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Note

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Deproteinized Mulberry Leaf Juice - A New Media for Growth of Micro-organisms

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Mulberry being a foliage crop is grown extensively for feeding of silkworms and are also used for cattle feeding. These leaves are highly nutritious, which contain various mineral elements and bio-molecules such as carbohydrates, proteins, lipids and other essential amino acids, etc. In the present study, deproteinized mulberry leaf juice was used for preparation of the medium for cultivation of various types of microbes. Results revealed that deproteinized mulberry leaf juice medium is best for isolation of fungi, bacteria and actinomycetes and this medium can be substituted with synthetic media, which are having the costly ingredients for isolation and identification of bacteria, fungi and actinomycetes. Further, this deproteinized mulberry juice medium can also be used in mass multiplication of useful/beneficial microbes to enhance soil microflora to improve soil fertility and to avoid root diseases. Perspective enterprises can take up the mass multiplication/large-scale production of useful microbes such as *Trichoderma, Rhizobium, Pseudomonas* and *Bacillus* to use in mulberry and in other agricultural crops using deproteinized mulberry leaf juice.

Key words: Mulberry leaves, Microorganisms, Medium, Rhizosphere, Mulberry garden

Introduction

Mulberry (*Morus* spp.) is cultivated in 2,82,244 hectares for rearing of silkworm (*Bombyx mori* L.) for production of silk in India. India is the second biggest country in silk production with an annual production of 14,048 tonnes raw silk. Indian silk is used in textile industries for production of silk garments. India earns Rs. 926.29 crores per annum as a foreign exchange by exporting the raw silk and silk garments (Anonymous, 1999). Mulberry being a foliage crop is grown extensively for feeding of silkworms. However, mulberry leaves are also used as cattle feed, for preparation of tea and even in pharmaceuticals (Singhal *et al.*, 2001). Leaf protein has now been widely recognized as a good quality of protein, which can be readily produced in many developing countries to meet the protein deficiency among their populations (Chand, 1982a). Brown juice popularly known as deproteinized juice is obtained as a by-product during the bulk extraction of protein from the plant sources. Deprotenized leaf juices of various plants have been used for the preparation of culture media for cultivation of many useful bacteria, fungi and actinomycetes especially species of *Saccharomyces, Streptomyces, Rhizobium, Penicillium,* etc. (Chand *et al.*, 1987; Chand, 1982b). Mulberry leaf is a highly nutritious leaf, which contains various mineral elements and biomolecules such as carbohydrates, proteins, lipids and other essential amino acids, etc. (Bongale and Chaluvachari, 1993; Chaluvachari and Bongale, 1995; Kichisaburo, 1997). As the mulberry leaves are very rich nutritively, it was thought for multidirectional use in general and to develop a cheaper growth medium for cultivation of microorganisms in place of synthetic media in particular.

Materials and Methods

Extraction of juice

Five hundred grams of fresh mulberry leaves of Victory 1 (V-1) was collected and chopped nicely after washing with distilled water. Then the juice was extracted from the chopped leaves by adding one-liter double distilled water

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by using pestle and mortar. The extracted leaf juice was heated at 80°C for 30 minutes to coagulate the insoluble proteins. After cooling to room temperature the juice was filtered through a doubled layered muslin cloth to separate out the coagulated protein portion. The soluble but uncoagulated fraction, popularly known as deproteinized leaf juice, which contains chiefly carbohydrates, amino acids, minerals, lipids, vitamins, etc., was collected and used for preparation of one litre medium.

Preparation of media

To deprotienized leaf juice 2% agar was added for solidification of the medium. Then the medium was sterilized at 15 lb pressure for 45 min in an autoclave. Twenty-five ml of lukewarm medium was poured into sterilized Petriplates (10 cm diameter) under aseptic condition in laminar airflow. These plates were used to study the growth of bacteria. Whereas for the growth of fungi, 0.5% sodium propionate was added to the lukewarm medium and for actinomycetes, 2% sodium propionate and 150 g/ml actidione (cycloheximide) an antibiotic were added to the lukewarm medium to arrest the growth of bacteria and fungi, respectively before pouring into plates.

