Research Article

Detection of Quorum Sensing in Bioemulsifier Producing Bacteria

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ABSTRACT

Bacteria produces many kinds of molecules that allow bacteria to communicate about population size, metabolic states or producing end products that initiate some activities such as bioluminescence. These signal molecules are generally called as autoinducers. Quorum sensing reveals the fact that bacteria have the capacity to assess the number of other components they can activate once the threshold number is reached. Acyl homoserine lactones are present mainly in gram negative bacteria and they control their own synthesis. In AHLs, the head group consists of homoserine lactones and the tail region determines the specificity of the receptor. Oligopeptide molecules are present mainly in gram positive bacteria. Their synthesis is dependent on ribosomes. In the present study it is hypothesized that the production of autoinducing peptides and biofilm formation are interlinked. The small peptides which may have antimicrobial activity will also act as signals for the bacteria to form biofilm when they reach considerable cell density.

Keywords: Auto inducers, AHLs, Gram specific strains, oligopeptides, signaling molecules, stationary phase, quorum sensing

INTRODUCTION

Bacteria have signalling mechanism to communicate between the cell communities. Gram-negative bacteria utilize acylated homoserine lactones (AHLs) as signalling molecules and gram-positive bacteria make use of small peptides (oligopeptides) as signalling molecules. These signal molecules enable specific intraspecies communication[1]. Quorum sensing in gram positive bacteria involves signal oligopeptides. The biofilm producing organisms are highly resistant to antibiotics[2]. The use of drugs that destroy the biofilm producing pathogens is the major challenge in the current research scenario. The eradication of disease and infection causing pathogens is very difficult particularly pathogens that produce biofilm. Blocking quorum sensing (Quorum-quenching) in can be one of the strategies to destroy the biofilm producing pathogens. Lot of research has been focused on finding natural quorum-quenching molecules that can be used along with new generation of antimicrobials[3]. Quorum quenching compounds has to be designed from natural sources. Various plant extracts have been reported to have antibiofilm activity of Acinetobacter spp[4,5]. Theoretically, the microorganisms reach the stationary phase due to the depletion of nutrients or accumulation of nutrients[6,7]. In this case the microorganisms should not have grown in the spent media. But a considerable microbial growth was observed in spent media. It indicated that the microorganisms release signaling molecules once they attain considerable cell density/number. The idea of the proposal is originated by taking the cue from the growth of microorganisms in spent culture. Though there are many reports available on autoinducing peptides of Gram positve bacteria[8]. Very few reports are available on autoinducing peptides of Gram negative bacteria[9]. This may also lead to identifying the anti-inducer molecules which can be targeted against virulence factors. Biofilm producing microorganisms are more resistant to antimicrobial substances[10,11]. In the present study it is hypothesized that the production of autoinducing peptides and biofilm formation are interlinked. The small peptides which may have antimicrobial activity will also act as signals for the bacteria to form biofilm when they reach considerable cell density.

MATERIALS AND METHODS

Estimation of growth profiles in broth and supernatant inoculated cultures

The overnight culture of A*cinetobacter M6*, KR559749 was inoculated in LB broth and maintained in two sets, in which one set is autoclaved and another set is not autoclaved and are performed in triplicate. The growth was monitored at 600 nm till they attained the stationary phase. When the cultures attained the stationary phase they were centrifuged at 10,000g at 4°C for 10 minutes and the supernatants were collected. The procedure was repeated by inoculating A*cinetobacter M6*, KR559749 in to their respective supernatants. The initial and final concentrations of carbohydrate and protein content with respect to the culture free broth was estimated using phenol-sulphuric acid and Folin-Lowry methods respectively[12].

Bioemulsifier production and quantification

The Bioemulsifier production was studied in LB medium by growing sample-M for 7 days in the respective *Acinetobacter* Supernatants. The supernatant was collected by centrifugation, dried, lyophilized and

analyzed for Bioemulsifier production by measuring total carbohydrate and protein contents.

Estimation of protein content by Folin-lowry method

The protein content of the supernatants was estimated by using Folin-Lowry method using BSA (100 µg/ml) as a standard protein concentration. Absorbance was measured at 660 nm by plotting the standard graph and the amount of protein present in the supernatants were estimated from the standard graph[12].

