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KINETICS STUDY FOR HEAT TOLERANCE OF LDPE DEGRADING STRAINS

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ABSTRACT

Some microorganisms are sensitive to heat. They do not survive if subjected to higher temperature for longer duration. In this research, two bacterial isolates were screened and identified as *Bacillus amyloliquefaciens* BSM-1 and *Bacillus amyloliquefaciens* BSM-2 which are able to degrade the low density polymers. The thermal inactivation study was done for both isolates at four different temperature and survival curves were obtained for a specific temperature. The thermal death constants values are indicating that BSM-2 was more heat resistant than the BSM-1. This may be due to the synthesis of heat shock protein by BSM-2. Thus isolate BSM-2 was showed more heat tolerance and survived in higher temperature for maximum time and able to degrade the high percentage of polymers.

KEYWORDS: LDPE degrading bacteria, Heat tolerance, Thermal death constant, Heat shock protein.

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INTRODUCTION

Heat is lethal to microorganisms, but each species has its own particular heat tolerance. During a thermal destruction process, the bacteria subjected to heat are killed at a rate that is proportional to the number of organisms present. The process is dependent both on the temperature of exposure and the time required at this temperature to accomplish to desired rate of destruction [1]. The theory that thermal death of bacteria follows the kinetics of a unimolecular reaction (first-order kinetics) has been discussed by many researchers [2-5]. A first-order reaction is one in which the rate is proportional to the number of molecules present. If thermal death of bacteria truly follows first-order kinetics, death must then result from inactivation of a single molecule or site per bacterial cell. Furthermore, the death rate should be highest at the start when the number of bacteria is highest [6]. Thermal death kinetics refers to the rate at which the microbes die at abnormal conditions of temperature. This character depends on the microbe's type, its call wall composition, heat shock proteins which enable few species to survive at even high temperatures. Several microbes have specific temperature range for their survival and help the scientific community to categorize them as psychrophiles, mesophiles and basophiles. The relation between specific death constant and rate of cell death was evaluated as: R_d (Rate of cell death) =K_d (Thermal death constant) X N (Number of viable cells).Kinetics for thermal inactivation calculations thus involve the need for knowledge of the concentration of microorganisms to be destroyed, the concentration of microorganisms that can remain behind, the thermal resistance of the target microorganisms and the temperature time relationship required for destruction of the target organisms [1]. Low density polyethylene (LDPE) is one of the most used polymers in the society. The worldwide utility of polyethylene is expanding at a rate of 12% per annum and approximately 140 million tones of synthetic polymers are produced worldwide each year [7]. With such huge amount of polyethylene getting accumulated in the environment, their disposal evokes a big ecological issue. It takes thousand years for their efficient degradation [8]. Thus microbial degradation is the natural process where living organisms are able to depolymerized this synthetic polymers into monomers. The enzymes produced by the microbes are responsible for this biodegradation. Thus microbes are the best tool by which this ecological threat may be reduced. The heat resistance of a particular microorganism is needed to select process parameters such as temperature and time for inactivation of the target pathogen [9]. Heat resistance of bacteria is affected by various factors such as growth temperature, stage of growth, bacterial strains, composition, and the pH of the heating medium [10, 11]. In addition, the higher temperature causes denaturation of those useful enzymes. Thus the present study focused on the heat tolerance ability of plastic degrading microbes which can resists for longer time in higher temperature to degrade this hazardous polymers because heat resistance of bacteria can be determined through thermal death kinetic tests.

MATERIALS AND METHODS

Sample Collection for Isolation of microorganisms

The LDPE plastic soil samples were collected from the municipal solid waste landfill area, Pallikaranai, Chennai, India. The soil samples were collected at depth of 2-3cm, in a sterile container and then air dried at room temperature and after this collected samples placed in refrigeration of further studies.

