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Research Article

IDENTIFICATION AND PREVENTION OF BACTERIAL CONTIMINATION ON EXPLANT USED IN PLANT TISSUE CULTURE LABS

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ABSTRACT

In the present study seven types of bacterial isolates were recovered from our explantsand these were *Pseudomonas, Escherichia coli, Proteus* sp, *Micrococcus* sp, *Staphylococcus aureus, Bacillus, Corynebacterium*sp.The use of field grown plants as a direct source of explant material for the production of 'clean' *in vitro* plantlets, presents a major challenge. To avoid the contamination from explant used in plant tissue culture labs different solvents were used for surface sterilization trials conducted using leaf explantsand in our study solventssuch as mercuric chloride and sodium hypochlorite reduced the bacterial contamination up to 95 % and detergent reduced bacterial contamination up to 90 % and other solvents also gave satisfactory results for better and economic sterilization technique in plant tissue culture lab.

Keywords: Contamination, Explants, Sterilization, Medicinal plants.

INTRODUCTION

Contamination with microorganisms is considered to be the single most important reason for losses during *in vitro* culture of plants. Such microorganisms include viruses, bacteria, yeast, fungi, etc.[1].These microbes compete adversely with plant tissue cultures for nutrients. The presence of these microbes usually resulting increased culture mortality but can also result invariable growth, tissue necrosis, reduced shoot proliferation and reduced rooting. Explants contamination is a function of several plant and environmental related factors such as plant species, age, explant source and prevailing weather condition. Despite the best timing and selection efforts it is almost impossible to eliminate contamination from *in vitro* grown plants. The cumulative result is an abundant waste of time, effort and material, which if not mitigated can have severe economic consequences,in order to design methods to decrease contamination from field grown medicinal plants, we first attempted to decrease overcontamination (identified by visible fungal or bacterial growth) of nodal explants. The majority of explants clean after these two first weeks *in vitro* remained clean and produced viable, growing plantlets. In this paper, we present a novel but simple sterilization method that decreased contamination of explants. The surface sterilization method described in this paper has significantly reduced contamination levels of leaf explants selected directly from field grown medicinal plants while reducing the time and the risks associated with the use of Organic solvents. During sterilization, the living materials should not lose their biological activity and only contaminants should be eliminated, therefore explants are surface sterilized only by treatment with disinfectant solution at suitable concentrations for a specified period. The disinfectants widely used are sodium hypochlorite, calcium hypochlorite, ethanol (or isopropyl alcohol), mercuric chloride, hydrogen peroxide, silver nitrate and bromine water Hypochlorite is known to be a very effective killer of bacteria, and evenmicro molar concentrations are enough to reduce bacterial populations significantly. However, little is known about the exact mechanisms of its bactericidal activity. When diluted in water the hypochlorite salts (NaOCl, Ca $(OCl)_2$) lead to the formation of HoClwhose concentration is correlated with bactericidal activity [2].Sodium hypochlorite, usually purchased as laundry bleach is the most frequent choice for surface sterilization. It is readily available and can be diluted to proper concentrations. A balance between concentration and time must be determined empirically for each type of explants because of phytotoxicity. Calcium hypochlorite is used mostly in Europe and the concentration generally used is 3.25%, it may be less injurious to plant tissues than sodium hypochlorite, Ethanol is a powerful sterilizing agent but also extremely phyto-toxic. Therefore, the explant is typically exposed to it for only a few seconds or minutes.

MATERIAL AND METHODS

Preparation of explant

The plants were collected from herbal garden of Himachal Institute of Pharmacy .The explantwas prepared with a clean knife.

Isolation and characterization

In order to isolate the bacteria, explant was inoculated in nutrient broth medium for 24 hrs at 37 ^{0}C. The developed bacterial contaminants transferred to nutrient agar (NA) medium plates. All the isolated Purified bacteria were observed under microscope after proper staining (Gram staining). Essential biochemical tests were carried out as per standard methods[3, 4].

Surface Sterilization

For surface sterilization explant was treated with different solvent for different time period and non-treated (without washing)explant were inoculated into different flasks containing nutrient broth medium for 24 hrs at 37^0 C. 1 ml of the developed bacterial contaminants from each flask was transferred to nutrient agar (NA) medium platesto determine CFU.

