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Potato Tubers as a Culture Medium For Phytopathogenic Bacteria and Fungi

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POTATO TUBERS AS A CULTURE MEDIUM FOR PHYTOPATHOGENIC BACTERIA AND FUNGI

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Tubers of the Irish potato were early recognized as a suitable medium for artificial culture of various organisms. Bacteriologists were first to use slices of tubers for culturing bacteria. Similarly, De Bary, the father of mycology, and Brefeld, the father of myceticulture, used both cooked and raw tubers as a substrate for culturing fungi. Later, Hallier observed that some fungi grew better on a medium containing both starch and sugar than on one containing starch alone. So, after his observations, the abundant starch content of the potato tuber was augmented by different sugars whenever occasion demanded it.

At the present time, potato tubers are probably more widely used in myceticulture, as a basic nutrient for making agar, than any other single plant material. Furthermore, potato agar is doubly useful since it is a good medium for culturing both bacteria and fungi thus serving a dual purpose and lightening the burdens of the investigator. Potato agar is clear, has a suitable acidity (pH 6 to 6.8), retains moisture fairly well and, with certain modifications, meets the major percentage of pathologists' needs.

Myceticulturists have employed numerous formulae for making potato agar, but these formulae have not been assembled so as to be directly available to investigators. Furthermore, these formulae have not been arranged for convenient citation in literature. So an attempt has been made to assemble the principal formulae for preparing potato agars, give references to literature and present detailed directions for preparing one formula, potato dextrose agar. This agar is especially useful to teachers of general science, botanists, myceticulturists, bacteriologists and investigators.

Formula 1. Potato dextrose agar:

Solution A. Potato decoction.	
1. Water, distilled	500 cc.
2. Potato tubers, peeled	200 gms.
Solution B. Water agar.	
3. Water distilled	500 cc.
4. Agar agar, powdered	17 gms.

Solution C. Potato dextrose agar.

5. Solution A mixed with B	1000 cc.
6. Dextrose, powdered	20 gms.

1. Wash, peel, and thinly slice 200 grams from a good-sized Irish potato. Do not expose peeled or sliced potatoes to the air any length of time, but submerge them at once in the 500 cc. of water to prevent discoloration.
2. Cook solution A in a steamer for 1 hour and in the same steamer melt together the ingredients in solution B which has been prepared, as stated above, in a separate receptacle.
3. Filter the potato decoction through washed muslin and restore to 500 cc. by adding more water, if necessary, to the cooked potato and filtering it into the under-volume decoction. If especially clear agar is desired, filter through filter paper.
4. When the agar agar in solution B is melted, add solution A, and return the mixture, potato agar, to the steamer after adding the dextrose as in solution C. Mix well by pouring several times from one jar into the other.
5. Prepare a filter and filter the agar as directed below:
   a. To a large funnel, fasten a rubber tube, pinchcock and glass tube outlet. Mount this apparatus on a ringstand for permanent use.
   b. Place white, washed excelsior so as to cover the inner surface of the funnel; on the excelsior, place a piece of clean washed muslin and on this a layer of clean cotton 8 or 10 inches square. It is generally best to split the cotton and place it in warm water, squeeze and work with the fingers until it is wet through before spreading it over the muslin; cover the cotton with a second layer of muslin.
   c. Heat this filter by passing hot water through it and allow it to drain before using. Heating prevents the agar's hardening quickly.
   d. Set the pinckcock and start the filter. Draw off the first 200 cc. of agar filtered, and return it to the filter. Keep the unfiltered agar as hot as possible. When clogged, the filter can be raised from the funnel by grasping the muslin; the upper pieces of muslin can be lifted with the excessive sediment, washed, and returned; also, partly congealed agar can be wrung through the filter.
   e. Clean the filter immediately after using; washing and drying the cloths and running hot water through the tubes.
6. Place about 10 cc. of agar in each of 50 test tubes.
7. Place the remaining agar in 250 cc. or 500 cc. flasks, filling but two-thirds of the volume.
8. Plug the test tubes and flasks with cotton, at once.
9. Sterilize (autoclave) 20 minutes at 15 lbs. pressure (120° C.); or better, steam 20 minutes the first day, 10 the second and 5 the third, reckoning from the time the steamer is hot.
10. After sterilizing, remove the wire basket containing the sterilized tubes of agar and set it at a slant so the agar in the tubes does not come nearer than one inch from the cotton plug. Allow the agar to harden in this position making agar slants.
11. Consulting references: Carter, Fritz, Hopkins, Thom, Jackson.

Formula 2. Potato dextrose agar:

This is prepared as described for Formula 1, only the potato decoction is filtered through filter paper or centrifuged before adding to B.

Formula 3. Potato dextrose agar:

This is prepared as described for Formula 1, only B and C are heated together. This may bring about the caramelizing of the dextrose sugar which is undesirable, but this method is often used.

