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A Potential Substitute for Agar in Routine Cultural Work on Fungi and Bacteria*

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Agar was first used by Fanny Eilshemius Hesse in 1881 (Hitchens and Leikind, 1939) as a solidifying agent in the preparation of bacteriological culture media. Subsequently, agar has been used in increasing quantities in microbiological laboratories all over the world. Agar is a gelatinous complex polysaccharide obtained from such marine algae as *Gelidiella* sp. and *Gracilaria* sp. It is purified for use in precise microbiological and biochemical studies. All grades of agar are used in laboratories; the specific need determining the grade. The cost of agar has increased in recent years and the market price in India currently ranges from Rs. 350 to Rs. 17,000 per kg depending on the grade's purity, and on whether the agar is locally produced or imported. We, therefore, decided to look for a cheaper, more easily available material that could be used as a substitute for agar in routine laboratory plant pathological studies on fungi and bacteria.

After testing many different materials, we selected granulated tapioca or tapioca pearls (commonly called *sabudana* or sago in India) as a possible substitute. Baked products obtained from the cassava [*Manihot esculenta* (Crantz)] starch are commercially known as tapioca. We faced several problems, particularly in the variation in quality and suitability among samples of granulated tapioca available in the markets; some grades being better than others in terms of their stability, and the paste-like consistency of the melted tapioca after it had been autoclaved. In India, granulated tapioca is mostly produced in small factories where strict quality control is not exercised. Finally, we obtained a small-grained tapioca called "*Motidana*, no. 2 quality" (100-grain weight 0.2 g) from Messrs Sri Lakshmi Sago Manufacturing Company, Vellapalem 533 434, East Godavari District, Andhra Pradesh. This product had properties similar to those of agar, in that it formed a gelatinous base that could be autoclaved and used to prepare tube slants and culture plates. Like agar, the solidified tapioca gel could be stored, then re-melted by heating and used for plate cultures.

We then tried a dextrose-tapioca medium for growing fungi, but found it to be unsatisfactory. We assumed that this was probably because tapioca contains less than 1% protein. We therefore supplemented the medium with chickpea dhal (decorticated split cotyledons) flour (*besan* in Hindi) which is readily available in grocery shops all over India. This medium which we called chickpea-dextrose-tapioca (CDT) was found satisfactory; several fungi and bacteria could grow on it. The inclusion of chickpea flour in the medium led to the deposition of a thin layer of flour scum on the inside walls of the tubes after autoclaving, lending an unclean look to the tubes, but the medium looked fine when it was poured into petri dishes from flasks. Because tapioca contains starch, we were expecting the medium to be liquefied by some organisms, but none of

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those that we tried, including the active dried yeast (*Saccharomyces cerevisiae*), caused any visible softening of the medium. The only disadvantage we found in using tapioca, instead of agar, was that the medium was more opaque and white than such media as potato-dextrose-agar (PDA), and this reduced the visibility of the initial growth of "white" fungi, such as some species of *Fusarium* and *Phytophthora*. However, this problem was overcome by adding a food coloring agent (brown, green or blue) to the medium, thus increasing the visual color contrast between the medium and fungal growth.

We describe below the step-wise procedures for the preparation of CDT using flasks and tubes :

Ingredients : Chickpea dhal flour 5 g; dextrose 20 g; granulated tapioca (*Motidana*, no. 2 quality) 150 g; distilled water 1000 ml.

i) **Using flasks**

1. Place 30 g granulated tapioca and 4 g dextrose in a 500 ml conical flask.
2. Add 1 g chickpea dhal flour to a 100 ml beaker, and 200 ml distilled water to a measuring cylinder.
3. Pour 20 ml of water from the cylinder into the beaker and suspend the flour by stirring with a spatula.
4. Add the flour suspension and the remaining 180 ml distilled water to the tapioca & dextrose in the flask (step 1).
5. Shake the flask for about 15 sec, plug with cotton wool, and autoclave at 121°C and 15 psi for 20 min.
6. Remove the flask from the autoclave as soon as the pressure returns to normal and the temperature to about 90°C.
7. Gently shake the flask containing the autoclaved CDT and pour about 15 ml medium into each sterile petri dish (10 cm dia) under aseptic conditions. The pH of CDT was 4.6.
8. The 200 ml medium from each flask should be sufficient to prepare 10-12 culture plates.

