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Control of *Aspergillus parasiticum* NCIM 898 infection in potato tubers using biosurfactant

A D Mule[1], Z P Bhathena[2]

1 Department of Microbiology, Bhavan's College, Andheri, Mumbai 400 058

2 Corresponding Author E. Mail ID : zarine_bhathena@rediffmail.com

Phone : +91-2226244871

Abstract: Increasing microbial infections in plant, seeks an innovative sustainable alternative that could be used to sustain food production by diminishing the viability and infectivity of the phytopathogen. *Sternophomonas maltophila* isolated from oil contaminated environment, was screened for its ability to produce biosurfactant assessed based on its potential to reduce surface tension. Biosurfactant property of *Sternophomonas maltophila* was confirmed by RBC haemolysis. Biosurfactant extracted by organic extraction method, was used in the control of aflatoxin producing potato tuber mold *Aspergillus parasiticum* NCIM 898. A direct biosurfactant application at 10% concentration was found to cause complete spore lysis and could be useful in eliminating this mold infection in potato tubers.

Key Words: Biosurfactant, *Sternophomonas maltophila*, Aflatoxin, *Aspergillus parasiticum* NCIM 898.

Introduction

The deleterious effect of chemical fertilizers, synthetic pesticides & growth regulators on environment, demands an innovative alternative that would sustain soil health along with food production. Use of biologically active and environment friendly substances for plant growth stimulation & protection can form an indispensable part of agricultural green technology to increase the crop productivity through innate mechanisms that protect plants from disease and pest invasion. Thus core strategies that increase crop productivity along with reduction in diseases form a key role in recent crop management systems as approximately 18% of the crop yield, valued at Rs 90,000 crore are lost in India due to pest attacks each year.

Though biological control, through use of antagonistic microorganisms (primarily bacteria and fungi) and the production of extra cellular metabolites such as antibiotics, hydrogen cyanide (HCN), and siderophores (Hornby, 1990) to control target phyto pathogens are known, its implementation is fraught with constraints, and successful application depends on a thorough knowledge of the ecology and biology of both the target pest and the biological control agent. Biosurfactant, being a biological natural product with the absence of any enzyme, acid, sledges, pesticide, petrochemical or gene altered materials can thus form an important part of bioprotective components that can be used to ensure good organic farming. Such a postulation can be based on its antimicrobial & anti-fungicidal properties besides its ability to form an efficient surface coating, through its emulsifying, stabilizing, wetting and foaming property (Desai & Banat, 1997).

With an annual global production of about 300 million tons contributing to around 7.5% to the world's production, potato is an economically important staple crop and increase in its productivity is an important aspect of Indian agricultural programme. This is specially required with respect to aflatoxin producing *Aspergillus* infections which colonize and contaminate tuber during growth or during storage following prolonged exposure to humid environments. Thus controlling *Aspergillus* infection forms a key step in maintaining export quality of potato as mycotoxin contamination causes economic losses in potato processing and export.

This paper uses novel approach of controlling Aspergillus *parasiticum* NCIM 898 by utilizing biosurfactant whose adhesive and coating properties would form a protective barrier around the tuber besides initiating an antimicrobial effect. Additionally its low toxicity, high biodegradability, better environmental compatibility (Georgiou *et al.*, 1990); activity at extreme temperatures, pH, and salinity along with its ability to be synthesized from renewable feedstock confers it with added advantages over the use of chemical surfactants.

Material and methods :

1. Isolation of biosurfactant producers

A biosurfactant producing isolate *Sternophomonas maltophila* was obtained during a preliminary screening programme where in stagnant waters harvested from oil rich ecological areas like garages, servicing centers, petrol pumps and industrial belts producing chemical surfactants, edible oil and

petroleum products were assessed.

The isolates were obtained primarily on Christova Mineral medium containing 2% paraffin oil after incubation for 48 hours and selected as biosurfactant producers based on the rational that paraffin utilization involves the production of biosurfactant. (Desai and Banat,1997). Assessment of biosurfactant producing isolate was also carried out using

A. Quantitative Haemolysis assay: (Dehghan-Noudeh *et al.,* 2005) In 5% human RBC suspension prepared in McIlvaine's buffer (0.2 ml) was incubated for 30 min at 37°C with an equal volume of metabolic filtrate obtained by growing *S. maltophila* in Christova's mineral medium supplemented with 2% paraffin oil for 96 hours, at 25°C. After incubation, the mixtures were spun in a micro centrifuge (Eltech) at 3000 rpm for 30s. 0.2 ml of the resulting supernatant was then added to 3 ml Drabkin's reagent (LOBA chemicals) to assess the amount of haemoglobin released. Appropriate positive and negative controls were set up. The absorbance of the samples was determined calorimetrically at 540 nm and % heamolysis calculated as:-

$$\text{Percentage haemolysis} = \frac{OD_{(test)} - OD_{(0\% \text{ control})}}{OD_{(100\% \text{ control})}} \times 100$$

b. Surface tension reduction activity (Desai and Banat, 1997, Singh and Desai, 1989)