Inoculation and incubation of plates

After solidification of the medium the plates prepared for isolation of bacteria, fungi and actinomycetes were exposed to air for 15 min at mulberry garden to study the microflora of air. The point of exposure was 5 to 6 ft. above the ground level. Another set of the plates were exposed at silkworm rearing house to understand the presence of micriflora in rearing house. Rhizosphere soil samples of mulberry also collected and serially diluted and 1: 10000 dilution was used for isolation of the rhizosphere microflora. All the sets of the plates were kept in incubation at 37°C for 48 hrs for growth of bacteria, and 28°C for 7 days for growth of fungi and actinomycetes. All the similar sets were also conducted in a synthetic medium Czapekís dox agar (Hi Media Co., India) for the comparison. All the observations of experimentation were done in triplicate and repeated twice. Each individual isolate of bacterium, fungus and actinomycete were transferred to the respective slants of mulberry leaf agar medium and synthetic medium for further studies. The isolated fungi were identified on the basis of their morphological and cultural characters with the help of published monographs/books/publications in different Journals (Gilman, 1965; Ellis, 1971; Booth, 1971; Domosch *et al.*, 1980; Ellis and Ellis, 1985; Nelson *et al.*, 1983; Rotem, 1994). Whereas the isolated bacteria and actinomycetes were identified based on their morphological, cultural and biochemical characters such as incubation temperature, Gram reaction (in case of bacteria), morphology, motility test, carbohydrate fermentation, gelatin hydrolysis, litmus milk reaction, catalase test, growth in nutrient broth, indole test, urease test, starch and casein hydrolysis. These characters will be compared with those of all recognized groups of bacteria and actinomycetes given in the Bergeyís manuals (Pridham and Tresner, 1974; Buchanan and Gibbons, 1979; Krieg, 1984) and other publications. These identified microbes were also compared with available collection in Mulberry Pathology Laboratory, CSRTI, Mysore, which have been identified earlier by International Mycological Institute, Kew (England).

Results and Discussion

Bacteria, fungi and actinomycetes were counted after required incubation period of 48 hrs and 7 days (fungi and actinomycetes), respectively. The average number of colony forming units (cfu) in 25 ml of mulberry leaf agar medium Czapekís dox agar medium (synthetic) were counted and presented in Table 1. More number of bac-

Table 1. Total number of isolated microbial population in deproteinized mulberry leaf juice agar medium and czapeks dox agar medium (synthetic)

Location of isolation	Media	Number of cfu/ 25 ml media		
		Bacteria	Fungi	Actinomycetes
Silkworm rearing house	Deproteinized leaf juice	6.66	6.00	2.33
	Czapek's dox agar	7.01	6.08	2.39
Mulberry garden	Deproteinized leaf juice	19.66	21.89	4.67
	Czapek's dox agar	20.01	22.01	5.04
Rhizosphere soil of mulberry garden	Deproteinized leaf juice	6.66	13.59	7.00
	Czapek's dox agar	6.99	13.90	7.20
CD (P = 0.05)		NS	NS	NS

Time of exposure 15 minutes. *Average of 3 replications. NS = Non significant.

Table 2. List of microbial genera/species isolated and identified in deproteinized mulberry leaf juice agar medium and czapeks dox agar medium (synthetic)

