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Research Article

Isolation and Identification of Low Density Polyethylene (LDPE) Degrading *Bacillus* spp. from a Soil of Landfill Site

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Abstract

The use of plastics are increasing since last few decades due to its versatility and Low Density Polyethylene (LDPE) is one of the most predominantly used and has contributed to the environmental pollution. As most of the available techniques to manage LDPE are not sound economically and environmentally, biodegradation of LDPE has a potential to solve the problem created by the LDPE wastes. Therefore, *Bacillus spp.* were isolated from soil and screened for their potential to degrade LDPE. Based on the clear zone around their colony in Mineral agar containing LDPE powder, six *Bacillus* spp. such as *B. carboniphilus, B. sporothermodurans, B. coagulans, B. neidei, B. smithii* and *B. megaterium* were screened for further determination of their LDPE degradation potential. After incubation of *Bacillus* spp. in Mineral agar and Mineral broth, both containing LDPE pieces at 30°C for 2 months, the weight of LDPE pieces were found to be reduced which was positively correlated with pH reduction. The rate of weight loss ranged from 16%-26% in Mineral agar and 8%-25% in Mineral broth. Therefore, *Bacillus* spp. have a potential to be applied for the biodegradation of LDPE and manage LDPE wastes without environmental pollution.

Keywords: Plastics; Polyethylene; LDPE; Biodegradation; *Bacillus* spp

Abbreviation

LDPE: Low Density Polyethylene; CI: Confidence Interval.

Introduction

Plastics are very useful material [1], which has been used in the advancement of technology and other social benefits [2]. Among several types of plastics, Low Density Polyethylene (LDPE) is one of the most abundant commercially produced synthetic polymers. LDPE is easily processed and characteristically inert which makes it appropriate for many industrial uses [3]. Therefore, production of plastics and its use are increasing but its degradation resistant property has contributed a lot to pollute the environment. As the currently available techniques to manage LDPE wastes are expensive and pollute the environment, efforts have been focused in the biodegradation of LDPE [4].

Biodegradation is the ability of microorganism to influence abiotic degradation through physical, chemical, or enzymatic action [5]. Exo-enzymes from microorganisms break down complex polymers to yield smaller molecules that can pass through semi-permeable outer membranes and then to be utilized as carbon and energy sources [6]. Microorganisms are ideally suited for the biodegradation of plastic as they possess enzymes and the small size make them able to be in contact with the surface of the plastic [7].

Some of the bacteria reported to degrade LDPE are *Bacillus* spp., *Staphylococcus* spp., *Streptococcus* spp., *Diplococcus* spp., *Micrococcus* spp., *Moraxella* spp. and *Pseudomonas* spp. [8-10]. Fungi like *Penicillium* spp. are also considered to have a potential to degrade polyethylene [11].

Bacillus spp. produce extracellular hydrolase enzymes including lipase, CMCase, xylanase, chitinase, and protease for the degradation of plastic bags [12]. *Bacillus* spp. has shown higher degradation (up to 23% in one month) of plastic [13] with the production of bio-surfactant to enhance the attachment to the surface [14]. *Bacillus subtilis, Bacillus mycoides, Bacillus megaterium, Bacillus subtilis, Bacillus amylolyticus* and *Bacillus cereus* can degrade polyethylene [15-17].

Plastics solely accounts for 8-12% of the total waste accumulated in landfill site of Nepal [18] which affect the surrounding environment. Each year, many terrestrial and aquatic animals die by ingesting plastics. In addition, the deposition of plastics into the agricultural land reduce the productivity. As *Bacillus* spp. has

already been reported to degrade LDPE, it is hypothesized that indigenous *Bacillus* spp. isolated from plastic containing soil samples can effectively reduce the weight of plastics via biodegradation. The identified potential strain of *Bacillus* spp. can be further applied for the plastic waste management.

Materials and Methods

Sample collection

Ten grams of soil at the depth of about 5 cm from ten different areas surrounding the LDPE bags in Sisdol Landfill site and Teku Dumping site were collected. These are major landfill site and dumping site respectively located in Nepal. The soil samples were stored in refrigerator until the experiment. The plastic samples were disinfected by using 70% ethanol before the experiment.

Sample processing

Soil samples were diluted to 10^{-2}, 10^{-4} and 10^{-6} dilutions and then heated at 70ºC for 10 minutes to kill the vegetative cells except spore forming *Bacillus* spp. The diluted samples (1 ml) were inoculated onto Nutrient Agar by spread plate technique and incubated at 30°C for 24 hours. After obtaining the isolated colonies of *Bacillus* spp., quadrant streaking was done and incubated at 30°C C for 24 hours to obtain the pure culture.

Screening of LDPE degrading Bacillus spp.

LDPE degrading *Bacillus* spp. were screened with the Mineral Agar containing 0.3% LDPE powder prepared in the laboratory [19]. The LDPE powder was prepared by heating LDPE with xylene followed by grinding and sieving. The composition of Mineral Agar was as follows (g/L): K_2HPO_4, 1; KH_2PO_4, 0.2; $(NH_4)_2SO_4$, 1; $MgSO_4 \cdot 7H_2O$, 0.5; NaCl, 1; $FeSO_4 \cdot 7H_2O$, 0.01; $CaCl_2 \cdot 2H_2O$, 0.002; $MnSO_4 \cdot H_2O$, 0.001; $CuSO_4 \cdot 5H_2O$, 0.001; $ZnSO4 \cdot 7H_2O$, 0.001; Agar 15; pH 7.0).

Pure culture of *Bacillus* spp. were inoculated into Nutrient broth and incubated at 30°C overnight. 0.1 ml of overnight culture was inoculated into the Mineral Agar and incubated at 30°C for 2 weeks. LDPE degrading *Bacillus* spp. were screened based on the growth and partial clear zone around its colony on Mineral agar.

