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Full Length Research Paper

Physical adsorption immobilization of antimicrobial peptide (bacitracin) producing bacillus strain GU215 on polystyrene film

Adnan Amin[1]*, Muhammad Ayaz Khan[1], Ali Zaman[3], Abdul Haleem Shah[4], Uzma Haroon[2], Sheikh Muhammd Farooq Azam[2] and Muhammad Yasin[1]

[1]Gomal Center of Biochemistry and Biotechnology (GCBB) Gomal University, D.I.Khan, KPK, Pakistan.

[2]Department of Chemistry, Gomal University, D.I.Khan KPK, Pakistan.

[3]Department of Microbiology, Gomal College of Veterinary Sciences (GCVS) Gomal University D.I.Khan, KPK, Pakistan.

[4]Department of Biological Sciences, Gomal University, D.I.Khan, KPK, Pakistan.

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The physical immobilization of *Bacillus* sp GU215 on polystyrene polymer film by adsorption was performed and bacitracin (antimicrobial peptide) production by both free and immobilized cells were investigated at optimum conditions. The immobilized cells presented immediate log time within 4 h, while the free cells showed a 24 h delayed response. Likewise, free cells of *Bacillus* spp GU215 showed maximum zones of inhibition (18 mm) within 72 h at 30°C temperature and pH 8, whereas polystyrene polymer immobilized cells presented largest 14 mm zones of inhibitions within 48 h at same conditions as free cells. We concluded that there existed a weak physical adsorption between polystyrene polymer film and immobilized bacterial cells that rendered earlier detachment of cells and resulted in a decrease in antimicrobial activity as compared to free cells.

Key words: Polystyrene, bacitracin producing cells, immobilization, adsorption, log time.

INTRODUCTION

The physiology of interactions between microorganisms and solid supports of different materials for fixation remained the goal of many researchers since last several decades. Two main research directions emerged from these studies: (i) the cell adhesion on solid surfaces (inclusive of growth and proliferation) and (ii) the killing action of surfaces (Mahfoudh et al., 2010).

These days, application of immobilized cells and enzymes have captured the interest of researchers (Moreno-Garrido, 2008) to search for cost effective, efficient, stable and non toxic immobilization vehicle (Li et al., 2008). Generally, the cell immobilization is successfully performed by a variety of methods including cross-linking, physical entrapment, covalent coupling and the natural or physical adhesion.

*Corresponding author. E-mail: dani_amin79@yahoo.com.

The cross-linking and physical entrapment methods are effective only with high cell concentrations; however enjoy a limited acceptance as a result of diffusional limitations associated with gel like immobilization media (Klein and Wagner, 1983). The covalent coupling involves a strong covalent adhesion of cells onto the support, thus yield a strong and mostly permanent fixation. However the toxicity of coupling chemicals originates limitations of loss of enzyme activity and cell viability (Costa et al., 2011). Besides all, the natural adhesion is not only an attractive technique for researchers due to natural biofilm formation and offer maximum levels of cell viability and biochemical activity (Klein and Wagner, 1983) but also provides safestable, community based environment to bacteria that is very essential for nomadic survival (Dunne, 2002).

The polystyrene (PS) is a well recognized adhesion material as it is not toxic, having low production cost and easy shapability (Guruvenket et al., 2004) Polystyrene

(PS) is commonly employed for cell immobilization due to the significant optical as well as mechanical properties aiming at enhanced production (Hafieezullah et al., 2010)

With keeping in mind the importance of polystyrene as immobilization support, the present work aimed to evaluate the bacitracin production by bacillus spp GU215 immobilized by physical adsorption on polystyrene film with optimized conditions of pH, incubation time and glucose concentration.

MATERIALS AND METHODS

Microorganism and fermentation media

An antimicrobial peptide (bacitracin) producing strain (bacillus sp GU251) isolated locally in Gomal Center of Biochemistry and Biotechnology (GCBB), Gomal University D.I.khan KPK Pakistan was used during the study. The bacillus strain was identified using biochemical means and preserved on agar slants at 4°C . This isolated bacterial culture was transferred at least two times followed by inoculation in fermentation media. The fermentation medium used investigation have (g/L); L-glutamic acid 5.0; $MnSO_4.H_2O$ 0.01, $MgSO_4.7H_2O$, Glucose 10, KH_2PO_4 0.5 $FeSO_4.7H_2O$ 0.01, K_2HPO_4 0.5; NaCl 0.01; $CuSO_4.7H_2O$ 0.01, $CaCl._2H_2O$ 0.015; pH 8).

