J. Exp. Zool. India Vol. 24, No. 2, pp. 000-000, 2021	www.connectjournals.com/jez	*ISSN 0972-0030*
DocID: https://connectjournals.com/03895.2021.24.000		eISSN 0976-1780

ENHANCEMENT OF PLASTIC DEGRADATION POTENTIAL OF *STREPTOCOCCUS PYOGENES* THROUGH THE ETHIDIUM BROMIDE

Chitranshu Pandey, Rishika Patel and Pallavi Sharma

MRD Lifesciences Pvt. Ltd., Vibhuti Khand, Gomti Nagar, Lucknow - 226 010, India.
email:

(Received 28 November 2020, Revised 30 January 2021, Accepted16 February 2021)

ABSTRACT : In several "short live" packaging applications, plastic gloves, plays an important role. Garbage bags and toys are the majority of waste plastic. For their constancy in our landscape, incorrectly discarded plastic products are a major pollutant to the environment, potentially life-damaging. One of most troublesome plastic in this regard, between synthetic plastics Polyethylene (PE) is Polyethylene waste typically exists in the lack of adequate treatment methods burned that cause's severe air pollution. Polyethylene found inert can be biodegraded, if necessary the strains of microbial are used. In this research plastic degradation was analyzed by 30 days inoculation in broth culture method. The bacterial species obtained associated with degrading plastic were identified as *Streptococcus pyrogen*. The efficacy of plastic degradation as evaluated in broth culture method. The work illustrated that mutated *Streptococcus pyrogen* have grater tendency to degrade the plastic when compared with wild *Streptococcus pyrogen* in optimized media.

Key words : *Streptococcus pyrogen*, plastic, polyethylene, LDPE.

How to cite : Chitranshu Pandey, Rishika Patel and Pallavi Sharma (2021) Enhancement of plastic degradation potential of *Streptococcus pyogenes* through the ethidium bromide. *J. Exp. Zool. India* **24**, 000-000. DocID: https://connectjournals.com/03895.2021.24.125

INTRODUCTION

Over several decades, polyethylene has been recognized as among the polluting substances to the world. This is extremely resistant to process of degradation. The use of polyethylene has exploded on the sides each year, the yearly production rate exceeds 25 million tonnes (Alexander, 1981). In addition to the regional need for Polyethylene also grows to 12 percent per annum and around 140 million tons of synthetic polymers manufactured annually (Kathiresan, 2003). Low density polyethylene (LDPE) is one of the polyethylene types and it is utilized for fabrication of different goods and materials. It is because of its characters , for example lightweight, wonderful effect resistance, incredibly versatile, easy to clean, thermoforming efficiency, meets food requirements Guidelines on handling, no absorption of moisture, and corrosion-and chemical resistant. Because of its greatest properties, LDPE is commonly used in the manufacture of packaging and different containers. Tubes, washing tubes, tubing, computer-based plastic bags and some test equipment. The plastic bag is the more common LDPE substance employed in domestic storage. With increased usage of polyethylene, its current accumulation in the environment was concern for the climate. Hydrophobic and inactive chemical rendering it very robust to biodegrade. It requires hundreds and potentially thousands of years to completely decompose into the environment. It was then calculated that 5-7 per cent of household garbage is made up of polyethylene (Chee *et al*, 2010). There has been a great deal of work to reduce polyethylene accumulation in both manufacturing processing and handling of polyethylene goods, and great attention was paid to this point of view on biodegradation (Seo *et al*, 2007; Yang *et al*, 2014). Browse for effective polyethylene disposal through biological methods are not concerned with chemistry, and biodegradation and Bio recycling are alternative. In reality, recycling is because of cost factor and the loss of mechanical properties not technically feasible. Biodegradation by Pramila and Ramesh (2015) is the safest form of polyethylene disruption. It produces less harmful chemicals and reveals bio-geo-chemical cycling potential of the substrate. Over the last 2 decades, many studies on microbial LDPE degradation have been documented and it is also mentioned some microbes. Grame +VE like *Bacillus weihenstephanensis*, *Bacillus*