Surface activity was measured using CDS-DuNouy ring tensiometer. 24 hr old *S. maltophila* suspension at OD_{540nm} of 0.5 was used to inoculate Christova mineral broth. The flask was incubated under shake conditions for 92 h and the metabolic filtrate harvested by centrifugation. Surface tension was measured by placing the platinum-iridium ring just below the surface of metabolic filtrate solution, which then rose through the surface of the aqueous solution until the ring broke. The amount of force required for breaking through the surface layer was recorded in dynes/cm^3, using Kruss K 10 tensiometer.

c. Emulsification index (EI): (Morikawa *et al.,* 1993)

4 ml of 72 h metabolic filtrate obtained after growth of *S. maltophila* on Christova mineral medium containing 2% paraffin as a carbon source was overlaid & mixed with 4 ml of oil substrates (paraffin oil) in a dilution glass test tube (16 by 150 mm).The height of the oil layer in the tube was measured in cm. The tubes were then vortex for 3 min and then shaken manually for 2 min to create an emulsion. The height of the emulsion layer was measured after 2 h & 24 hr and emulsification index (EI) calculated using the following formula:-

EI (%) = (height of emulsion layer)/ (height of the oil plus emulsion layer) X 100.

2 Biosurfactant Production, Extraction and Purification : (Rahman and Gakpe, 2008)

Potent bacterial isolates *S. maltophila* was adjusted to a final density of 1X 10^2 cfu/ml in 50 ml of Christova's Mineral medium with 2% paraffin. The flask was incubated at 30°C for 96 h under shaker condition of 100 rpm.

Metabolic filtrate harvested after centrifugation was adjusted to pH 2 using 6 N HCl. After an overnight incubation at 4°C equal volumes of chilled mixture of chloroform and methanol (2:1) was added to obtain the biosurfactant containing organic layer, which was dried at 40°C for 3 hours. The extraction process was repeated thrice and the dried mass obtained weighed and its protein content was determined by Lowry *et al.* (1951) method. The extracted biosurfactant was then subjected to adsorption chromatography (Saini *et al.*, 2005) using silica gel solid-phase column (mesh size 200-300 mesh, 1cm diameter, 6 cm length) saturated with solvents of gradually increasing polarity in the order of chloroform, acetone, chloroform**:** methyl alcohol (2:1), and methyl alcohol: Water (2:1). The surfactant harvested was eluted by sterile glass distilled water overnight to remove the remaining cations, while smaller fractions of proteins were removed by adding chilled iso-butanol (10%) and by centrifugation at 4000 rpm for 10 min. Supernatant was then stored after lyophilization at 4°C and used as stock surfactant.

3. Sporolytic activity

Zoospores of *Aspergillus parasiticus* NCIM 898 were harvested using the method described by Zhou and Paulitz (1993), where in the fungi grown for 72 hours on Sabourauds agar supplemented with 1% $CaCO_3$ was flooded with 20 ml of sterile distilled water supplemented with 0.1% Tween 80. After 2 h the zoospore were harvested by centrifugation and stored in saline at 4°C.

1 ml of purified biosurfactant of *S.maltophilia* prepared in concentration of 2%, 4%, 6%, 8%, and 10% was mixed with 1 ml of *Aspergillus parasiticus* NCIM 898 spore suspensions adjusted to minimum infectivity index of 1 X10^3 spores/ ml (Kasprzyk, 2002) and incubated for time intervals of 10, 40, 60, 80, 100 & 120 mins respectively. The spore biosurfactant mixture was then observed for the integrity / bursting of the spore under phase contrast microscope (Olympus CX 31 Asbestos Phase Contrast Microscope). Viability of spores was further confirmed by plating the spore biosurfactant mixture on Sabouraud's Agar. Percentage decline in spore viability due to the treatment of

biosurfactant was assessed using appropriate controls.

4. Sporolytic activity of biosurfactant to control potato tuber wilt infestation

Mid size potato tubers collected from Borivali market, were washed twice with sterile distilled water, followed by immersing in 2% sodium hypochlorite solution for 10 min to ensure surface sterilization. Excess sodium hypochlorite was removed by repeated washing with sterile distilled water. The tubers were then randomly divided into two groups, such that each labeled test and control group contained at least ten uniformly sized tubers. The test tubers were then sprayed with 3 ml of 1 $X10^3$ spores/ ml, suspension of *Aspergillus parasiticus* NCIM 898 using spray pump. After an interval of 5 min the tubers were then sprayed with 10 ml of 10% biosurfactant suspension. In contrast, the control group was sprayed only with *Aspergillus parasiticus* NCIM 898 spore suspension. Additionally, a negative control group containing only surface sterilized potato tuber without any spray treatments was also included

After 1 hr the excess liquid within each group if any was decanted and the potato tubers were incubated at 25°C for 1 week interval and graded for infectivity according to Percent Disease Index (PDI) (Rahman *et al.,* 2008) (Table 1). Additionally, percentage decline in infectivity due to the treatment of biosurfactant was assessed through determination of total Aspergillus count /cm^2 on Sabouraud's Agar.