Formula 4. Potato dextrose agar, double strength:

Prepared as for Formula 1, only 400 grams of potatoes are used.

Formula 5. Potato dextrose agar, filtered decoction:

Prepared as for Formula 1, only the potato decoction is filtered through filter paper and added to solution B without further filtering.

Formula 6. Potato dextrose agar, increased dextrose:

Jackson, Fusarium.

About the same as for Formula 1, only add 50 cc. of dextrose.

Formula 7. Potato dextrose agar, Fusaria:

Solution A.

1. Sliced potatoes, Irish	400 gms.
2. Distilled water	1000 gms.

3. Steam 30 minutes at 15 lbs. pressure, then strain.

Solution B.

4. Soak the agar agar in the water overnight.

5. Agar agar	25 gms.
6. Distilled water	1000 cc.

7. Melt by autoclaving for 20 minutes at 15 lbs. pressure.

8. When melted, remove and add: dextrose 25 gms. and solution A to B.

9. Place in the desired containers and sterilize at 15 lbs. pressure for 10 minutes after the autoclave has been heated.

Formula 8. Potato dextrose agar, "46":

Fusaria, etc. Appel and Wollenweber.

1. Potato juice	1000 cc.
2. Cane sugar	100 gms.
3. Agar agar	15 gms.

4. Add 10 gms. of citric acid after sterilizing.

Formula 9. Potato dextrose agar, for mushrooms, Lambert:

1. Water, distilled	2000 cc.
2. Potato, sliced	400 gms.
3. Dextrose	40
4. Agar agar, flour	30

5. The destrose was added as in Formula 1.

Formula 10. Potato dextrose agar, Hopkins:

1. Water, tap	1000 cc.
2. Pared potatoes	200 gms.
3. Agar agar	20
4. Dextrose	10

Formula 11. Hard potato dextrose agar; for sclerotia, Stout:

1. Water	1000 cc.
2. Potatoes, sliced	200 gms.

3. Cook one hour; drain; add:

4. Glucose	20

5. Agar agar 30
6. Cook one hour, then filter.

Formula 12. Potato dextrose agar, double concentration. Togashi; Webb and Fellows:

Solution A. Potato dextrose solution.

1. Potatoes, peeled 600 gms.
2. Dextrose 60
3. Water, redistilled 1000 cc.

Solution B. Glucose-peptone solution.

4. Glucose 20 gms.
5. Peptone 10
6. Monopotassium phosph. 0.25 gms.
7. Magnesium sulphate 0.25
8. Water, redistilled 1000 cc.

Formula 13. Potato dextrose agar. Sclerotium, Rosen:

1. Water, dist. 1000 cc.
2. Potatoes, peeled 500 gms.
3. Dextrose 20
4. Agar agar 20
5. Boil the potatoes until ready to fall apart; filter the fluid several times through closely woven cloth.

Formula 14. Potato agar, Mohedra:

1. Potatoes, peeled 200 gms.
2. Water, distilled 1000 cc.
3. Agar agar 15 gms.
4. Autoclave 30 minutes at 15 lbs. pressure.
5. Strain through muslin and discard the residue.

Formula 15. Potato agar, Fred:

Solution A. Potato decoction.

1. Water top 1000 cc.
2. Potatoes, peeeled and sliced 20 gms.
3. Cook 1 hour in the steamer, decant, restore the decoction to 1000 cc. then add:

Solution B. Agar solution.

4. Agar agar 30 gms.
5. Glucose 20
6. Heat in the steamer until the agar agar is in solution; filter, then follow the usual procedure as in Formula 1.

Formula 16. Potato mush agar, Mohedra:

1. Potatoes, peeled 400 gms.
2. Agar agar 15
3. Water, distilled, to make 1000 cc.
4. Autoclave, 30 min. at 15 lbs.
5. Force all through muslin then tube and follow the usual procedure.

Formula 17. Potato mush agar, Brown, 1929:

1. Potatoes, peeled, boiled to a mush in water 200 gms.
2. Squeeze through muslin
3. Make up to 1000 cc. (Distilled water)

Formula 18. Potato mush agar, Brown:

Solution A. Potato mush.

1. Potatoes, peeeled 200 gms.
2. Cover with water and boil to a mush.
3. Cool, press through muslin or a fine sieve.

Solution B. The agar.

4. Agar agar 10 gms.
5. Water 1000 cc.
6. Stir until the agar is melted, then add solution A to B and follow the usual procedure.
7. Note: he also employed 400 gms. of potato to the litre as a medium. 1926, p. 382.