spp., *Staphylococcus* spp., *Streptococcus* spp. and *Diplococcus* spp. have documented degradation of LDPE (Kathiresan, 2003; Kim and Rhee, 2003). While some gram negatives of *Pseudomonas fluorescens*, *Pseudomonas aeruginosa* and *Acinetobacter ursingii*, isolated from contaminated plastic soil, can degrade LDPE (Kumar *et al*, 2007). *Moraxella* spp., *Burkholderia cepacia* and *Escherichia coli* do have capacities to degrade LDPE (Milstein *et al*, 1994). Studies of polyethylene decomposition, using isolated bacteria from oil polluted soil. Two chosen isolates minimize polyethylene weight by up to 1.3 per cent after one month Incubation times (Dey *et al*, 2012). In Banyumas, bacteria isolated from landfill soil reduces the polyethylene weight of 2.33 per cent after 1 month of incubation (Shimao, 2001). Bacteria isolated from the waste disposal site are worthy to the polyethylene degradation (Usha *et al*, 2011). Pseudomonas exhibits very good capability to biodegrade polyethylene *Brevibacillus* and *Rhodococcus* followed with a weight loss of 40.5%, 37.5% and 33% accordingly (Starnecker and Menner, 1996).

This initial research was then conceived to obtain possible bacterial isolates to destroy polyethylene from landfill. This paper explains selected bacteria's ability to degrade LDPE and the outcomes are presented to works reported elsewhere.

METHODOLOGY

Sample collation and bacterial isolation

Garbage soil sample (waste disposable site dumped with polythene bag and plastic cup) was collected from the site near MRD LifeSciences Institute in Gomati Nagar, Lucknow. Bacterial colony was isolated by serial dilution on nutrient agar media, colonies were purified by streak plate method. There were 10 different colonies found on nutrient agar media plate and temporarily named as CPR01901 to CPR01910, these are differently identified on basis of their colony morphology.

Screening of plastic degrading bacteria

Pre-weighted 1 cm diameter disks made from plastic bags were transferred aseptically to the flask consisting 50 ml of broth medium, various bacterial species were inoculated in these flask separately. Control was sustained in bacteria-free medium with plastic disks. There were separate flasks held for each Treatments and placed in shaker incubator. The plastic discs were obtained after 30 days of shaking, thoroughly washed Utilizing distilled water, dried in shade and then measured to the final weight. Weight loss calculations were made of plastics (Jumaah, 2017).

Treatment with EtBr

Isolated bacterial species was treated with different concentration of EtBr i.e. 4 µg/ml, 6 µg/ml, 8 µg/ml, 10 µg/ml, 12 µg/ml (Shekhar and Chitranshu, 2020).

Media optimization

Media was optimized by one time at a factor for isolated bacterial species. Different sources of carbon, nitrogen, sources, temperature were evaluated and optimized results for isolated bacterial growth were noticed (Botre *et al*, 2015).

Biochemical test

To identify the isolated bacteria, the different biochemical test like gram staining, catalase, hemolysis and PYR test were performed and results of these test followed to Bergy's manual (Young, 1926).

RESULTS

Sample collection and bacteria isolation (Fig. 1).

Fig. 1 : Soil sample was collected and bacteria were isolated from soil through serial dilution and spread plate method. This figure depicts: a. Site of soil sample, b. serial dilution followed by spread plate method.

Screening of bacteria for polythene degradation (Table 1).

Treatment of bacterial CPR01903 with EtBr (Fig. 2).

Effect of EtBr treatment on degradation of plastic through CP01903 (Fig. 3)

Effect of nitrogen on growth selected colony CPR01903 (Fig. 4)

Table 1 : Summarization of degradation of weight of plastic after treated with different isolated colonies. Data illustrate that colony CPR01903 have good ability to degrade the given plastic.