The cell suspension

To a well grown (at least 4days) bacterial slant, about 2 ml sterilized distilled water was added and mixed gently to produce a turbid cell suspension. This was transferred to about 48 ml of nutrient broth in an Erlenmeyer flask. The flaks containing well grown bacterial suspension was incubated in orbital shaker incubator at 120 rpm at 30°C for at least 72 h. The broth culture was later ce ntrifuged and the pellets so formed were washed using 0.9% NaCl and 2% KCl. The Washed pellets were resuspended in sterilized 0.9% NaCl. This cell suspension was used as stock culture for immobilization procedures.

Antimicrobial activity of bacitracin

The antibacterial activity of bacitracin was determined using agar diffusion method (Barefoot and Krauthammer, 1983). Shortly the fresh samples were taken from fermentation broth in micro centrifuge tubes and centrifuged for 15 min at 12,000 rpm. Samples from inoculated fermentation media were taken an aseptically and centrifuged in micro centrifuge at 12,000 rpm for 15 min. About 5 µl cell free supernatant was transferred using micropipette to pre formed wells (6 mm diameter) in sterilized agar plates that were seeded earlier with lawn of the *Staphylococcus aureus* (ATCC# 6538). After sample application, agar plates were incubated at 30°C for 24 h and activity was determined by zones of inhibition around wells.

Measurement of cell density

The cell density of fermentation media was determined by taking the optical density (OD 600 nm) using a UV spectrophotometer (Shimadzo, Japan). The dry cell weight (DCW) was measured by taking about 18 ml of cell culture in centrifuge tubes by weight. The cell culture was centrifuged at 12,000 rpm for 15 min. The supernatant obtained was washed three times with sterilized demineralized water and dried in oven at 70°C for 24 h followed by determination of weight.

Optimization of various parameters for maximum antibiotic production

The bacitracin production was optimized in reference to time of incubation (0-96 h), PH of fermentation media (6-8) and glucose concentration (2-5%).

Immobilization on polystyrene

The polystyrene films were prepared by dissolving 0.8 g of polystyrene into 100 ml chloroform with continuous agitation. After the chloroform has been evaporated, 2 × 2 polystyrene chips were made using sharp sterile cutter. The polystyrene chips (about 100 of size 2 × 2 mm) were first rinsed with spirit, follo wed by three times washing with sterilized distilled water. The polystyrene chips were transferred aseptically into 20 ml culture broth and 5 ml microbial suspension in a 50 ml flask and incubated for 24 h in orbital shaker at 120 rpm and 30°C temperatures for im mobilization. After immobi-lization has taken place, the polystyrene chips were washed three times with sterilized distilled water and transferred to fermentation media. The inoculated fermentation media was incubated in orbital shaker incubator at 120 rpm up to 72 h at 30°C (Hafieezullah et al., 2010).

RESULTS

Bacitracin producing strain

The bacitracin (antimicrobial peptide) producing bacillus strain was identified as bacillus spp GU215 using biochemical and cytomorphological methods (MacFaddin, 2000; Krier and Holt, 1984).

Bacitracin production by free and polystyrene immobilized cells

During present investigation the bacitracin production by free and polystyrene immobilized *Bacillus* spp GU215 was studied using batch fermentation mode. The widest zones of inhibition (18 mm) presented by free cells after at pH 8 in 72 h. A progressive expansion in the zone of inhibition was observed in accordance with increase in concentration of glucose (carbon source) by 4%, that suffered a steady decline in activity with further increase in glucose concentration (Figures 1 and 2).

Nevertheless the immobilized cells presented significantly higher antibacterial activity compared to free cells. Maximum activity of polystyrene immobilized cells (13 mm) was observed in 72 h, at PH 8. Similar as free cells widest zones of inhibitions were observed at 4% glucose concentration (Figures 3 and 4).

