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OPTIMIZATION OF SURFACTANT AND POLYMER CONCENTRATION TO ALTER THE MORPHOLOGY OF MATT FORMING FUNGAL CULTURES

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Abstract: General growth of fungus in a broth will be in the form of a pellet or matt, which can be transformed to fine suspension by adding various surfactants and polymers at different concentrations. Different surfactants like Brij – 35, Brij – 92, Cetrimide, Pluronic F – 68, Sodium Lauryl Sulphate, Triton X – 100 and Tween – 80 and polymers like Agar, Sodium Alginate, Sodium CMC, Gelatin concentrations were optimized to transform matt forming fungi to fine suspension. Effect of these polymers and surfactants were carried on *Cunninghamella echinulata* NCIM 693, *Curvularia lunata* NCIM 716 and *Rhizopus arrhizus* NCIM 997. Most of the surfactants and polymers altered the morphology of the three fungi to fine suspension but it was observed that with Triton X100 at concentration ranging 0.015 – 0.02 % the morphology of all the three fungi was transferred from matt to fine suspension and same results were obtained with agar at concentration ranging 0.03 – 0.04 %. Finally the fine suspensions of these fungal cultures were transferred to the media without surfactant or polymer and incubated in a shaker incubator at 28 ^{0}C and a shaking speed of 120 RPM and was observed that there was no morphological change in the characteristics of fungi studied

Keywords: Triton X – 100, Agar, *Cunninghamella echinulata, Curvularia lunata, Rhizopus arrhizus*

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INTRODUCTION

Microbial biotransformation plays important role in predicting mammalian metabolism of drugs and chemicals [1-5] which is an important parameter in the evaluation of safety and efficacy of the drugs and also in the discovery and development of new leads [6-8], in the biodegradation of toxic environmental pollutants to non-toxic substances [9, 10], for introducing chemical functions into inaccessible sites of molecules[11], for stereoselective synthesis of chiral lactones and epoxides[12], for enantioselective reduction of ketones to produce chiral alcohols of remarkable optical purity[13] and for hydrolysis of racemic mixture to yield pure enantiomers e.g., hydrolysis of racemic ibuprofen amide to s(+) ibuprofen[14]. Fungi had provided various oxidation, reduction, dealkylation, deacylation, hydrolysis products and are well reported [1, 15].

General growth of fungus in a broth will be in the form of a pellet, which can be transformed to fine suspension by adding various surfactants and polymers at different concentrations (Venisetty and Ciddi, 2003). By doing this the rate of bioconversion of a drug using fungus can be enhanced as the surface area available for the fungus will be increased. Added advantage of surfactant addition is, they alter the membrane permeability of fungus and make the drug more freely enter into fungus cells and make the drug easily available to intra cellular enzymes for biotransformation. The use of surfactants[10, 16-18] and solubilizers[19] increases the solubility of the substrates in aqueous media and further enhances the biotransformation.

It has been reported that the addition of water soluble polymers like agar and polyvinyl alcohol were found to suppress the pellet formation of fungus extensively, and the mycelial growth became flocculent and filamentous whereby ebastine oxidation to active metabolite carebastine increased more than 40%[6]. Higher concentrations of these polymers did not effect the growth of cells but reduced the yeilds of carebastine. It has also been reported that surfactants at low concentration stimulate the biodegradation of sorbed hydrocarbons[20]. Therefore it is essential to optimize the concentration of the surfactants and polymers.

MATERIALS AND METHODS

Agar was purchased from Rankem, Mumbai; Pluronic F – 68, Tween – 80, Sodium Lauryl Sulphate, Brij 35, Brij 92, Sodium Carboxy Methyl Cellulose and Potato Dextrose Broth were purchased from Himedia, Mumbai; Triton X-100 and Sodium Alginate were purchased from Loba Chem India, Mumbai; Cetrimide and Gelatin were purchased from S.D. Fine, Mumbai. Deionised water collected by Millipore Elix 3 water purifier was used for the study. Shaker Incubator, Newbrunswik Scientific, Innova 4230 was used for this study. Pictures were taken by Hewlett and Packard digital zoom camera. Cunninghamella echinulata NCIM 693, Curvularia lunata NCIM 716 and Rhizopus arrhizus NCIM 997 were purchased from National Collection of Industrial Microorganisms, Pune, India. Cultures are sub cultured for every 60 days and