Location of isolation	Media	Fungi	Bacteria	Actinomycetes
Silkworm rearing house	Deproteinized leaf juice	Aspergillus olevacious A. niger *Rhizopus* spp.	*Bacillus* spp. *Pseudomonas* spp. *Micrococus* spp.	*Streptomyces* spp.
	Czapek's dox agar	Aspergillus olevacious A. niger *Rhizopus* spp. *Penicillium* spp.	*Bacillus* spp. *Pseudomonas* spp. *Micrococus* spp.	*Streptomyces* spp.
Mulberry garden	Deproteinized leaf juice	Fusarium oxysporum F. solani A. olevacious A. flavous Alternaria alternata Cercospora moricola	*Pseudomonas* spp. *Xanthomonas* spp. Bacillus spp.	*Streptomyces* spp. *Nocardia* spp.
	Czapek's dox agar	Fusarium oxysporum F. solani A. olevacious A. flavous Alternaria alternata Helminthosporium tetramera Cercospora moricola	*Pseudomonas* spp. *Xanthomonas* spp. Bacillus spp.	*Streptomyces* spp. *Nocardia* spp.
Rhizosphere soil of mulberry garden	Deproteinized leaf juice	F. oxysporum F. solani A. niger *Alternaria* spp. Trichoderma harzianum *Rhizobium* spp. *Penicillium* spp.	*Bacillus* spp. *Pseudomonas* spp. Micrococus spp.	*Streptomyces* spp. *Nocardia* spp.
	Czapek's dox agar	F. oxysporum F. solani A. niger *Alternaria* spp. Trichoderma harzianum T. viridae *Rhizobium* spp. *Penicillium* spp.	*Bacillus* spp. *Pseudomonas* spp. Micrococus spp.	*Streptomyces* spp. *Nocardia* spp.

teria, 19.66 cfu/25 ml medium was recorded in mulberry garden followed by 6.66 cfu/25 ml in rearing house and rhizosphere soil (dilution 10^4). Fungi cfu/25 ml was high in mulberry garden sampling (21.89) followed by 13.59 in rhizosphere soil and 6.0 in rearing house. However, maximum number of actinomycetes was observed (7.0/25 ml medium) in rhizosphere soil, followed by 4.67 in mulberry garden and minimum in rearing house (2.33/25 ml medium). In case of Czapekís dox agar (synthetic) medium taken for comparison, the microbes *viz.* bacteria, fungi and actinomycetes were recorded as 7.01, 6.08 and 2.39 cfu/25 ml medium, respectively in silkworm rearing house while in mulberry garden 20.01 bacteria, 22.01 fungi and 5.04 actinomycetes (cfu/25 ml medium) were observed. However, rhizosphere soil of mulberry garden 6.99, 13.90 and 7.20 bacteria, fungi and actinomycetes were recorded, respectively. There was no significant difference ($p = 0.05$) in respect of number of cfu/25 ml of deproteinized leaf agar media and Czapekís dox agar.

Different types of fungi, bacteria and actinomycetes were identified and presented in Table 2. The commonly encountered fungi were *Aspergillus olevacious, A. niger, A. flavous, Rhizopus, Rhizobium, Fusarium oxysporum, F. solani, Cercospora moricola, Alternaria alternata, Alternaria* species, *Penicillum* species and *Trichoderma harzianum.* Among them, *F. oxysporum, A. alternata* and *C.*

moricola are pathogenic to mulberry (Phillip *et al.*, 1994). Both the bacteria like Gram - ve and Gram + ve were identified as species of *Bacillus, Pseudomonas, Micrococus* and *Xanthomonas,* whereas the identified actinomycetes were belonged to the genus of *Streptomyces* and *Nocardia.* However, in both the media similar types of the microbial genera and species were observed but in case of synthetic medium, where *Helminthosporium tetramera* and *Penicillium* spp were recorded additionally (Table 2).

The present study indicates that the deproteinized mulberry leaf juice can be substituted with synthetic media, which are having the costly ingredients for isolation and identification of pathogenic and beneficial bacteria, fungi and actinomycetes. Mahanta (1974) reported that the pea extract medium is suitable for the cultivation of nitrogen fixing bacteria. In the present study, by using this cheaper medium one can ascertain the microbiological qualities of air in the rearing houses and in mulberry gardens to take up proper management methods to avoid contamination and disease proliferation.

Thus, the present study clearly indicates that the deproteinized mulberry leaf juice medium can be used for culturing of various types of microbes. Further, this deproteinized mulberry juice can also be used in mass multiplication of useful/ beneficial microbes to enhance soil microflora to improve soil fertility and to avoid root diseases. Perspective enterprises can take up the mass multiplication/ large-scale production of useful microbes such as *Trichoderma, Rhizobium, Pseudomonas* and *Bacillus* to use in mulberry and in other agricultural crops.

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