0.041	0.04	Day 0
Rep 2	0.389	0.391	0.101	0.098	0.143	0.142	2.041	2.12	0.04	0.04	1/17/23 @ 4pm
Rep 3	0.385	0.362	0.106	0.101	0.15	0.147	1.897	2.077	0.041	NA	
Rep 1	0.178	0.176	0.043	0.046	0.053	0.052	2.01	2.031	0.039	0.038	Day 1
Rep 2	0.187	0.186	0.046	0.044	0.05	0.051	1.984	2.074	0.039	0.039	1/18/23 @ 12 pm
Rep 3	0.187	0.196	0.042	0.045	0.052	0.052	2.069	2.092	0.039	0.039	
Rep 1	0.162	0.166	0.038	0.041	0.087	0.09	2.06	2.039	0.039	0.039	Day 2
Rep 2	0.169	0.167	0.04	0.06	0.088	0.045	2.075	2.055	0.044	0.039	1/19/23 @ 11:45 am
Rep 3	0.163	0.165	0.073	0.037	0.045	0.045	2.067	2.04	0.039	0.039	
Rep 1	0.476	0.453	0.142	0.117	0.148	0.157	2.16	2.077	0.118	0.039	Day 3
Rep 2	0.488	0.453	0.115	0.113	0.158	0.157	2.122	2.091	0.041	0.044	1/20/23 @ ??
Rep 3	0.535	0.519	0.119	0.106	0.154	0.189	2.146	2.086	0.126	0.064	

After the first run of solutions, several changes were made to the solutions. Smaller quantities of the base ingredients were used for the tested solutions, 20 microliters of inoculant was utilized rather than the original 10, baking soda was used to raise the pH of the solutions, and some tweaks were made to try and better match the VR media.

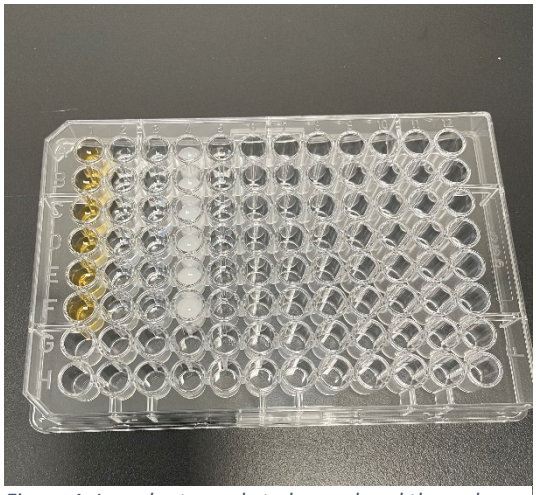

Figure 1. Inoculants ready to be analyzed through spectometry

Below are the solutions used for the second run of the experiment, with the fifth solution again being the original VR media.

Solution 1 (S1) (250mL)

0.025 g NaCl
0.1 g bakers yeast
0.05 g epsom salt
0.1 g baking soda
1 tbsp molasses
Distilled H2O to 250 mL

Starting PH: 5.68
PH after baking soda: 6.92

Solution 2 (S2)

0.025 g NaCl
0.1 g bakers yeast
0.05 g epsom salt
0.5 g baking soda
2 g granulated sugar
Pedialyte to 250 mL

Starting PH: 5.44
PH after baking soda: 6.56

Solution 3 (S3)

0.025 g NaCl
0.1 g bakers yeast
0.05 g epsom salt
1.6 g baking soda
2 g granulated sugar
gatorade to 250 mL

Starting PH: 3.05
PH after baking soda: 6.55

Solution 4 (S4)

0.025 g NaCl
0.1 g bakers yeast
0.05 g epsom salt
3 g granulated sugar
coconut milk to 250 mL

Starting PH: 7.71

Solution 5 (VR) - Control

0.025 NaCl
0.05 MgSO4
0.1 yeast extract
0.1625 K2HPO4
2.5 g mannitol
H2O to 250mL

Starting PH: 5.47

Table 2. Absorbance time series of DIY Trial 2.

OD 600	S1	S1 control	S2	S2 control	S3	S3 control	S4	S4 control	VR	VR control	
Rep 1	0.275	0.218	0.088	0.099	0.234	0.26	2.059	2.051	0.039	0.038	Day 0
Rep 2	0.266	0.228	0.082	0.099	0.23	0.239	2.085	2.046	0.039	0.039	1/23/23 @ 4:10 pm
Rep 3	0.294	0.214	0.084	0.106	0.246	0.248	2.067	2.041	0.04	0.037	
Rep 1	0.261	0.183	0.046	0.046	0.308	0.197	2.079	2.18	0.037	0.037	Day 1
Rep 2	0.273	0.184	0.048	0.046	0.209	0.207	2.134	2.151	0.039	0.038	1/24/23 @ 4 pm
Rep 3	0.213	0.192	0.047	0.048	0.206	0.198	2.116	2.168	0.038	0.038	
Rep 1	0.157	0.15	0.047	0.054	0.16	0.153	1.985	2.013	0.091	0.044	Day 2
Rep 2	0.161	0.158	0.046	0.047	0.163	0.163	1.989	2.022	0.097	0.039	1/25/23 @ 4 pm
Rep 3	0.156	0.157	0.046	0.048	0.161	0.162	2.007	2.009	0.093	0.039	
Rep 1	0.169	0.163	0.043	0.047	0.175	0.168	1.968	2.069	0.075	0.039	Day 3
Rep 2	0.165	0.163	0.043	0.044	0.184	0.172	2.075	2.043	0.088	0.04	1/26/23 @ 4 pm
Rep 3	0.173	0.169	0.042	0.052	0.165	0.16	2.051	2.044	0.078	0.04	

After the testing, the solutions were then plated using VR media mixed with agar to solidify the mix. The rhizobia were streaked out and left to grow to evaluate the presence and health of the bacteria.