Result and Discussion

The prevalence of potent biosurfactant producing microorganisms from oil contaminated sites were studied from selected ecosystems based on the rationale that a high oil concentration increases the predominance of surface active agent producing organisms. Liquid paraffin which is a common agent used in fuels, paints, pigments, dyes and inks, wax, medicine (laxative), culinary, toiletries and cosmetics was used as source of hydrocarbon for primary screening as biosurfactants are associated with the uptake of hydrophobic carbon substrates like paraffin (Lang and Wullbrandt, 1999). Additionally paraffin utilization involves breaking of carbon chain C_{14}-C_{18}, which occurs at the interphase of water and insoluble hydrocarbon, (Cresenzi, 1995) and its dispersion is enhanced through production of biosurfactant. (Rosenberg, 1988). Thus using paraffin as the sole source of carbon in mineral Christova's medium enabled recovery of biosurfactant producing isolates.

Though most of the sampling sites showed high heterotrophic load ranging between 10^4 to 10^8 cfu/g, (Table 2) maximum localization of biosurfactant producers were obtained from soil samples collected from an Edible Oil Industry. This is similar to the results of Balogun and Fagade (2008) who reported a high heterotrophic load which reduced at least by two to three log when assessed for population that were biosurfactant producers. Tabatabae *et al.,* (2005) also reported total heterotrophic cell counts of oil well within the range of 10^3-10^5 cfu of which only 10^2 cfu were found to be potent biosurfactant producers.

Thus though four different samples were assessed and 19 different isolates were obtained , petrol pump sample showed maximum isolates. This could be related to the prevalence of hydrocarbon and oil in the

Table 1: Percent Disease Index (PDI) of potato tuber infection (Rahman *et al* ., 2008)

Sr. No.	Areas affected per tubers	*Infectivity Index*
1.	None or very few lesions on the tubers.	0
2.	More than 1% but less than 10% lesions on the tubers.	3
3.	More than 10% but less than 25% lesions on the tubers.	10
4.	More than 25% but less than 50% lesions on the tubers.	25
5.	Half of the foliage destroyed	50
6.	More than 50% but less than 75%. of the foliage destroyed	75
7.	More than 75% but less than 90% of the foliage destroyed	90
9.	Tuber completely destroyed	100

Table 2: Primary screening of isolates from various sampling sites based on paraffin utilization

Sr. Number	Soil samples collected from	Total heterotrophic load cfu/gm	Total paraffin utilizers cfu/gm	Number of OTU
A.	**Soil Near Garage**			
	Sai Car Servicing Center & Garage	$2.63X10^{8}$	$2.11X10^{4}$	8
B.	**Soil Sample Near Oil Containing Ecosystem**			
	Edible Oil Industry	$2.98X10^{5}$	$8.14X10^{4}$	11
C.	**Soil Near Petrol Pump**			
	Borivali Petrol Pump	$3.64X10^{4}$	$5.64X10^{2}$	14
D.	**Environmental sample**			
	Stagnant puddle water	$5.71X10^{4}$	$2.11X10^{4}$	6

ecosystem. A bacterial community in contaminated site tends to be dominated by the strains that survive the zenobiotic entity or is able to utilize it for its growth. In response to paraffin oil in the Christova's medium the organisms which are already acclimatized to the hydrocarbons in spilled engine oil, grease, petrol, and diesel could utilize paraffin as a sole source of carbon and hence could be obtained in higher numbers as compared to those obtained from other sampling sites.

Bernheimer and Avigad (1970) reported that the biosurfactant produced by *Bacillus* could lyse blood and since then has been used to quantify surfactins (Moran *et al.*, 2002) and rhamnolipids . Johnson and Boese-Marrazzo, 1980 and Carrillo *et al.* (1996) found an association between haemolytic activity and surfactant production, and recommended the use of blood agar for observing lysis as a primary method to screen for biosurfactant activity. Against the Cx ratio of 1.6 and 1.55 and % heamolysis of 22% and 24% obtained with standard strains *Bacillus subtilis* MTCC 2423, *Pseudomonas aeruginosa* MTCC 2297 respectively, our isolate showing greater % heamolysis values (Table 3).

Denger and Schink (1995) reported a direct correlation between surface activity and emulsification activity such that emulsification index has been used as a efficient screening method to detect surface active molecules (Denger and Schink, 1995; Makkar and Cameotra, 1998). Emulsions being unstable do not form spontaneously, and energy input through shaking, stirring, homogenizing, or spray processes are frequently needed. Surface active substances (bio-surfactants) can increase the kinetic stability of emulsions so that, once formed, the emulsion does not revert back into their separate phases over a considerable period of storage, as the polar groups within the molecule orient toward water, while its non-polar fraction are oriented toward the oil. Thus surfactant molecule lowers the interfacial tension between the oil and water phases. Surface tension at the air/water and oil/water interfaces can be most accurately measured using a tensiometer where in addition of the metabolite in air/water or oil/water systems at increasing concentrations, can cause a reduction of surface tension up to a critical level, above which amphiphilic molecules associate readily to form supramolecular structures like micelles, bilayers, and vesicle. Lang & Wullbrandt (1999) reported that the surface tension of distilled water is 72 dynes/cm^3, and addition of surfactant like molecules can lower this value to 30 dynes/cm^3 & beyond. Thus, our bacterial isolate *Sternophomonas maltophila* which showed higher haemolysis (88%) , potent surface tension reduction (72.36%), and higher emulsification activity (Singh and Desai, 1989) was used for further studies.