RESULTS AND DISCUSSION

Turbidity of microbial enrichment broths wastaken as a primary indicator for microbialgrowthSeven types of bacterial isolates were recovered from our explants andidentified by their colony characteristics and gram staining (Table no.1) and biochemical testing (Table no.2)and these were *Pseudomonas,Escherichiacoli, Proteus* sp, *Micrococcus* sp, *Staphylococcus aureus, Bacillus* sp, *and Corynebacterium* sp. and surface sterilization of explants was done by various organic solvent and detergent from which the best sterilizing agent were 3% mercuric chloride, 3% sodium hypo chlorite, 3% hydrogen per oxide and the detergent also shows good result while bromine water , 70 % alcohol , and silver nitrite were satisfactory(Table no.3)

Our observations were in accordance with the study of [5]Presence of bacteria was noticed in the transverse section of the rhizome underthe microscope. In their study seven bacterial strains were isolated from the contaminatedculture. Four of them were gram positive and the rest were gram negative. Thegram positive isolates were *Cellulomonasuda, C. flavigena, Corynebacterium, paurometabolum* and *Bacillus megaterium.* The gram negative isolates were*Klebsiella*sp,*Erwiniacypripedii*and *Pseudomonas* sp. All of them were non sporeformer except *Bacillus megaterium*. In our study surface sterilization with the help of different solvent was successful but the most successful were sodium hypochlorite and

mercuric chloride reduced bacterial contaminations up to 95%, while the contamination was reduced up to 80 % by bromine water, detergent while alcohol and other solvents were also reduced bacterial contaminations up to certain extent which is similar to[6] in their research reducing contamination through two-step two reagent procedures, as described by is a laborious and drawn-out process. The use of ethanol or the combination of ethanol and other disinfectants is very expensive. In a typical tissue culture laboratory, ethanol, sodium hypochlorite and sucrose are a few of the major investments and any means of reducing this cost would be significantly useful especially in the developing countries. Additionally simplifying existing technique will have benefit in timesaving. Theysuggested that prudentselection of explants from the healthy parent plants coupled with an effective surface sterilization method should be the goal in avoiding culture contamination. In conclusion, results of this study have demonstrated that the use of locally produced bleach containing 3.5% hypochloritefor 30 min is as effective as the regular 2-step 2-reagent technique. Consequently, we would recommend its usage because of its simplicity and economy.

Table 1: Gram Staining and Colony Charecterization

Recovered Isolates	Gram Staining	Colony Characterization
Isolate no .1	Gram positive , rod	Abundant, opaque ,white waxy growth
Isolate no .2	Gram negative , rod	White , moist , glistening growth
Isolate no .3	Gram positive , cocci	Abundant, opaque, golden growth
Isolate no .4	Gram negative , rod	Thin, blue-grey, spreading growth
Isolate no .5	Gram positive, cocci	Soft, smooth , yellow growth
Isolate no .6	Gram positive , rod	Grayish , opaque , white waxy growth
Isolate no. 7	Gram negative , rod	Abundant ,thin ,white growth with medium turning green

Table 2: Biochemical Testing

Isolate	Indole	MR	VP	Citrate	Urease	Nitrate	Catalase	Identified organism
1	-	-	-	-	-	+	+	*Bacillus sp.*
2	+	+	-	-	-	+	+	*E.coli sp.*
3	-	+	-	-	_	+	+	*Staphylococcus sp.*
4	+	+	-	+	+	+	+	*Proteus sp.*
5	-	-	-	-	+	-	+	*Micrococcus sp.*
6	-	-	-	-	-	+	+	*Corynebacterium sp.*
7	-	-	-	+	-	+	+	*Pseudomonas sp.*

Table 3: Surface Sterilization of Explant

Solvent used	Time	O.D	Colony count
$AgNO_3$	5 min	0.722	292
Alcohol	15 min	0.324	108
Bromine water	5 min	0.405	128
Detergent	30 min	0.184	49
H_2O_2	5 min	0.095	21
$HgCl_2$	5 min	0.006	2
Sodium hypochlorite	5 min	0.064	17
Without washing	0 min	1.818	612

Down under are shown the figures of the N.B. containing the explants treated with different chemicals after incubation for 24 hrs at 37^0C.

Fig. 1: Control

Fig. 2: Silver Nitrate ($AgNO_3$)