Identification of Bacillus spp.

The identification of *Bacillus* spp. was done by conventional microbiological techniques by following Bergey's manual of systematic bacteriology [20]. The techniques involved microscopy, cultural characteristics and biochemical tests such as Catalase test, Oxidase test, motility test, Methyl Red test, Voges-Proskauer test, H_2S production, Indole production, Nitrate reduction, Citrate and Propionate utilization, hydrolysis of casein, gelatin and starch, acid production from various carbohydrates (L-arabinose, D-glucose, Glycogen, D-mannitol, D-mannose, Salicin, Starch, D-xylose), and growth at varying pH, salinity and temperature.

Determination of LDPE degradation potential of Bacillus spp.

Mineral agar medium was prepared in the laboratory with above-mentioned composition and pre-weighed pieces of LDPE were added on it. Then, 0.1 ml overnight culture of *Bacillus* spp. were inoculated and incubated at 30°C for 2 months. Similarly, Mineral broth medium was prepared with the composition of Mineral agar without addition of agar. Fifty milliliter of Mineral broth was transferred into the conical flasks and one-gram LDPE pieces (0.5 X 0.5 cm^2) were transferred into it. Then, 5 ml of overnight culture of *Bacillus* spp. were inoculated into the same conical flasks and incubated at 30°C at 150 rpm for 2 months in shaker incubator. Negative control was maintained by adding LDPE pieces to Mineral agar and Mineral broth without inoculation of the *Bacillus* spp. and incubated at the same condition. Viability assessment of *Bacillus* spp. was done at regular interval.

After two months of incubation, pH of the mineral broth was determined. The LDPE pieces were collected, washed thoroughly using 70% ethanol and dried at room temperature. Then, LDPE pieces were weighed for final weight. From the data collected, degradation potential of Bacillus spp. were determined by calculating weight loss of the plastics by using the equation as follows:

$$\text{Degradation potential (\%)} = \frac{\text{weight loss of the sample}}{\text{original weight of sample}} \times 100$$

Statistical analysis

Data presentation and analysis was done by computer program Rx64 3.5.1. Paired t-test assuming unequal variances was carried out to test the significance of correlation coefficient and difference between mean values.

Results

LDPE degrading *Bacillus* spp. were screened based on their capability to grow in Mineral agar containing LDPE powder and clear zone around their colony. Among them, six *Bacillus* spp. were screened for further identification and determination of degradation potential. Based on microscopy, cultural characteristics and biochemical tests, the organisms were identified as *B. carboniphilus, B. sporothermodurans, B. coagulans, B. neidei, B. smithii and B. megaterium*.

The degradation potential of *B. carboniphilus, B. sporothermodurans, B. coagulans, B. neidei, B. smithii* and *B. megaterium* were 34.55%, 36.54%, 18.37%, 36.07%, 16.40% and 34.48% respectively in Mineral agar and 25%, 21%, 16%, 14%, 8% and 21% respectively in Mineral broth. The degradation rate of LDPE in Mineral agar was significantly higher than in Mineral broth (CI =95%) (Figure 1 and 2). In addition, the correlation between weight loss of LDPE and pH reduction was significant (CI =95%) (Figure 3).

Figure 1: Biodegradation of pieces of LDPE by B. coagulans in Mineral agar after 2-months of incubation at 30°C.

Figure 2: LDPE Biodegradation potential (%) of Bacillus spp. in Mineral agar and Mineral broth.

Figure 3: Positive correlation between LDPE biodegradation potential (%) and pH reduction (%) with r(0.05, 10) = 0.92 and P-value<0.05.

Discussion

Previous studies had reported *B. smithii* to degrade Polylactic acid with the production of PLA depolymerase enzyme [21] but the same organism has degraded LDPE in this study. Therefore, same organism may have a potential to degrade different types of plastics; provided the exposure to plastics for sufficient time. Except *B. megaterium*, other *Bacillus* spp. have not been reported to degrade LDPE in previous studies [22].

In this study, the weight loss of LDPE is positively correlated with reduction of pH. The pH of the medium decreases with respect to the degradation of plastics due to the increase in –COOH concentration in the medium [23]. The reduction of pH validates that the culture is metabolically active and the utilization of plastics by the organisms for their growth [24]. Although the reduction of pH is an indicator of LDPE degradation, it is also associated with the reduction of microbial population in the media. Thus, the optimum pH need to be maintained throughout the degradation process for optimum degradation [25], which was not done in this study.

The degradation of LDPE was found higher in Mineral agar medium than in Mineral broth medium. Some of the screened *Bacillus* spp. were aerobic. The growth of such strains should be rapid in agar medium than in broth even though the broth was in shaking incubation. There was no significant difference between the LDPE degradation potential of the *Bacillus* spp. isolated from two

different sites. Thus, the degradation potential is independent of the location if there is existence of LDPE since long time.

Most of the studies have provided standard growth media containing essential nutrients for the particular organisms [24]. The use of standard growth media can increase the microbial mass prior to the degradation. With the hypothesis of the potential of *Bacillus* spp. capable of using LDPE as a sole source of carbon, Mineral media was used as in some other studies [19,25]. Therefore, the low population of *Bacillus* spp. may have caused the low degradation of LDPE.

Conclusion

Even though it takes a long time for the degradation process, LDPE are actually biodegradable when optimum growth condition was provided. Therefore, it can be concluded that the *Bacillus* spp. have an ability to degrade LDPE. Although LDPE are biodegradable, it should not be promoted for use but *Bacillus* spp. can be promoted for the management of LDPE wastes deposited in an environment.

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