Formula 19. Potato nutrient agar, Cook and Taubenhaus:

1. Pare, steam and mash a quantity of potatoes.
2. Mashed potatoes 250 gms.
3. Water, tap 1000 cc.
4. Filter through cotton; the drained liquid is potato decoction.
5. Potato decoction 500 cc. Add:
6. Agar agar 15 gms.
7. A nutrient solution prepared as follows: 500 gms.
   a. Potassium phosphate (diabasic) 1 gm.
   b. Magnesium sulfate 1
   c. Sodium chloride 1
   d. Ammonium sulfate 2
   e. Calcium carbonate 2
   f. Water, tap 1000 cc.
8. For fungi as Penicillium, Aspergillus, Cephalothecium.

Formula 20. Potato-prune agar; mucors and other weak parasites:

A. Potato decoction, Formula 1 500 cc
B. Prune decoction
   a. Prunes, dried 13 Number
   b. Water 250 cc.
   c. Steam 30 min.
C. Melted agar
   a. Water 250 cc.
   b. Agar agar 18 gms.
   c. Steam until melted
D. Mix A, B, and C.

Formula 21. Potato starch medium:

1. Potato starch, dry 3 gms.
2. Cover with water 10 cc.
3. Steam 30 minutes

Formula 22. Potato nutrient substitute, Brown, p. 387:

1. Water 1 L.
2. Agar agar 15 gms.
3. Peptone 1.8
4. Asparagin 1.8
5. Glucose 2.0
6. Potato starch 40 gms.
7. K Cl 0.15
8. $MgSO_4$, 7 H_2O 0.75
9. K_3PO_4 1.35
10. $FeCl_3$, 1 drop per litre. Trace.

Formula 23. Potato synthetic agar, Brown, p. 387:

1. Glucose	2 gms.
2. Asparagin	2 gms.
3. K_3PO_4	1.25
4. $MgSO_4$, 7 H_2O	0.75
5. Agar agar	15
6. Water	1 litre

Formula 24. Potato malt agar, Verplancke:

1. Malt extract	2 gms.
2. Potato dextrose agar	1 litre
a. Potatoes	390 gms.
b. Water	1 L.
c. Dextrose	10 gms.
d. Agar agar	20

3. Note: page 405, he added 10 gms. of potato starch to the above for certain color reaction of Fusaria.

Formula 25. Glycerined potato plugs, Sartory, p. 10:

1. Potato plugs submerged for 4-5 hours in
2. Glycerine 6-15 per cent, then
3. Place in tubes and sterilize.

Formula 26. Alkalined potato plugs, Sartory, p. 11:

1. Potato plugs submerged overnight in
2. Three per cent caustic soda solution
3. Then sterilized.

Formula 27. Acidulated potato plugs, Sartory, p. 11:

1. Potato plugs submerged overnight in
2. Lactic acid, 3 per cent, then
3. Sterilized.
4. Note: either citric or hydrochloric acid may be used.

Formula 28. Raw, sterile potato plugs:

1. In the bottom of each 12 test tubes, place a small wad of saturated cotton, plug and steam sterilize.
2. Use a cork borer with a diameter smaller than that of a test tube; also, make a wooden plunger for it. To sterilize, dip the cork borer in alcohol and ignite; dip the plunger in formaldehyde solution and drain dry.
3. Disinfect the surface of a large potato tuber and place it on a sterile piece of glass.
4. Dip the blade of a long bladed knife in alcohol, ignite, and cut a slice from the potato, about 4 cm. thick.
5. Under aseptic conditions, cut plugs with the sterilized cork borer from this slice of tuber and with the plunger, shove one in each test tube, plug.
6. Incubate for 3 days, before inoculating; the sterility will then manifest itself.
7. Some prefer to cut plugs from a whole tuber, then cut a bevel on the upper side where the inoculum is placed.
8. If the work is done with care in an atmosphere of formaldehyde fumes, better success will be obtained.

Formula 29. Cooked potato plugs, Entomophthora, Sawyer:

1. Prepare as in Formula 28, only sterilize for 15 minutes at 10 lbs. pressure.
2. However, no care need be taken to keep the plugs sterile. Some prefer to make rectangular sections of the tuber.

Formula 30. Cooked sliced potatoes:

Slices of potato tubers were placed in Petri dishes and steamed.

Formula 31. Raw sterile sliced potatoes, Phytophthora, Knight; also, bacteria:

Slice the potatoes as described in Formula 28, then under aseptic conditions, place the slices in Petri dishes, Allow them to incubate for 3 days for testing the sterility.

Formula 32. Potato plugs in Petri dishes:

Follow the directions in Formula 28, only store the plugs in sterile Petri dishes.

Formula 33. Sliced potatoes with nutrient solution:

Boulanger sowed spores of truffles on slices of potatoes in Petri dishes with nutrient solutions.

Formula 34. Potato juice gelatin, Clinton:

1. Water	500 cc.
2. Potato	50 gms.
3. Gelatin	50

Formula 35. Potato, orange glucose agar, Malroth; Penicillium, Phomopsis; growth and sporulation:

1. Potato glucose agar	1000 cc.
2. Orange extract	50 cc.

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