The medium should be kept hot while pouring it into petri dishes to avoid its gelling in the flask. To keep the medium hot, maintain the temperature in the autoclave at about 90°C. Remove a few flasks at a time from the autoclave and pour the medium they contain into petri dishes. The autoclaved medium can be stored for up to one month at room temperature (25-30°C). When required, the medium can be re-melted in an autoclave at 121°C and 15 psi for 10 min and poured into petri dishes. However, it is preferable to use freshly prepared medium. Petri dishes containing CDT can be stored in sealed containers in a refrigerator for at least 2 weeks without deterioration.

ii) **Using tubes**

1. Take 50 test tubes (18 mm dia x 150 mm length) in an aluminum basket.
2. Weigh 600 mg granulated tapioca (it is convenient to use a small container, e.g., the cap of a glass vial). Use the cap as an approximate measure to expedite addition of tapioca to tubes.
3. Add one measure (600 $\pm$ 80 mg) of tapioca to each test tube.
4. Take 1 g chickpea dhal flour and 4 g dextrose in a 500 ml beaker.
5. Add 200 ml distilled water and suspend the flour and dextrose.
6. Pour 4 ml of this suspension into each test tube, stirring the suspension frequently with a spatula.
7. Plug the tubes and autoclave at 121°C and 15 psi for 20 min.

8. Remove the basket of tubes from the autoclave as soon as the pressure returns to normal and the temperature to about 90°C.
9. To prepare solidified medium slants, place the tubes on a slope stand.
10. These slants can be stored for up to one month at room temperature (25-30°C), or for longer in refrigerator, without deterioration.

We describe below the results of some of the tests we carried out, particularly the comparison of tapioca-containing media with PDA which is the most commonly used medium for initial isolations of fungi and many bacteria.

(i) **Isolations from infected tissues** : The fungal pathogens listed below were successfully isolated using both CDT and PDA:

Host	Disease	Tissue	Fungus
Chickpea	Ascochyta blight	Seed	*Ascochyta rabiei*
Groundnut	Early leaf spot	Leaf	*Cercospora arachidicola*
Pearl millet	Leaf spot	Leaf	*Helminthosporium* sp.
Pigeonpea	Fusarium wilt	Stem	*Fusarium udum*

Bacterial isolates were also obtained on CDT and PDA from mung bean leaves infected with bacterial leaf spot.

ii) **Growth of various fungi and bacteria on CDT** : One-5 mm mycelial disc from a 4-day-old agar culture of the test fungus was placed in each petri dish containing CDT or PDA. Visual observations were recorded after 3-15 days. In general, CDT supported as good a growth as did PDA of *Alternaria alternata, Fusarium oxysporum* f. sp. *ciceri* (Foc), *Fusarium solani, Fusarium udum,* and *Phytophthora drechsleri* f. sp. *cajani* (Pdc). However, in young cultures the periphery of Foc, *F. udum,* and Pdc colonies was not easily visible on CDT because of the opacity of the medium. Growth of *Exserohilum turcicum* and *Ascochyta rabiei* was better on CDT than on PDA. Growth of Pdc and *A. rabiei* was slow on PDA after one week, but these fungi continued to grow profusely on CDT. Fewer pycnidia and conidia of *A. rabiei* were produced on CDT than on PDA. Excellent growths of *Sclerotium rolfsii* and *Rhizoctonia bataticola* were obtained on CDT and PDA. However, sclerotial formation by *S. rolfsii* was delayed and only a few, large sclerotia were produced on CDT.

Aspergillus flavus and *A. niger* also grew on CDT; but sporulated less profusely on CDT than on PDA. *Bradyrhizobium* strain IC 7029 of groundnut and three bacterial isolates from mung bean were multiplied on CDT and PDA. But *Bradyrhizobium* strain IC 3100 of pigeonpea did not multiply on either medium.

