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Development and evaluation of a cost-effective alternative media for the culture of plant pathogens

Jayashree Bhattacharjee, S. G. Borkar and Debashre Bhattacharjee

Department of Plant Pathology and Agricultural Microbiology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri- 413 722 (Maharashtra)
e-mail: jayashreebhatt89@gmail.com

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Abstract

The study was conducted in search of a cost-effective alternative medium for the culture of both fungal and bacterial plant pathogens at Post Graduate Institute, M.P.K.V., Rahuri during 2012-13. Out of 3 solidifying agents evaluated at various combinations, 1 percent agar + 2 percent tapioca was found best as it formed solid transparent medium which is effective for bacterial streaking as well as fungal disc placement, therefore the quantity of agar, used in the routine medium reduced by 50 percent. Potato (200 g/L) as a source of carbohydrate and commercial sugar (2 percent) as a source of sucrose were effective in that medium for growth of both fungus and bacteria. The medium cost Rs. 52.22/L compared to Rs. 103.78/L in case of using potato dextrose agar (PDA) and Rs. 119.67/L in nutrient agar sucrose (NAS). Therefore, it was suggested that, tapioca-potato-sugar-agar (TPSA) could be a cost-effective alternative medium for working with both fungus and bacteria of pant origin.

Key words: Low-cost alternative media, solidifying gelling agents, tapioca, agar, plant pathogens

In the developing countries, microbiological research is hindered by the high cost and scarcity of effective culture media (Adesemoye and Adedire, 2005). Therefore, it is essential to develop and evaluate locally available alternative media in microbiological research to reduce the cost involvement. Agar is the main solidifying agent used in microbiological research. It is very expensive and not always affordable particularly in developing countries. Moreover, due to over exploitation of marine algae for agar production, many agar producing algal species are seriously under the threat of extinction (Jain *et al.*, 1997). Furthermore, agar has adverse effect on health (Debargh, 1983). Therefore, it is of utmost importance for possible replacement of agar with alternative media alternatives. Tapioca powder, obtained from roots of cassava (Manihot esculanta Crantz) is would be a potential substitute for agar in microbiological media. It is an acidic polysaccharide like agar and contains 2 percent protein, 2.1 percent lipid and 77 percent carbohydrate (Dabai and Muhammad, 2005). It is odourless, sticky, paste in clarity and less in impurities (Maliro and Lemeck, 2004); it forms gelatinous matrix which can be autoclaved, stored and thereafter melted by heating (Kasanadze, 2000). Potato, maize and rice could be alternative sources of carbohydrate whereas mung and pigeon pea for protein sources in microbiological media (Abbas *et al.*, 2007). Commercial sugar can be used in place of sucrose and glucose in microbiological media (Badoni and Chauhan, 2011). Keeping these points in priority, the present study was conducted on development and evaluation of alternative media which would be cost-effective for culturing both fungus and bacterial plant pathogens.

Materials and Methods

The study was conducted in the Department of Plant Pathology and Agricultural Microbiology, Post Graduate Institute, M.P.K.V., Rahuri during 2012-13. Tapioca, isubgol and agar were compared for their solidifying ability (Table 1 and 2); potato, maize and rice for carbohydrate sources and mung and pigeon pea for alternative protein sources (Table 3) in order to find out the best alternative.

Preparation of test medium

A medium was prepared using 20 g tapioca, 200 g potato, 20 g sugar and 10 g agar in 1000 ml of distilled water and name as Tapioca-potato-sugar-agar (TPSA). For the preparation, potato was first boiled, peeled and sieved through muslin cloth. Tapioca (2%), agar (1%) and sugar (2%) were added into that. Then, this mixture was boiled till homogenously mixed, sieved through muslin cloth and poured into the flasks, autoclaved and used for evaluation using selected plant pathogens after adjustment of pH to 7.0.