preserved at 4^0 C after growth. Potato dextrose broth was prepared by dissolving 24gms of Potato Dextrose powder in 1 liter of de-ionized water. pH of the media was adjusted to 5.6. 10ml of media was taken in each 50ml conical flask and added different concentrations of various surfactants and polymers. Physico-chemical properties of the surfactants used are tabulated (Table 1.). After inoculation of the fungi aseptically, the flasks were placed on shaker incubator with temperature adjusted to 28°C and shaking speed at 120 rpm. Screening experiments were started at surfactant/polymer concentrations 1, 2, 3, 4 % and based on the change of morphology the concentrations were optimized by either increasing or decreasing until the fungal morphology was altered. Photographs of the cultures were taken for every concentration of polymers and surfactants studied. Finally the fine suspensions of fungal cultures were transferred to the media without surfactant or polymer and incubated in a shaker incubator at 28^0C and a shaking speed of 120 RPM to assess any change in the characteristic of fungus.

RESULTS AND DISCUSSIONS

Fine suspension of Cunninghamella echinulata NCIM 693 is achieved with surfactants, Brij 92, Brij 35, Pluronic F - 68, SLS, Triton X100, Tween 80 and polymers, Agar, sodium alginate, Sodium CMC, Gelatin at concentration range of 0.01 – 0.012 %, 0.05 – 0.06 %, 0.8 – 0.9%, 0.004 – 0.006 %, 0.015 – 0.02 %, 2.6 – 2.8 % and 0.02 – 0.03%, 0.11 – 0.13 %, 0.16 – 0.18 %, 0.08 – 0.1 % respectively. Cetrimide did not alter fungal morphology in all the concentrations tested, but shown antifungal activity at 0.001 %. SLS has shown fungal dispersion below 0.008% and at which it has shown antifungal activity (Fig. 1).

Fine suspension of Curvularia lunata NCIM 716 is achieved with surfactants like Brij 92, Brij 35, Pluronic F - 68, SLS, Triton X100, Tween 80 and polymers like Agar, Sodium alginate, Sodium CMC, Gelatin at concentration range of 0.05 – 0.06 %, 0.06 – 0.07 %, 3.0 – 3.2%, 0.003 –0.005 %, 0.015 – 0.02 %, 4.3 – 4.5 % and 0.04 – 0.05%, 0.65 – 0.7 %, 0.28 – 0.3 %, 0.28 – 0.3 % respectively. Cetrimide did not alter fungal morphology in all the concentrations tested, but shown antifungal activity at 0.0005 %. SLS has shown fungal dispersion below 0.008% and at which it has shown antifungal activity (Fig. 2).

Fine suspension of Rhizopus arrhizus NCIM 997 is achieved with surfactants like Brij 92, Brij 35, Pluronic F - 68, SLS, Triton X100, Tween 80 and polymers like Agar, Sodium alginate, Sodium CMC, Gelatin at concentration range of 0.06 – 0.08 %, 0.07 – 0.09 %, 3.8 – 4.0%, 0.003 –0.005 %, 0.015 – 0.02 %, 3.8 – 4.0 % and 0.04 – 0.05%, 1.2 –1.3 %, 0.4 – 0.45 % respectively. Cetrimide did not alter fungal morphology in all the concentrations tested, but shown antifungal activity at 0.001 %. SLS has shown fungal dispersion below 0.008% and at which it has shown antifungal activity (Fig. 3).

In most of the cases it was noticed that higher the Critical Micellar Concentration(CMC) of the surfactant, lesser the concentration required to achieve a fine suspension of fungal cultures.

The fine suspension cultures of all three strains when transferred to a media without surfactant and polymer showed the formation of matt again indicating that there is no change in the characteristics of the fungus by adding low concentrations of surfactants and polymers.

CONCLUSION

From the results obtained, it was observed that with Triton X100 at concentration ranging 0.015 – 0.02 % (minor range for all three cultures tested) the morphology of all the three fungi was transferred from matt to fine suspension and same results were obtained with agar at concentration ranging 0.03 – 0.04 %. Hence it is considered that in biotransformation studies using fungi one can get a fine suspension of fungi by addition of these agents in the said concentrations. An added advantage of using Triton X 100 is that it is a surfactant which increases the solubility of the drug and also enhances the permeation of the drug into the cell and facilitates further increase in biotransformation with intracellular enzymes.