2.Biosurfactant Production, extraction and purification

At low pH value biosurfactants become insoluble which can be then precipitated and re-dissolved in organic solvents owing to the hydrophobic ends in the molecule (Swaminathan, 2011). Based on its molecular weight, chemical nature, form and solubility biosurfactants have been extracted by use of various organic solvents like methanol & chloroform (Bryant, 1990), hexane (Morikawa *et al.,* 1993 and Barkay *et al.,*

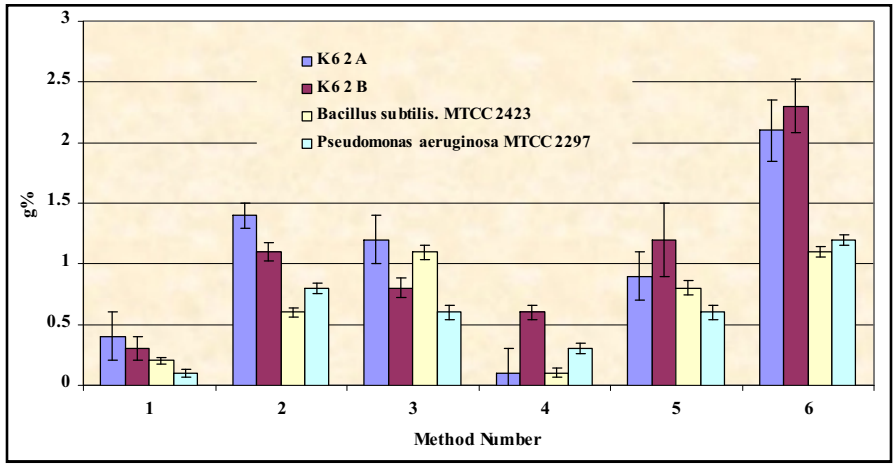
Table 3: Screening of Garage soil sample for Biosurfactant activity

Number of Isolates	Cx Ratio	%Haemolysis	EI(24 h)	% Surface Tension Reduction
A. Soil Near Garage				
SS 1	1.21	2	3	3
SS 2	1.36	1	4	2
SS 3	1.78	14	8	7
B. Soil Sample Near Oil Containing Ecosystem				
K 1	0.9	1.24	14	10
K 2	1.55	1.35	5	2
K 3	1.36	1.32	4	1
K 4	1.98	52	6	16.66
K 5	1.54	1.54	3	4.1
K 6 A	1.96	88	28	72.36
C. Soil Near Petrol Pump				
S 1	1.23	5	5	9
S 2	1.65	10	7	6
S 3	1.85	12	6	2
S 4	1.69	18	2	4
S 5	1.27	4	5	7
S 6	1.95	22	2	6
S7	1.36	1.32	4	4
S9	1.85	12	6	2
D. Environnemental sample				
GP 1	0	0		
GP 2	1.65	54	20	68.88
GP 3	1.85	26	3	10.21
E. coli (-ve Control)	0	2	0	2
Bacillus subtilis. MTCC 2423	1.5	22	12	60
Pseudomonas aeruginosa	1.55	24	16	55
MTCC 2297				

1999), dichloromethane (Cooper *et al.,* 1981), pentane (Macdonald *et al.,* 1981) have been used to extract. Hence selection of organic solvent needs to be standardized as suitable solvent plays important role in efficiency of the downstream recovery process the results of which are shown in Fig. 1**.** Chilled mixture of Chloroform: Methanol (2:1) caused maximum extraction of biosurfactant in organic layer for all the four different isolates studied though yields from isolate *Stenotrophomonas maltophilia* were approximately 2 folds more than that obtained from standard biosurfactant producer *Bacillus subtilis* MTCC 2423 & *Pseudomonas aeruginosa* MTCC 2297.

Methanol and chloroform in various ratios have been routinely used to extract lipids and thus forms an ideal extraction solvent system. In spite of that variation in the extraction efficacy was detected. Cooper *et al.* (1981) used Chloroform-methanol-water mixture

Figure 1. Biosurfactant extraction using organic extraction process



Key

Method Number	Reference	Organic Solvents Used
1.	Cooper *et al* 1979	Chloroform- methanol- water (2:1:1)
2.	Bryant 1990	Ethyl acetate methanol Silicic acid (1:2:1)
3.	Morikawa 1993 & Barkay *et al* 1999	Hexane CH_3 $(CH_2)_4CH_3$
4.	Cooper *et al* 1981	Dichloromethane (CH_2Cl_2)
5.	Macdonald *et al* 1981	Pentane (C_5H_{12})
6.	Desai & Banat 1997	Chloroform: Methanol(2:1)

[$CHCl_3$-CH_3OH-H_2O] to extract surfactant from *Corynebacterium lepus*, however, very little biosurfactant was isolated by this method in our study This could be attributed to the use of water in which chloroform largely remains immisicible and the actual ratio of Chloroform-methanol-water used in the experiment. It is reported that the system of lipid extraction could be best designed by maintaining the chloroform, methanol and water in the combined phases to 8:4:3 (by volume), otherwise selective losses of lipid may occur. In our experimental setup chloroform, methanol and water was maintained in 10:5:7 (by volume) as described by Cooper and Goldenberg (1981) and could be one of the reasons of not yielding surfactant for the isolate under study.