Assay of plant pathogens

Among the fungal pathogens, leaf-spot causing pathogens, namely *Colletotrichum gloeosporioides, Alternaria alternata* and *Helminthosporium bipolaris;* root pathogens,

Table 1. Effect of solidifying agents and its concentration on the solidification and consistency of the medium

Medium No.	Solidifying agent with concentration (percent)			Solidifying ability	Consistency and transparency of the medium
	Tapioca	Isubgol	Agar		
1. Tapioca	0	0	1	±	Transparent semi-solid
Isubgol	0	0	2	+	Solid transparent
2. Tapioca	1	0	1	±	Semi-solid transparent
Isubgol	2	0	1	+	Solid transparent
3. Tapioca	2	2	1	+	Solid non transparent
Isubgol	3	3	1	+	Solid non-transparent
Agar	2	1	1	+	Solid non-transparent
4. Tapioca	2	2	0	+	Semi-solid non-transparent
Isubgol	3	2	0	+	Semi-solid non-transparent
Agar	3	3	0	+	Semi-solid non-transparent
5. Tapioca	2	0	0	±	Semi-solid transparent
Isubgol	3	0	0	±	Semi-solid transparent
Agar	10	0	0	±	Semi-solid transparent

+ solidified medium; ± semisolid medium

Table 2. Performance of solidified gelling agent for bacterial streaking and fungal disc placement

Name of medium and composition (percent)	Performance/ sustainability for bacterial streaking and growth		Performance/Sustainability for fungal disc placement and growth.	
	Streaking of bacteria	Growth of bacteria	Placement of fungal disc in petriplates	Growth of fungal pathogen in petriplates
Agar (2)	Good for bacterial streaking	No proper bacterial growth	Good for placement	Meagre fungal growth
Agar (1) + Tapioca (2)	Good for bacterial streaking	Good bacterial growth	Good for placement	Good fungal growth
Agar (1) + Tapioca (2) + Isubgol (2)	Not good for bacterial streaking	No proper growth	Good for placement	Good fungal growth
Tapioca (2) + Isubgol (2)	Not good for bacterial streaking	No proper growth	Good for placement	Good fungal growth

namely *Sclerotium rolfsii*, *Fusarium oxysporum*, and *Rhizoctonia solani* and a bio-controlling agent, *Trichodermma viride* were tested. Bacterial pathogens namely *Xanthomonas axonopodis* pv. punicae infecting pomegranate and *Xanthomonas axonopodis* pv. citri infecting citrus were tested.

Analysis of data

Observations on mycelia growth (cm) of fungal leaf spot pathogens and root pathogens were recorded after 48 hours. Data were analysed by SPSS 11.2 and differences between the means were compared using Duncan's multiple range test after ANOVA at 5% level of significance.

Results and Discussion

In this study, a combination of 1% agar with 2% tapioca was found good as it formed a solid transparent medium (Table 1 and 2) which supports bacterial streaking as well as placement of fungal disc. It reduced 50 percent quantity of agar used in the routine medium. Potato (200 g/L) was most effective among the carbohydrate sources tried (Table 3), it induced good growth of bacterial and fungal pathogens on incubation at 30°C. The medium was found working without additions of protein sources. As protein sources are costly, they were not promoted further in order to reduce the cost involvement in the alternative media. Common sugar (2%) was effective as a source of sucrose and glucose. Present findings are in agreement with the earlier reports (Abbas *et al.*, 2007; Badoni and Chauhan, 2011) where also development and evaluation of cost effective alternative media were done for culture and maintenance of various microbes. The suitability of cereal derivatives in microbiological media had also been reported (Adesemoye and Adedire, 2005; Omemu, 2008)

The performance of TPSA on fungal pathogens compared to conventional potato-dextro seagar (PDA) is presented in Table 4 and 5. The medium supported excellent growth of *Alternaria* and *Sclerotium* with higher sporulation. There was no significant difference ($p \leq 0.05$) in the growth of mycelia (diameter) of leaf-spot pathogens as well as root pathogens obtained on TPSA and PDA at 48 h which indicated TPSA is equally as good as PDA.