Isolation and screening of polyethylene degrading microbes

To isolate the LDPE degrading bacteria, the plating was done through spread plate technique using 10^{-6} dilution on synthetic medium agar plates containing 0.3% LDPE

powder. The composition of medium was as follows: (g/l: K_2HPO_4 1, KH_2PO_4 0.2, $(NH_4)_2SO_4$ 1, $MgSO_4$ $7H_2O$ 0.5, NaCl 1, $FeSO_4.7H_2O$ 0.01, $CaCl_2$ $2H_2O$ 0.002, $MnSO_4$ H_2O 0.001, $CuSO_4$ $5H_2O$ 0.001, $ZnSO_4$ $7H_2O$ 0.001, Agar 15) with pH 7.0. The plates were incubated for 24h at 37°C. The colonies grown on these plates were purified and maintained on nutrient agar slant at 4°C.

Characterization and Identification of Positive isolates

Among microbial colonies, the LDPE degrading bacterial isolates were isolated and repeatedly subcultured to get the pure colonies for the identification. The LDPE degraders were characterized by morphologically, biochemical test and molecular identification. The morphology of the isolates were done using Gram staining and endospore staining. The biochemical tests were performed as by Bergy's Manual of Systematic Microbiology [12]. For molecular identification, strains were subjected for 16s rRNA analysis. The genomic DNA of the strain was amplified by Polymerase chain reaction using universal primers fP1 (5'-GAGTTTGATCCTGGCTCA-3') and rP2 (5'-ACGGCTACCTTGTTACGACTT-3') as described by Weisberg et al [13]. Amplified nucleotide sequences were determined with National Center for Biotechnology Information (NCBI) BLAST database. The nucleotide sequence of the 16S rRNA gene was compared with published 16S rRNA sequences using BLAST search at GenBank data base of NCBI.

Determination of thermal death kinetics of bacteria

This study was carried out for the particular isolates at four different temperatures. 2ml cultures of 24 hr fresh broth suspension of each isolates were taken in 16 test tubes. It was divided into four groups and incubated at four different temperatures i.e. (50°C, 70°C, 90°C and 110°C). One tube was taken out every 15 min interval from each group and plating was done using spread plate technique after serial dilution. Time duration was taken up to 1hr. All plates were incubated at 37°C for 24hrs. Colonies were counted using HiMedia digital colony counter. From this, CFU (colony forming unit), final and initial concentration were obtained and finally survival curve and thermal death constants, K_d values for each temperature were determined.

RESULTS AND DISCUSSION

Isolation and identification of microorganisms

Two bacterial isolates were identified on the selective screening medium supplemented with LDPE. The strains were identified as BSM-1 and BSM-2. BSM-1 was having 97.6% homology to *Bacillus amyloliquefaciens* NBRC15535 strain (NR_041455.1) while BSM-2 shown 99.6% homology to *Bacillus amyloliquefaciens* FZB42 strain (NR_075005.1). The identified *Bacillus amyloliquefaciens* BSM-1 and *Bacillus amyloliquefaciens* BSM-2 were shown maximum utilization of synthetic medium.

Heat tolerance of LDPE degrading bacteria

Thermal death constant is useful in determining the sensitivity of bacteria to change in temperature. In this study, the survival curve for *Bacillus amyloliquefaciens* BSM-1 (Figure 1) and *Bacillus amyloliquefaciens* BSM-2 (Figure 2) were indicates the thermal destruction of bacteria follows a first-order kinetics. The graph was obtained by plotting the log CFU (colony forming unit)/ml as the ordinate and time as the abscissa. Thermal death constants were determined at four different temperatures (50°C, 70°C, 90°C and 110°C) for each strain, but at 50°C for both the bacteria were having enumerable CFU counts, thus the graph was not obtained at that particular temperature. The values of thermal death constant indicates that the bacteria are with larger death constant values having a lower activation energy are more susceptible to temperature than the bacteria with lower death rate constant (Figure 3). Thermodynamically, less heat resistance phenomena is more favorable. The K_d values

pattern of BSM-1 was exact reversed to that of BSM-1. The thermal death constant for BSM-1 initially was low at 70°C, but later it increases with temperature. For BSM-2, K_d values at 70°C was maximum, as temperature increases, it became reciprocal to the temperature. According to this, BSM-1 was showed less heat tolerance ability, but in case of BSM-2, it was more resistant to heat at higher temperature.