Purification

Though the metabolic filtrate used for the harvest of biosurfactant was free from the bacterial cells, presence of some cellular debris and proteins of the nutrient broth can form major impurities as they precipitate with chloroform -methanol along with the biosurfactant. Hence purification of the biosurfactant was undertaken with respect to the removal of protein from the biosurfactants extracted by organic solvent method by the potent isolate. Biosurfactant on re-precipitating yielded 37.5% protein reduction while, purification of biosurfactant through adsorption chromatography yielded 87.5% protein reduction without much loss of its lipid content

3. Sporolytic activity of *S. maltophilia* biosurfactant by Phase contrast microscopy

Fungal spore germination marks the resumption of vegetative development leading to the formation of a new individual or colony. Germination, usually follows

a period of dormancy, and is said to have occurred with the development of a new hypha. Thus, controlling fungal infection at spore germination level is economically crucial step, as it not only saves crop but also prevent the possible spread of the pathogen.

Though fungal spores are microscopic propagates that lack embryo specialized for dispersal or dormant survival, spores produced by sexual process (zoospore & ascospores) are usually functional in dormant survival. Such spore populations are challenging as their control is not possible by convenient techniques (Kasprzyk *et al.,* 2002) and hence seek special attention. The spore cell wall is thicker than somatic cell, have relatively lower water content and respiration rate with high content of energy storage material such as lipid glycogen and trehalose (Kasprzyk *et al.,* 2002) which makes its survival easier and elimination crucial. Thus means that effectively control of their germination on crop play a crucial role in management of various plant pathogens.

Qualitative assessment of lysis of the zoospores of *Aspergillus parasiticus* detected through heamocytometer (Table 4 & Fig. 2) using a phase contrast microscopy at 2% concentration of biosurfactant was not effective as it did not cause any spore lysis even over a time period of 120 mins. The time kill response of the biosurfactant at 4% concentration was also at 120 min as the 94 % reduction in the viability of the spore was detected while at 6% & 8% concentration the biosurfactant was sporolytic at lower time period of 80 mins (92% effective reduction) & 60 min (89% effective reduction) respectively. 10% biosurfactant was the most successful amongst the lot as it could lyse 100% of the spore in shortest time of 10 min and thus can be the most effective concentration to be used in crop care formulations. Though very little information with respect to the proposed mechanism for zoospore lysis upon exposure to the biosurfactant is known, motility of zoospore was found to be affected followed by an explosion of zoospore. It is reported that membranes of *Blastocladiella emersonii* zoospores contains 49% lipid with most of them being glycolipids and neutral lipids, and the interplay of the surfactant with such molecules could be the means of its effective lysis.

4. Sporolytic activity of biosurfactant of *S. maltophilia* **to control potato tuber wilt infestation**

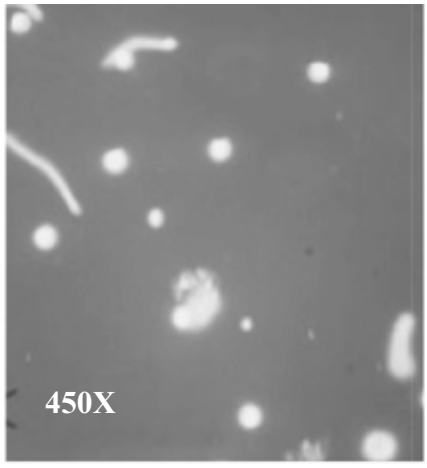
Potato (*Solanum tuberosum* L.) is one of the four major food crops of the world as it produces more dry-bio mass, has well balanced protein and produces more calories from unit area of land and time than other major food crops besides containing essential nutrients such as proteins and minerals like calcium, phosphorus iron, and vitamins (B1, B2, B6 and C). However, risk of aflatoxin contamination due to *Aspergillus parasiticus* infestation need to be controlled if this crop potential is to be achieved. Harvested potato tubers are consistently infested with aerial spores of *Aspergillus spps* during storage and assessment of post harvest technology to reduce such infestation through spray of biosurfactant

Table 4 Integrity of *Aspergillus parasiticus* NCIM 898 spores obtained after challenging with biosurfactant of *S. maltophilia*

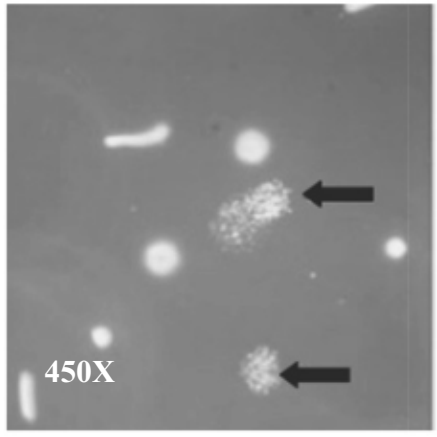
Sr. No.	% Biosurfactant	← Time interval in min →					
		10	40	60	80	100	120
1.	2%	X	X	X	X	X	X
2.	4%	X	X	X	X	X	√
3.	6%	X	X	X	√	√	√
4.	8%	X	X/√	√	√	√	√
5.	10%	√	√	√	√	√	√