It cannot be concluded that CMC of a surfactant alone is playing an important role as there are several factors which like surface tension, viscosity, cloud point, Hydrophilic Lipophilic Balance (HLB) etc., which can alter the morphology of the matt forming fungi.

TABLE 1. PHYSICO-CHEMICAL PROPERTIES OF SURFACTANTS USED

SURFACTANT	AVG. MOL. FORMULAE	CHEMICAL NAME	AVG. MOL. WEIGHT	CMC	HLB	CLOUD POINT(°C)
Brij - 35	$C_{58}H_{118}O_{24}$	Polyoxyethylene(23)lauryl ether	1198	0.09 mM	16.9	**> 100**
Brij – 92	$C_{22}H_{36}O_3$	Polyoxyethylene(2)oleyl ether	348	0.077 mM	4.9	**> 100**
Cetrimide	$C_{17}H_{38}BrN$	Cetyl trimethyl ammonium bromide	336	0.01 mM		
Pluronic F-68	$C_{402}H_{785}O_{186}$	Polyethyleneoxide(78) Polypropeleneoxide(30) Polyethyleneoxide(78)	8750	0.04 mM	29	**> 100**
Sodium Lauryl Sulphate	$C_{12}H_{25}OSO_3^-Na^+$	Sodium Dodecyl sulphate	288	7 – 10 mM	40	**> 100**
Triton X-100	$C_{33}H_{60}O_{10}$	Polyoxyethylene(10)isooctylphenyl ether	625	0.2-0.9 mM	13.5	**64**
Tween – 80	**$C_{64}H_{124}O_{26}$**	**Polyethylene Sorbitan Monooleate**	**1310**	**0.012 mM**	**15**	**64**

CMC: Critical Misecellar Concentration. HLB: Hydrophilic Lipophilic Balance

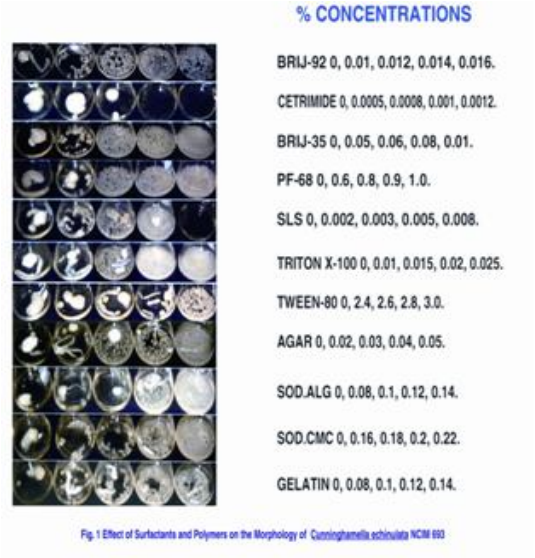

Fig. 1 Effect of Surfactants and Polymers on the Morphology of Cunninghamella echinulata NCIM 693

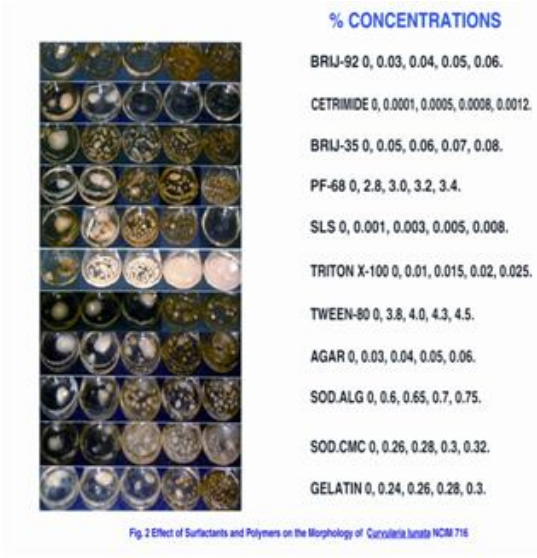

Fig. 2 Effect of Surfactants and Polymers on the Morphology of Curvularia lunata NCIM 716

Fig. 3 Effect of Surfactants and Polymers on the Morphology of Rhizopus arrhizus NCIM 997

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