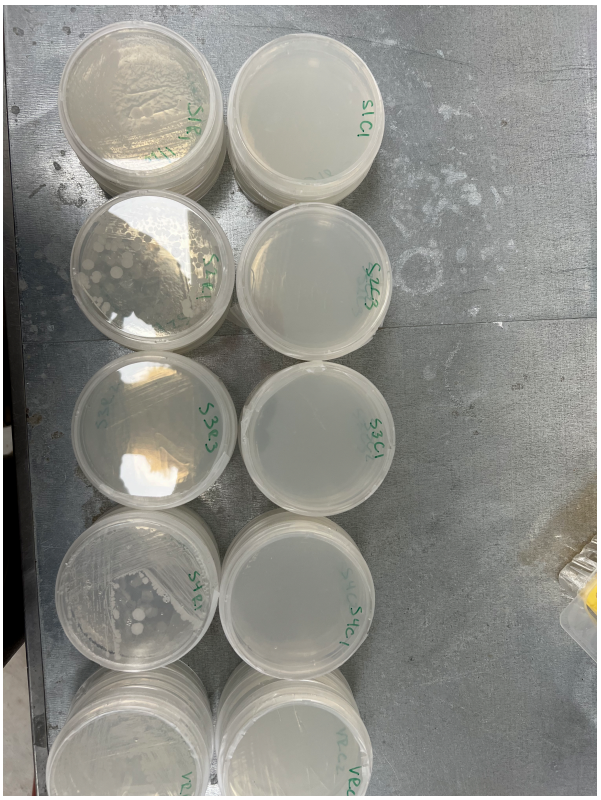

Figure 2. Results of DIY trial 1. All inoculants were spread on agar plates to observe rhizobial growth. All DIY broths were successful in growing rhizobia except for S3 seen in the middle of the image.

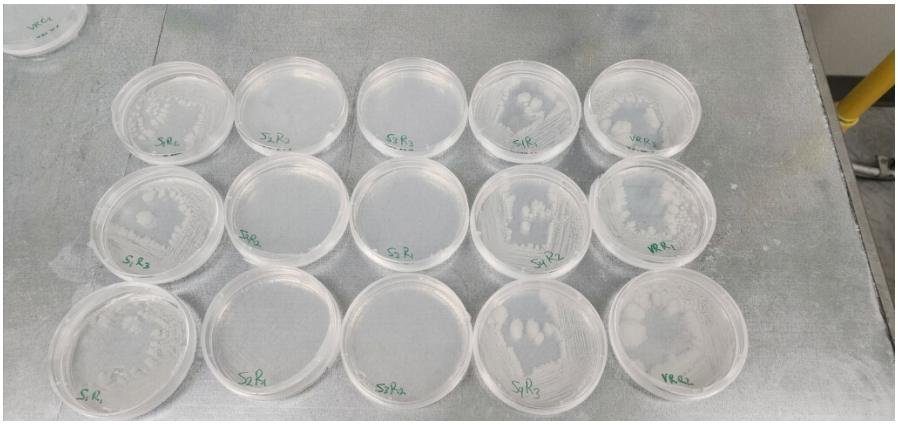

Figure 3. Results of DIY trial 2. All inoculants were spread on agar plates to observe rhizobial growth. The primary change in trial 2 was to raise the pH to a more neutral level. The results were the same as the previous trial, hence a pH adjustment is not necessary.

Conclusions

Figure 2 and Figure 3 demonstrate that rhizobia can effectively be grown on various media formulations. Solutions 1,3 and 4 were all successful media. S2, which include a Gatorade product as the primary solvent had a very low pH, which may have inhibited rhizobial growth. Even after the pH was increased to more neutral, we did not witness any rhizobial growth. Hence, this formulation is not recommended. On the other hand, Pedialyte did allow some growth but overall neither solution was satisfactory.

Overall, the most successful DIY solutions were S1 and S4. S1 is the simplest solution containing salt, Epsom salt, molasses, baker's yeast and bottled water. S4 was a simple mix of salt, sugar, Epsom salt, baker's yeast and coconut milk. It is unclear if other milk-based substitutes would be as effective. The pH of this solution is slightly more basic and opaque. As with S1, absorbance measurements are not a useful measure of bacterial growth and plating is the only means to determine growth.

With proper sterilization techniques and some readily available equipment (InstantPot, scale, glass or plastic incubation containers), this experiment demonstrates that it is technically feasible to culture rhizobia at home. Further tests are required to demonstrate proper inoculation and nodule formation through the use of these solutions.

Acknowledgements:

This material is based upon work that is supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, under agreement number 2018-38640-28416 through the North Central Region SARE program under project number LNC18-405. USDA is an equal opportunity employer and service provider. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.