(√) → spore lysed
(X) →no spore lysis

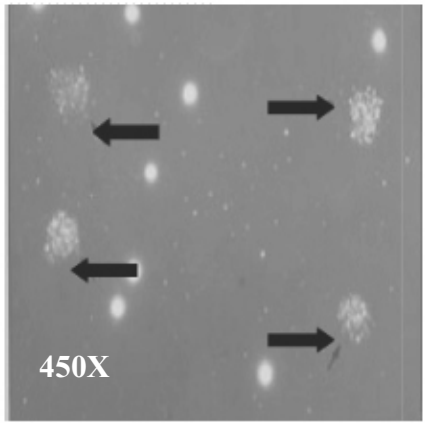
Figure 2: Effect of biosurfactant on spore lysis of *Aspergillus parasiticus* NCIM 898



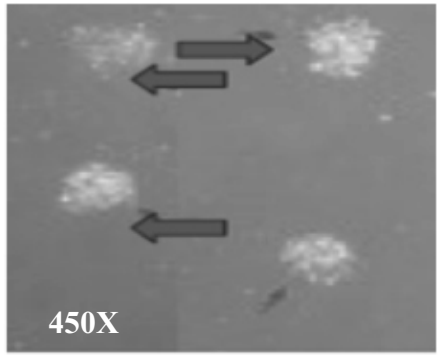
8% biosurfactant /10 min
SPORES INTACT



8% biosurfactant /40 min
FEW SPORES STARTED BURSTING



8% biosurfactant /60 min
SPORES COMPLETELY BURST



10% biosurfactant /10 min
SPORES COMPLETELY BURST

Table 5 Time kill response of various concentration of biosurfactant *S.maltophilia* on viability of *Aspergillus parasiticus* spores

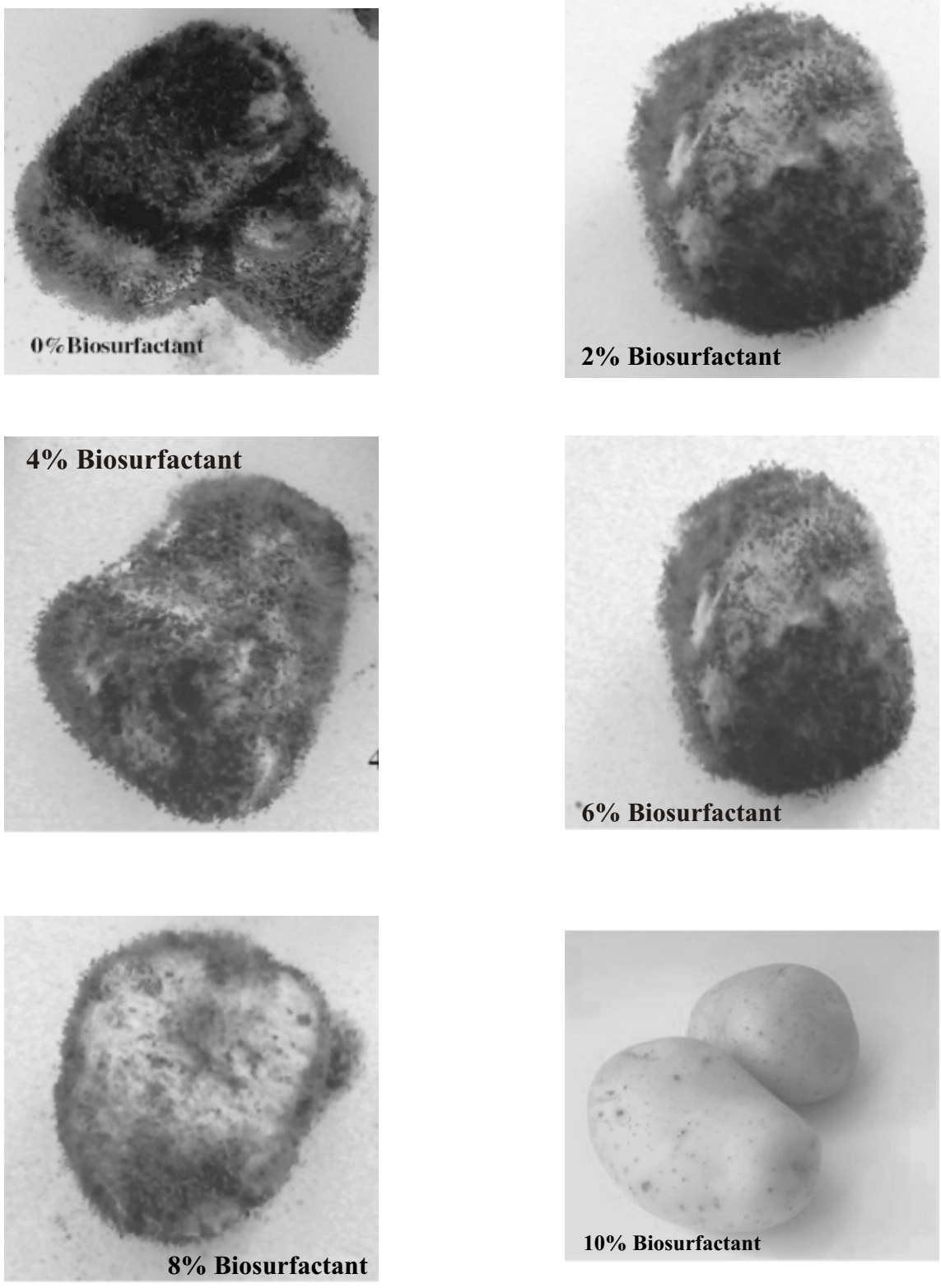
	Time of Exposure (in min) of biosurfactant					
	10	40	60	80	100	120
Concentration of Biosurfactant	**Percentage reduction in Viability of Spore**					
2%	6	6	13	14	14	18
4%	8	28	42	58	78	94
6%	24	42	84	92	100	100
8%	37	61	89	100	100	100
10%	100	100	100	100	100	100