Table 3. Efficacy of alternative carbohydrates and protein sources on preparation and performance of basal medium

Carbohydrate source in media	Protein source (2 %)	Sugar source (2 %)	Growth characteristics		
			Xanthomanas sp	*Fusarium*	*Alternaria*
Potato (250 g/L)	Pigeon pea	Sugar	Typical yellow growth	Good growth	Good growth with sporulation
Maize (2%)	Mung	Sugar	Typical yellow growth	Good growth	Good growth with sporulation
Maize (2 %)	Pigeon pea	Sugar	Albino growth	Scanty growth	Scanty growth
-	Mung	Sugar	Light yellow growth	Scanty growth	Scanty growth
-	Pigeon pea	Sugar	Albino growth	Scanty growth	Scanty growth
Potato (250 g/L)	Mung	-	Typical yellow growth	Very good growth	Good growth
Potato (250 g/L)	Pigeon pea	-	Typical yellow growth	Scanty growth	Scanty growth
Rice (2%)*	Mung	Sugar	Good growth	Good growth	Good growth
Rice (2%)*	Pigeon pea	Sugar	Bacterial growth spreading from streak	Fungal growth with contamination	Fungal growth with contamination
Potato	-	Sugar	Bacterial growth spreading from streak	Fungal growth with contamination	Fungal growth with contamination
			Typical yellow growth	Good growth	Good growth with sporulation

Basal media: (2% tapioca +1% agar) *non transparent granular media.

Table 4. Performance of Tapioca-Potato-Sugar-Agar (TPSA) vis-à-vis routine Potato-Dextrose-Agar (PDA) medium for growth of fungal plant pathogens.

Sl. No.	Medium	Mycelia growth (cm) of fungal leaf-spot pathogens (at 48 h)			Mycelia growth (cm) of root pathogens (at 48 h)		
		Colletotrichum	*Alternaria*	*Helminthosporium*	*Sclerotium*	*Fusarium*	*Rhizoctonia*
1.	Tapioca-Potato-Sugar-Agar	3.0[a]	3.5[a]	3.0[a]	8.5[a]	3.0[a]	3.2[a]
2.	Potato-Dextrose Agar	3.0[a]	3.5[a]	2.5[a]	8.5[a]	3.0[a]	3.2[a]

[a]statistically not significant ($p \leq 0.05$)

The performance of TPSA on bacterial pathogens is presented in Table 6. The growth of X. a. pv. punicae was more mucoid in TPSA than in nutrient agar sucrose (NAS), whereas in *X. a* pv. citri, it was yellow raised in TPSA compared to less yellow in NAS. This finding empirically has proven that TPSA would be a better option for the growth and sporulation of fungal as well as bacterial pathogens of plant. This study conform the findings of Nene and Shiela 1994; and others (Nene *et al.*, 1996; Debai and Muhammad, 2005).

Table 5. Performance of Tapioca-Potato-Sugar-Agar (TPSA) vis-à-vis routine Potato-dextrose-agar (PDA) medium on growth of bio-control agent.

Sl. No.	Medium	Growth of bio-control agent *Trichoderma viride* (at 48 h)
1.	Tapioca-Potato-Agar-Sugar	Full plate growth
2.	Potato-Dextrose-Agar	Full plate growth

The cost of TPSA was calculated as Rs. 52.22/ L compared to Rs. 103.78/L in case of PDA and Rs.119.67/L in NAS (Table 7). This indicated that the cost of TPSA is half of the cost of PDA and less than half of the cost of NAS. Earlier, Mateen

Table 6. Efficacy of host plant leaf extract in the basal medium for growth of *Xanthomonas sp.*

Sl. No.	Medium	Growth of *Xanthomonas*			
		X. a. pv. punicae		*X. a. pv.* citri	
		Colour	Type of growth	Colour	Type of growth
1.	Nutrient Agar	Less yellow	Less mucoid	Less yellow	Mucoid
2.	Tapioca-Potato-Sugar-Agar	Yellow	Mucoid	Yellow raised	Less mucoid

Table 7. Comparative cost of routine fungal/ bacteriological media vis-à-vis new universal medium.