Tube cultures of five fungi on CDT were stored at 30°C. The fungi were viable for more than 3 months. CDT can be used for short-term preservation of fungal cultures.

iii) **Soil plating** : Fungi and bacteria were detected from field soil on CDT and PDA. However, the colonies were not easily visible on CDT. To check whether tapioca can be used instead of agar in preparing selective media, modified Czapek Dox media with agar or tapioca were used for selective isolation of *Fusarium* spp. (Singh and Chaube, 1970). *Fusarium* spp. were isolated from Alfisol and Vertisol samples on both media - with agar and with tapioca. Not unexpectedly, *A. flavus* and a few bacterial colonies were also observed on both the

media. *A. niger* was observed on the tapioca medium but not on the agar medium.

iv) **Inhibition zone technique for testing fungicides** : A spore suspension of *A. alternata* containing about 1 x 10^4 ml^{-1} conidia was poured into petri dishes containing CDT or PDA. The excess spore suspension was decanted, and three 5-mm filter paper discs dipped in 0.25% Dithane M 45® (mancozeb 75% WP) were arranged equidistantly on the surface of the medium in each petri dish. After 3 days, inhibition zones were observed around the filter paper discs. The inhibition zones, however, were smaller on CDT than on PDA plates, indicating the slower diffusion of fungicide through CDT.

In another test, seeded CDT was prepared by adding spore suspensions of *A. alternata, A. flavus,* and *A. niger* to the cooled molten medium. The seeded media were poured into petri dishes. Good growth of all the three fungi was observed indicating that spores were apparently viable in the molten media. The seeded tapioca-based media can be used to evaluate fungicides.

v) **Growth of fungi on potato-tapioca medium :** Because some fungi were not easily visible on CDT, we used potato-dextrose-tapioca (PDT) medium (200 g potatoes, 20 g dextrose, 150 g granulated tapioca, 1000 ml distilled water). This medium was prepared just as we normally prepare PDA, except that tapioca was substituted for agar. The pH of PDT was 4.9 whereas that of PDA is 5.5. PDT slants were prepared following the procedure already described for CDT. One measure of tapioca (600 mg) was placed in each test tube and 4 ml of potato-dextrose broth added.

Both *S. rolfsii* and *R. bataticola* grew excellently on PDT and PDA. Fewer sclerotia of *S. rolfsii* but more sclerotia of *R. bataticola* were produced on PDT than on PDA. There was profuse mycelial growths and greater production of conidia by *A. alternata* on PDT than on PDA. Growth of Pdc, *E. turcicum,* and *A. rabiei* was also better on PDT than on PDA. However, fewer pycnidia and conidia of *A. rabiei* were produced on PDT than on PDA. The growth of Foc, *F. solani,* and *F. udum* on PDT were similar to or slightly less than on PDA. The mycelia of *A. flavus* and *A. niger* grew well on PDT, but they sporulated less than on PDA. The visibility of fungal growth was much better on PDT than on CDT. The opacity of PDT could be further reduced by using only 130 g/l granulated tapioca.

vi) **Isolations from infected plant tissues on PDT** : We successfully isolated the following fungi on PDT and PDA:

Host	Disease	Tissue	Fungus
Chickpea	Root rot	Root	*Fusarium* sp
Groundnut	Early leaf spot	Leaf	*Cercospora arachidicola*
Pigeonpea	Fusarium wilt	Stem & root	*Fusarium udum* sp.
Sorghum	Anthracnose	Leaf	*Colletotrichum* sp

Some fungi, e.g. *Alternaria* sp. from infected seeds of chickpea and *Phoma* sp. from infected seeds of sorghum were also isolated on PDT and PDA.

We feel confident that tapioca can be substituted for agar in media and for routine culture work. Even if the prevailing price of the most inferior grade of agar is taken as Rs. 350 per kg and the market price of tapioca as Rs. 15 per kg, the amount saved per litre of medium would be Rs. 4.75. Most laboratories in India, however, buy agar that costs more than Rs. 1000 per kg.

The critical factor which requires the attention of granulated tapioca manufacturers is standardization of the grade that is most suitable for media preparation. Scientists who wish to try tapioca as a substitute for agar could ask for a "good" sample (150 g) from the authors. We can also provide them with a list of tapioca-producing factories so that they can make direct enquiries. It is possible to use the granulated tapioca available in the market (100-grain weight approx. 2.4 g); the medium obtained may not be ideal, but it will surely serve the purpose.

Cassava starch has been used instead of potatoes in agar culture media for fungi (Ramakrishnan *et al.*, 1975; Somathiti and Naranong, 1987; Awuah, 1989). We report for the first time the use of tapioca as a substitute for agar in microbiological media. We believe that the use of tapioca as a substitute for agar may find application in fields other than plant pathology, such as soil, medical, and veterinary microbiology, especially in India and other tropical countries where tapioca is readily available.

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