S. No.	Colony	Weight of plastic in gm before inoculation	Weight of plastic in gm after inoculation and incubation of 48hrs
1.	CPR01901	0.019	0.019
2.	CPR01902	0.019	0.018
3.	CPR01903	0.019	0.015
4.	CPR01904	0.019	0.017
5	No bacterial colony	0.019	0.019

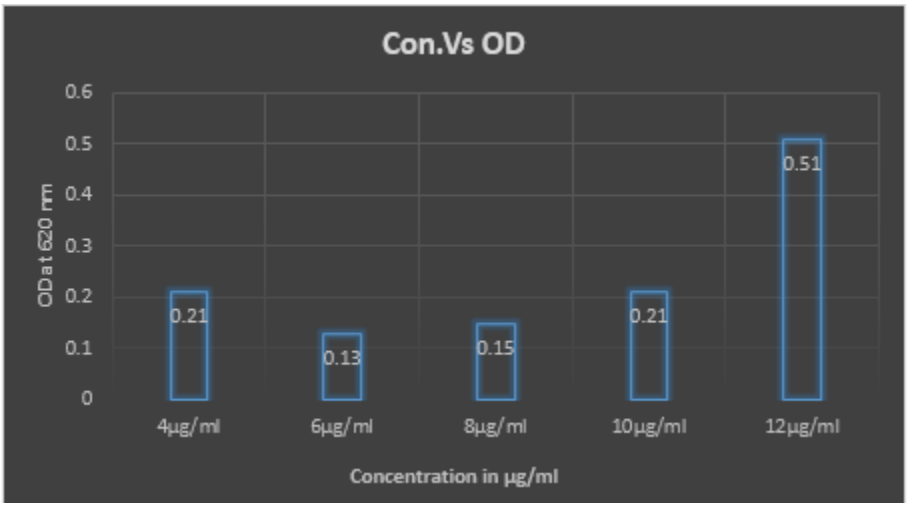

Fig. 2 : Concentration of EtBr verses OD at 620 nm. Data illustrated that optimum growth of CPR01903 was seen at 12 µg/ml.

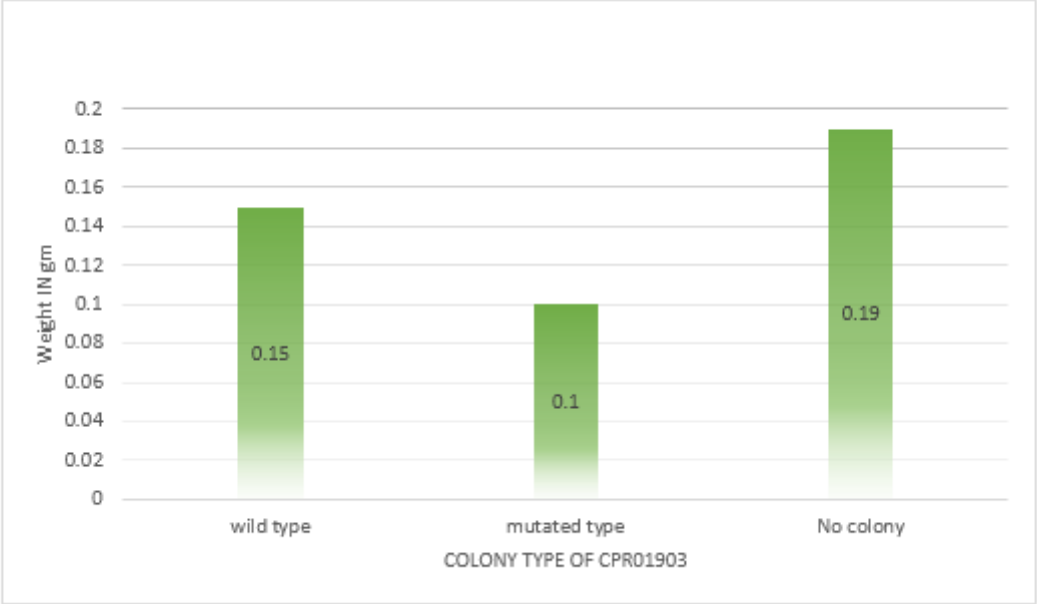

Fig. 3 : Figure inferred that colony (CPR01903) mutated with EtBr (12 µg/ml) have elevated capacity to degrade the plastic.