Estimation of carbohydrate content by Phenol-sulphuric acid method

The carbohydrate content of the supernatants was estimated by using phenol-sulphuric acid method using glucose (100 µg/ml) as a standard sugar solution concentration. Specific volumes were taken into different test tubes. Each tube volume was made up to1000 µl using distilled water. To each of the standard and test sample tube, 0.05 ml of 80% phenol was added followed by 5 ml of concentrated sulphuric acid and allowed to stand at room temperature for 10 min. Then tubes were kept in water bath at 30°C for 20 min and were cooled to room temperature and observed OD at 490 nm[12].

RESULTS AND DISCUSSION

Estimation of growth profiles in broth and supernatant inoculated cultures

In our experiments the obtained results show that the gram negative *Acinetobacter* strain, when inoculated in their respective supernatants (spent media) showing less growth in autoclaved supernatant when compared to the without autoclaved supernatants (Figure 1) justifying the statement that the AHLs which are responsible for growth regulation gets denatured when supernatants are subjected to autoclave there by providing a favorable environment for the bacteria to grow restricts the growth of the bacteria to continue further even when there is nutrient availability in supernatant through the mechanism of quorum sensing. Normally the presence of AHLs, auto inducers of gram negative bacteria restricts the growth of the bacteria to continue further even when there is nutrient availability in supernatant through the mechanism of quorum sensing.

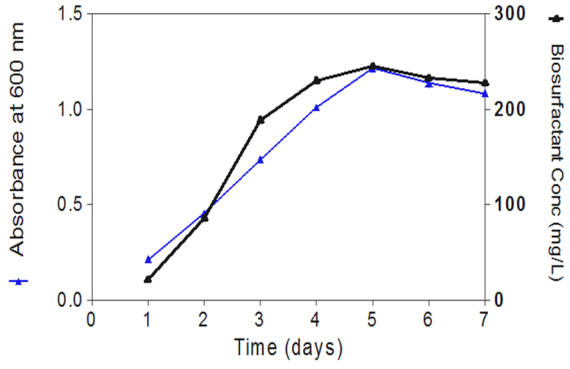

Figure 1: Growth profile of the gram negative Acinetobacter (with autoclave) in the supernatant

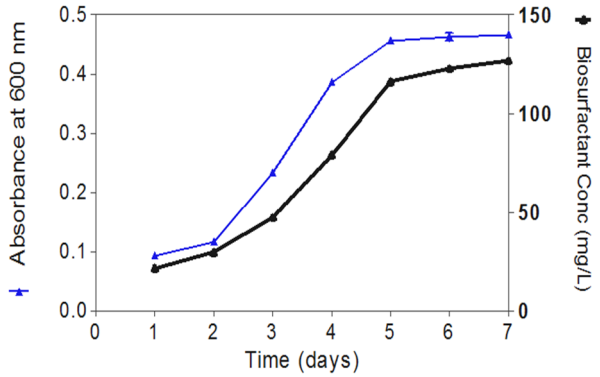

Figure 2: Growth profile of the Acinetobacter (without autoclave) in the supernatant

Estimation of protein content by Folin-lowry method

The initial concentration of protein in the broth before the inoculation of culture is 540 µg/mL. It is observed that there is a little considerable amount of protein utilization when the cultures were grown in broth and there is not any considerable utilization of protein in the supernatants.

Estimation of carbohydrate content by Phenol-sulphuric acid method

The initial concentration of carbohydrate in the nutrient broth before the inoculation of is 18.2 µg/mL. It is observed that the carbohydrate utilization is not so appreciable when the cultures were grown in broth as well as in the supernatants.

CONCLUSION

Generally most of the micro organisms grow comfortably in the nutrient broth to some period of time till there is a rich nutrient availability. When there is a depletion of nutrients in the medium, the cells communicate among themselves by quorum sensing by which they stops the growth and attains stationary phase. So assuming that there is a limited nutrient availability in the medium, we have tested the presence of carbohydrates and proteins in the spent media by phenol sulphuric acid and Folin-Lowry methods. On assuring that there is a presence of carbohydrates and proteins in the medium, we have cultured same organisms in their respective supernatants which have shown the results that there is not any considerable growth in the without autoclaved supernatant representing that the auto inducers are present in the supernatant which are limiting the growth of cultures even in the presence of nutrients in the supernatant. On the other hand, the cultures which are inoculated in the autoclaved supernatants have shown a considerable growth in their respective supernatants representing that there are no auto inducers to limit their growth in the medium, so that they have grown by utilizing the nutrients in the supernatant. Further we have to focus on the concept that whether these AHLs or oligopeptides shows any specificity or any other organism can grow in the supernatant of another organism.

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