Sl. no.	Name of the medium	Purpose	Component /L(g)	Cost (Rs)	Cost of medium/L (Rs)
1.	Potato-Dextrose-Agar (PDA)	Fungal pathogens	Potato- 200 g Dextrose- 20 g Agar- 20 g	5.00 8.58 90.2	103.78
2.	Nutrient-Agar-Sucrose (NAS)	Bacterial pathogens	Peptone- 5 g Beef extract- 3g Sucrose- 20 g Agar- 20 g	15.13 9.34 5.00 90.2	119.67
3.	Tapioca-Potato-Sugar-Agar (TPSA)	Fungal and bacterial pathogens	Potato- 200 g Tapioca- 20 g Sugar- 20 g Agar- 10 g	5.00 1.50 0.72 45.00	52.22

et al. (2012) also reported guar gum as a suitable and inexpensive substitute to agar in routine microbiological testing.

To conclude, TPSA could be a universal medium for the culture of both fungal and bacterial plant pathogens. It is also a cost half as compared to PDA and NAS. Its ingredients-tapioca powder, sugar, agar can be mixed and stored in plastic jars for a considerable period like commercially available culture media and media can easily be prepared by adding it to potato broth thereby saving time.

References

Abbas, H. M. and Shah, H.U. 2007. Proximate and mineral composition of mung bean. Sarhad *J. Agric.* **23**(2) : 463-466.

Adesemoye, A. O. and Adedire, C. O. 2005. Use of cereals as basal medium for the formulation of alternative culture media for fungi. *World J. Microb & Biotech.* **21**(3) : 329-336.

Badoni, A. and Chauhan, J. S. 2011. Some of cheaper alternatives to ms media for in-vitro culture of potato. *Libyan Agri. Res. Center J. Int*. **2**(4) : 161-167.

Dabai, Y.U. and Muhammad, S. 2005. Cassava starch as an alternative to agar-agar in microbiological media. *Afr. J. Biotechnol.* **4**(6) : 573-574.

Debargh, P. C. 1983. Effects of agar brand and concentration on the tissue culture medium. *Physiol. Plant.* **59**(2) : 270-276.

Jain, N., Gupta, S. and Babbar, S.B. 1997. Isubgol as an alternative gelling agent for microbial culture media. *J. Plant Biochem. Biotechnol.* **15**(6) : 129-131.

Kasanadze, A.K. 2000. Replacement of agar by cassava flour in microbial media. B.Sc. dissertation University of Malawi, Bunda College of Agriculture, Lilongwe.

Maliro, M. F. and Lameck, G. 2004. Potential of cassava flour as a gelling agent for plant tissue culture. Bunda College of Agriculture; Liongwe. *Afr. J. Biotechnol.* **3**(4) : 244-274.

Mateen, A., Hussain, S., Rehman, S.U., Mahmood, S., Khan, M.S., Rashid, A., Sohail, M., Farooq, M. and Shah, S.J.A. 2012. Suitability of various plant derived gelling agents as agar substitute in microbiological growth media. *Afr. J. Biotechnol*. **11**(45) : 10362-10367.

Nene, Y. L. and Shiela, V. K. 1994. A potential substitute for agar in routine cultural work on fungi and bacteria. *Indian J. Mycol. Plant Pathol.* **2**(24) : 159-163.

Nene, Y. L., Shiela, V. K. and Moss, J. P. 1996. Tapioca is a potential substitute for agar in tissue culture media. *Curr. Sci.* **70**(7) : 493-494.