S. maltophilia was undertaken the results of which are shown in Table 5 & Fig. 3 Treatment of the tubers with increasing concentration of biosurfactant caused a graded reduction in infectivity index. Though, 2% concentration was not quite effective in changing the infectivity index increase in its concentration caused its viable count as well as infectivity index to decrease, such that at 10% biosurfactant concentration infectivity index observed as 0 and 100% reduction in recovery of *Aspergillus parasiticus* NCIM 898 from potato tuber was detected . Though 10% biosurfactant treatment was very effective in preventing any fungal infestation, exposure to 8% biosurfactant was partially effective as it caused tubers to retain large uninfected areas averaging between 2 mm to 15 mm in diameter which were smooth with darkened skin devoid of any pycnidia though occasionally brown-black, powdery cellular masses lining the skin (Fig. 3) were observed. Tissue bits removed aseptically from such lesion borders yielded on potato dextrose agar few colonies of *Aspergillus parasiticus* NCIM 898, indicating 10% biosurfactant was required to effectively prevent infestation by 1 $X10^3$ cfu/ ml of *Aspergillus parasiticus* spores. Though the exact mechanism of biosurfactant action against pathogenic fungi is still unclear however there are reports of use of *Serratia marcescens* & *Pseudomonas aeruginosa* mediated biosurfactant molecule to eliminate fungal infection. It is believed that these organisms produce systemic elicitors which play role in degradation of fungal cell wall. (Lotan & Fluha, 1990) and thus prevent its infestation.

Thus application of S. maltophilia biosurfactant could be effective crop management method where in due to its ability to lyse spores, fungal infection could be terminated without causing undue stress to the plant. The root of this technology lies in the principle ability of the surfactant to reduce surface tension and break cell wall of the spore, causing intracellular leaking and inactivity and should be kept in mind in formulations that are used for crop protection and management.

The identification of biosurfactant-induced rupture of the plasma membrane as a new type of antagonistic mechanism, coupled with knowledge of the specific target, i.e., zoospores, and the common occurrence of epiphytic biosurfactant-producing bacteria in nature, have significant implications for the biological control of zoosporic plant pathogens. This is especially useful in controlling pathogens that cause destructive foliar diseases. The presence of free water, which is essential for zoospore liberation, spread, and motility, would also provide the optimum environment for the timely solubilization and dispersal of in situ produced biosurfactant. Thus, this study , constitute a cautious but optimistic view that biological control of zoosporic plant pathogens can be achieved by management of the physical and chemical environment, through activities that enhance the introduction or naturally occurring biosurfactant- producing bacteria.

Figure 3. Effect of biosurfactant on *Aspergillus parasiticus* NCIM 898 on percent disease index on *Solanum tuberosum Linnaeus* (potato tubers)



References :

Balogun A. and Fagade O. (2008): Screening for Surface-active Agent Producing Bacteria from Diesel Oil Polluted Tropical Soil, *World Appl. Sci J*. **36,** 930-933.

Barkay T., Navon-Venezia, S., Ron E.Z., and Rosenberg E. (1999): Enhancement of solubilization and biodegradation of polyaromatic hydrocarbons by the bioemulsifier alasan. *Appl Environ Microbiol* **65,** 2697-2702

Bernheimer A and Avigad L. (1970): Nature and properties of a cytolytic agent produced by *Bacillus subtilis*. *J Gen Microbiol*; **61**, 361-9.

Bryant O. **(**1990): Improved method for the isolation of biosurfactant glycolipids from *Rhodococcus* sp. strain H13A. *Appl. Environ. Microbiol*.**56,** 1494–1496.

Cairns W. L., D. G. Cooper, Zajic J. E., Wood J.M., Kosaric N. **(**1982): Characterisation of *Nocardia amaree* as a potent biological coalescing agent of water-oil emulsions. *Appl. Environ. Microbiol*. **43,** 362–366.

Carrillo P.G., Mardaraz C., Pitta-Alvarez S.I. and, Giulietti A.M. (1996): Isolation and selection of biosurfactant-producing bacteria. *World J. Microbiol. Biotechol.*, **12**, 82-84

Cooper D. G., MacDonald C. R., Duff S. J. B, Kosaric N. (1981): Enhanced production of surfactin from Bacillus slubtilis by continuous product removal and metal cation additions. *Appl. Environ. Microbiol.* **42,** 408-412.