DISCUSSION

The immobilization of secondary metabolite producing

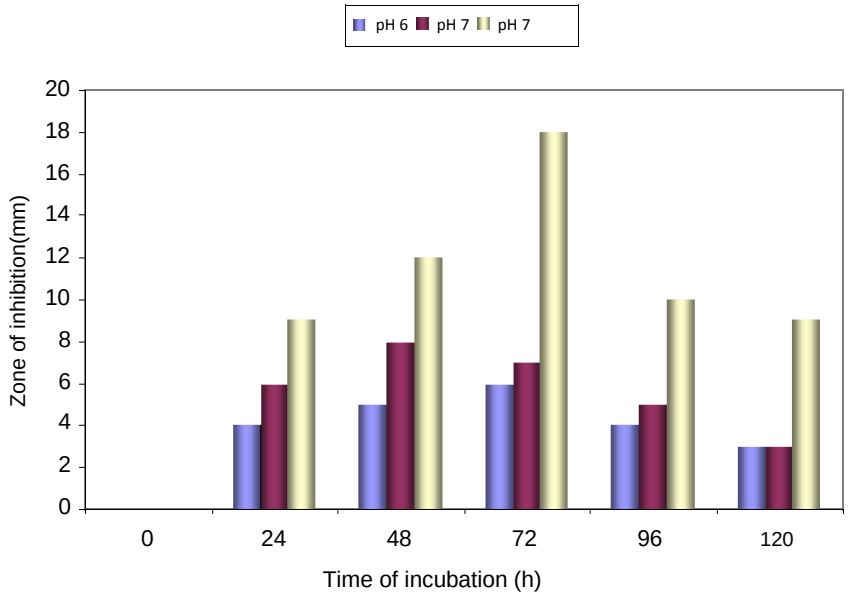

Figure 1. Maximum antimicrobial peptide (Bacitracin) production by free cell at various pH (6-8) and time of incubation (0-120 h).

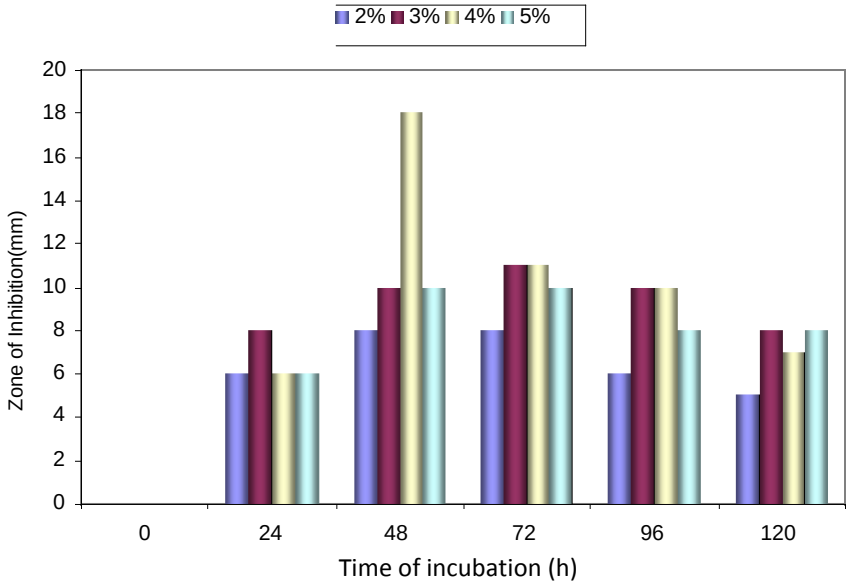

Figure 2. The optimized antimicrobial peptide (Bacitracin) production by free cell at various glucose concentration (2-5%) and time of incubation (0-120 h).

bacterial cells onto a solid support is well known since decades among researchers and is successfully practiced in industry (Schallmey et al., 2004; Hamedi et al., 2005; Mendo et al., 2004). To the best of our knowledge, no data is available regarding the immobilization of bacillus spp onto polystyrene film by physical adsorption. Present research is probably the first to investigate the effect of immobilization on antimicrobial peptide production by bacillus spp GU215, using polystyrene film by physical adsorption, and optimization of various growth parameters.

During present investigation, enhanced activity of

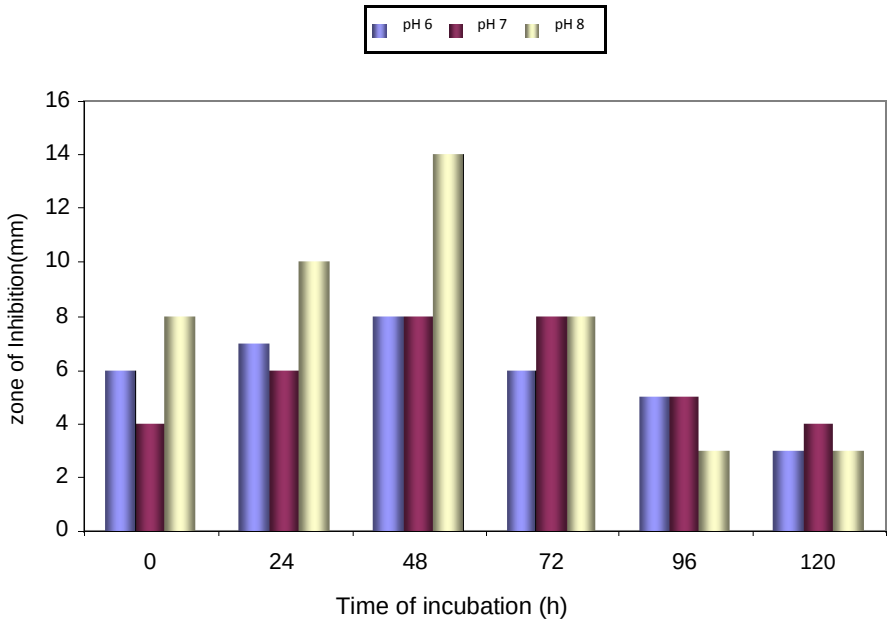

Figure 3. The maximum antimicrobial peptide (Bacitracin) production by polystyrene immobilized at various PH (6-8) and time of incubation (0-120 h).