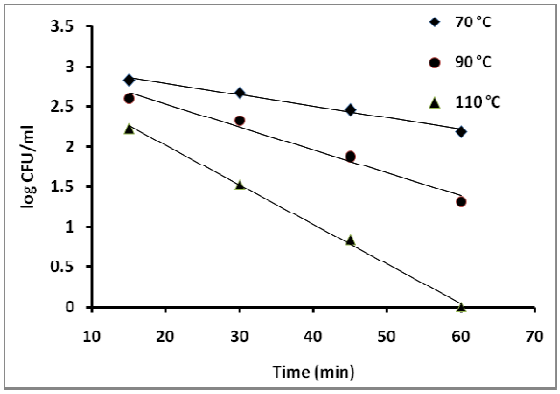

Figure 1
Survival curve for Bacillus amyloliquefaciens BSM-1 at different temperatures

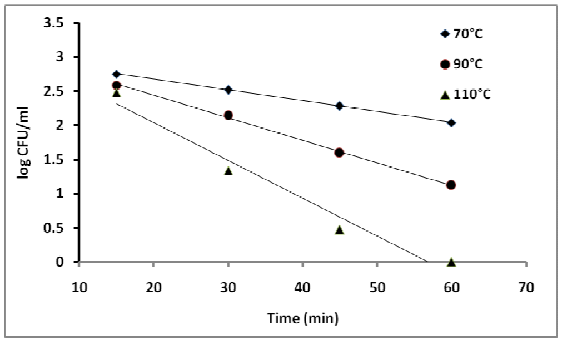

Figure 2
Survival curve for Bacillus amyloliquefaciens BSM-2 at different temperatures

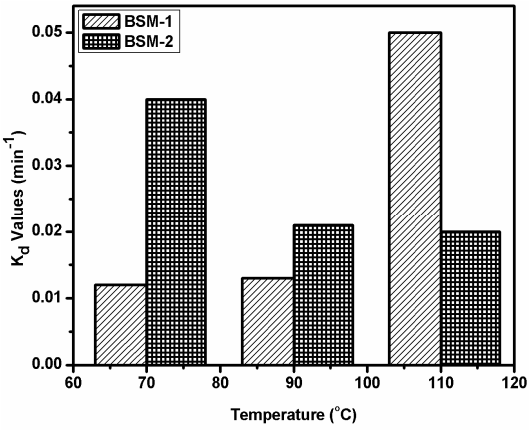

Figure 3
Thermal death constants for Bacillus amyloliquefaciens BSM-1 & BSM-2 at different temperatures

Temperature is one of the most important environmental factors influencing physiological functions in an organism [14]. When the cells are exposed to the stress conditions it usually triggers the expression of the specific type of proteins. Heat shock proteins (HSP) are a group of proteins induced by heat shock. Their expression is increased when cells are exposed to elevated temperatures or other stress [15]. This increase in expression is transcriptionally regulated. The dramatic up regulation of the heat shock proteins is a key part of the heat shock response and is induced primarily by heat shock factor (HSF) [16]. HSPs are found in virtually all living organisms, from bacteria to humans. As a consequence, the heat shock proteins are also referred to as stress proteins and their up regulation is sometimes described more generally as part of the stress response [17]. The mechanism by which heat-shock or other environmental stressors activates the heat shock factor has been determined in bacteria. During heat stress, outer membrane proteins (OMPs) do not fold and cannot insert correctly into the outer membrane. They accumulate in the periplasmic space. These OMP's are detected by DegS, an inner membrane protease, that passes the signal through the membrane to the sigmaE transcription factor. Further the expressed protein helps the cell to resist the death of cell due to elevated temperature [18]. In the present study the above mechanism finds its way to give heat resistance to the BSM-2 and this protein made the isolates to survive in harsh environment (high temperature) and simultaneously degrade the low density polyethylene.

CONCLUSION

Determinations of thermal death kinetics are necessary to know the heat resistance of bacteria at elevated temperature. The results of present study can be used to predict that higher the death constant values are more prone to thermal death and the lower values indicates more susceptible to heat. The LDPE degrading bacteria *Bacillus amyloliquefaciens* BSM-2 with low K_d values survived for longer duration at high temperature than the BSM-1 with high K_d values and thus causes more biodegradation in stress environment.

Conflict of Interest: Conflict of interest declared none

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