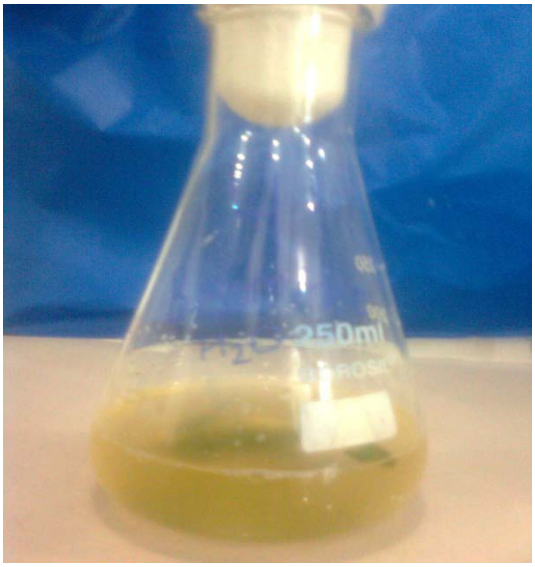

Fig. 3: Bromine Water

Fig. 4: 70% Alcohal

Fig. 5: Detergent

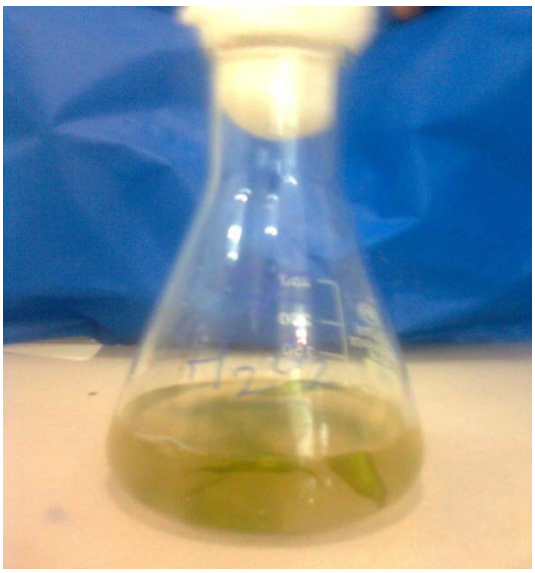

Fig. 6: Hydrogen Peroxide (H_2O_2)

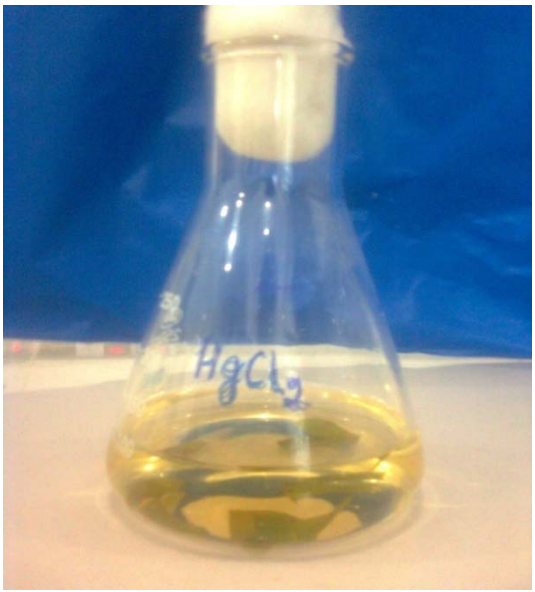

Fig. 7: Mercuric Chloride ($Hgcl_2$)

Fig. 8: Sodium Hypochlorite

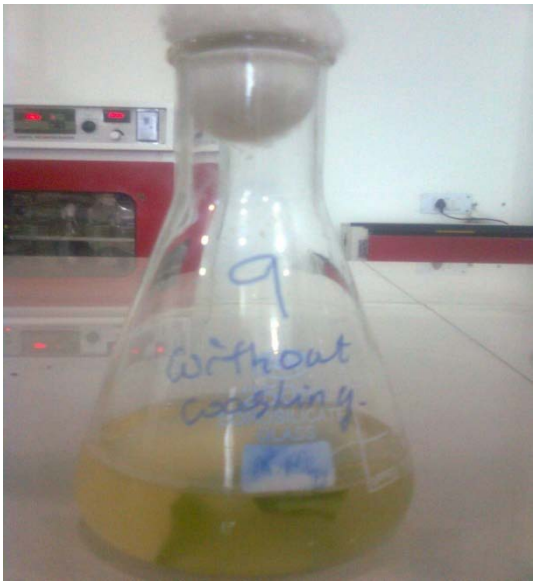

Fig. 9: Without Washing

CONCLUSION

Contamination with microorganisms hinders the growth of micropropagated plant because they use nutrient from the media as their energy source. To avoid this sterilization is necessary for proper growth and development of plant. Our study conclude that these organic solvents economic, simple and better sterilizing agents to use for sterilization in PTC lab.

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REFERENCES

1. Omamor IB, Asemota AO, Eke CR and Ezia EI. Fungal contaminants of the oil palm tissue culture in Nigerian Institute for Oil Palm Research (NIFOR). Afr. J. Agric. Res. 2007;2(10): 534-537.
2. Nakagarwara S, Goto T, Nara M, Ozawa Y, Hotta K. and Arata Y. Spectroscopic characterization and pH dependence of bacterial activity of the aqueous chlorine solution. Anal. Sci.1998; 14: 691-698.
3. Collins CH,Lyne PM. Microbiological Methods. 5th ed. London: Butterworths Co. Publishers. Ltd;1984p. 56 – 113.
4. Krieg RN,Holt JG. Bergey's manual of systematic Bacteriology volume 1. Baltimore (U.S.A) Williams and Wilkins Company;1984p. 308 - 429.
5. Habiba U, Sharmin R, Mihir LS, Khan MR, Syed H.Endogenous Bacterial Contamination during In vitroCulture of Table Banana: Identification and Prevention. Plant Tissue Cult.2002; 12 (2): 117-124.
6. Matthews R, Duncan EJ. A method to reduce microbial contamination in In vitro culture. Third Caribbean Biotechnology Conference. 1993, 160-166.