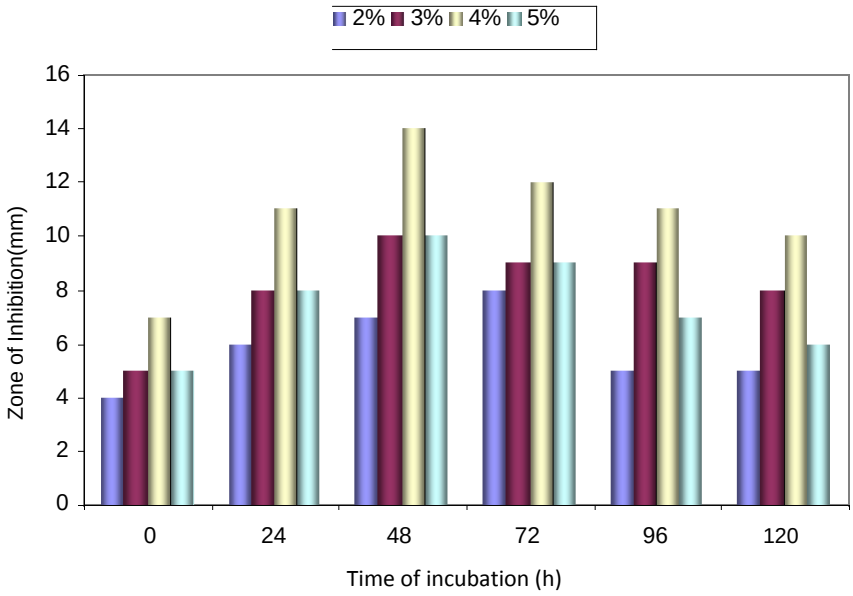

Figure 4. The optimized antimicrobial peptide (Bacitracin) production by polystyrene immobilized cell at various glucose concentration (2-5%) and time of incubation (0-120 h).

immobilized cells was noticed as compared to free bacillus cells. The lag time of the polystyrene immobilized cells began immediately (after 4 h of incubation) as observed previously by many researchers using different immobilization media (Costa et al., 2011; Chung et al., 2003; Ahmed et al., 2009). The relatively earlier exposure of immobilized cells to log time can be the result of induced stress during immobilization (Prakasham et al., 2002). It was surprisingly observed that the immobilized cells not only presented less activity than the free cells

but it only persisted for a very brief time i.e 48 h only. A peer literature review revealed that the reduced activity of immobilized cells onto polystyrene surface is due to absence of cell binding factors (Kooten et al., 2004; Mitchell et al., 2005). Polystyrene, like the other common synthetic polymers does not possess the components for specific cell surface receptors resulting in poor cell attachment that resulted in cells de sorbption (Sano et al., 1993; Gupta et al., 2002).

The effect of PH on secondary metabolite production has been studies by many researchers and it was evident that change in the pH mainly interferes the synthesis of secondary metabolite in the bacterial cells (Solé et al., 1997). During current study both free and immobilized cell showed maximum activity at alkaline pH, although the bacillus strain was also responsive to other pH levels.

However reduced activity was reported at compara-tively acidic environment. This corresponds to natural habitat of bacillus spp as reported in numerous studies that involved *bacillus spp*. (Hafieezullah et al., 2010; Zasloff, 2002; Bushra et al., 2007).

Never the less, presence of carbon source in the fermentation media is very essential for the optimum antibiotic production for both free and immobilized cells (Mendo et al., 2004; Janabi, 2006; Esikova et al., 2002). It was evident during the investigation that increase in the glucose concentration directly increases the metabolism of the microorganism and hence ends up in improved activity. In both free as well as immobilized cells, a progressive increase in activity was observed irrespective of pH levels till a peak level was achieved at 4% glucose concentration followed by steady decline in the activity. Nearly similar trend was reported by previous reports (Awais et al*.,* 2010; Muhammad et al*.*, 2009).

Conclusion

It is concluded that the immobilization of bacillus spp GU215 by adsorption on polystyrene film did not supported bacitracin for enhanced time due to weak attractions. Further studies are in progress to investigate the covalent immobilization of bacillus spp GU215 on polystyrene.

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