Denger K. and Schink B. (1995): New halo- and thermotolerant fermenting bacteria producing surface-active compounds. *Appl. Microbiol. Biotechnol*., **44**, 161-166.

Desai A.J. and Banat I.M. (1997): Microbial Production of Surfactants and Their Commercial Potential *Microbiol & Mol Biol. Rev.* **61**:1, 47-64.

Dehghan-Noudeh, G.M. Housaindokht, B. Sedigeh, F. Bazzaz (2005): Isolation, Characterization, and Investigation of Surface and Hemolytic Activities of a Lipopeptide Biosurfactant Produced by *Bacillus subtilis* ATCC 6633.

Georgiou G., Lin S.C., Sharma M.M. (1990): Surface active compounds from microorganisms. *BioTechnol* **10**: 60-65.

Hornby D. (1990): Biological Control of Soil-Borne Plant Pathogens. Ed. (CAB International, Wallingford, U.K.).

Johnson M.K. and Boese-Marrazzo D. (1980): Production and Properties of Heat Stable Extracellular Hemolysis from *Pseudomonas aeruginosa*. *Infect.Immun*. **29**, 1028-1033.

Kasprzyk, Lehvaslaiho H., Lijnzaad P., Melsopp C., Mongin E., Pettett R., Pocock M., Potter S., Rust A., Schmidt E., Searle S., Slater G., Smith J., Spooner W., Stabenau A., Stalker .J, Stupka E., Ureta-Vidal A., Vastrik I., and Clamp M. (2002):The Ensembl genome database project. *Nucleic Acid Research*, **30**, 38–41.

Lang S. and Wullbtandt D**.** (1999): Rhamnose lipids – biosynthesis, microbial production and application potential. *Appl. Microbiol. Biotechnol.* **51**, 22-24.

Lotan T. and Fluha R., (1990): Xylanase, a novel elicitor of pathogenesis - related proteins in tobacco, uses a non-ethylene pathway for induction. *Plant Physiology*, **93**, 811-817.

Lowry O., Rosebrough N., Farr A., Randall R. (1951): Protein measurement with the folin phenol reagent. *J. Biol. Chem*. **193**, 265-275

MacDonald R., Cooper D. and Zajic J. **(**1981): Surface-active lipids from *Nocardia erythropolis* grown on hydrocarbons. *Appl. Environ. Microbiol*. **41,** 117-123.

Makkar R. and Cameotra S. (1997): Biosurfactant production by a thermophilic *Bacillus subtilis* strain.*J. Ind. Microbiol. Biotechnol*, **18**, 37-42.

Makkar R. and Cameotra S. (1998): Production of biosurfactant at mesophilic and thermophilic conditions by a strain of *Bacillus subtillis. J of Microbiol and Biotechnoly.* **20,** 48-52.

Moran A.C., Alejandra Martinez M., Sineriz F., (2002) Quantification of surfactin in culture supernatant by hemolytic activity. *Biotechnol. Lett.* **24**: 177–180.

Morikawa M., Daido H., Takao T., Murata S., Shimonishi Y., Imanaka T. (1993): A new lipopeptide biosurfactant produced by *Arthrobacter* sp. strain MIS 38. *J. Bacteriol*., **175 :** 6459-6466.

Rahman P.K.S.M. and Gakpe E. (2008) : Production, Characterisation and Applications of Biosurfactants-Review. *Biotechnology*. **7**:2, 360-370.

Rosenberg E., Rubinovitz C., Gottlieb A., Rosenhak S., and Ron E. Z. **(**1988): Production of biodispersan by *Acinetobacter calcoaceticus* A2. Appl. *Environ. Microbiol*. **54,** 317–3.

Saini H., Khehra M., Sharma D., Chadha B., Chimni S., (2005): Comparative studies on potential of consortium and constituent pure bacterial isolates to decolorize azo dyes. *Water Res*. **39,** 5135-5141.

Singh M., and Desai J.D. **(**1989): Hydrocarbon emulsification by *Candida tropicalis* and *Debaryomyces polymorphus*. *Indian J. Exp. Biol*. **27,** 224–226.

Swaminathan M.S. (2011): World food security hunger and health : An overview. Millennium Goal Conference, Canada.

Tabatabaee A., Assadi M.M., Noohi A.A., Sajadian V.A. (2005): Isolation of Biosurfactant Producing Bacteria from Oil Reservoirs. *Iranian Journal of Environment Health Science Engineering,* **2**(1), 6-12.

Velikonja J. and Kosaric N. (1993): Biosurfactant in food applications, 419–446. *In* N. Kosaric (Ed.), Biosurfactants: production, properties, applications. (Marcel Dekker Inc., New York, N.Y.).

Zhou T. and Paulitz T.C. **(**1993): In Vitro and in Vivo Effects of *Pseudomonas* spp. on *Pythium aphanidermaium:* Zoospore Behavior in Exudates and on the Rhizoplane of Bacteria - Treated Cucumber Roots *Phtopatho.* **83**, 872—876.