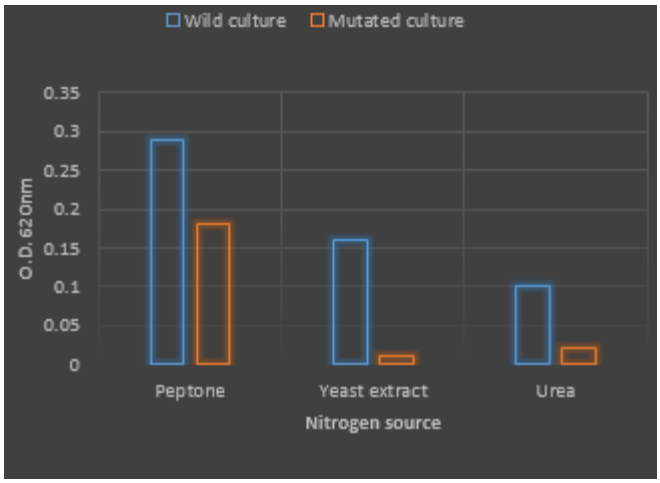

Fig. 4 : Effect of nitrogen source on wild and mutated CPR01903. outcome depicted that peptone is good nitrogen source for wild and mutated type CPR01903.

Effect of different concentration of peptone on wild and mutated bacteria (Fig. 5).

Effect of carbon source on wild and mutated CP01903 (Fig. 6).

Effect of different concentration of carbon (Fig. 7).

Table 2 : Degradation of plastic in optimized media through mutated bacteria.

S. no.	Sample	Original weight (gm)	Weight of plastic after inoculation (gm)			
			24hrs	48hrs	96hrs	120hrs
1.	Plastic 1	0.030	0.028	0.025	0.022	0.018
2.	Plastic 2	0.028	0.026	0.024	0.020	0.016
3.	Plastic 3	0.035	0.032	0.030	0.028	0.014
4.	Plastic 4	0.026	0.024	0.022	0.019	0.011

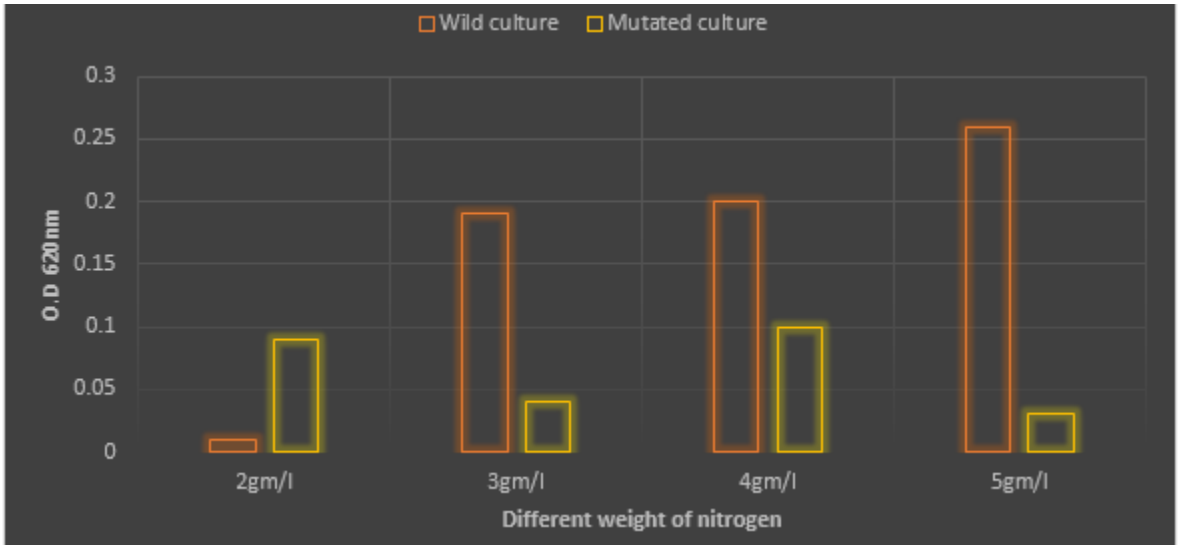

Fig. 5 : 5gm/l and 4gm/l showed good concentration for wild and mutated type bacteria, respectively.

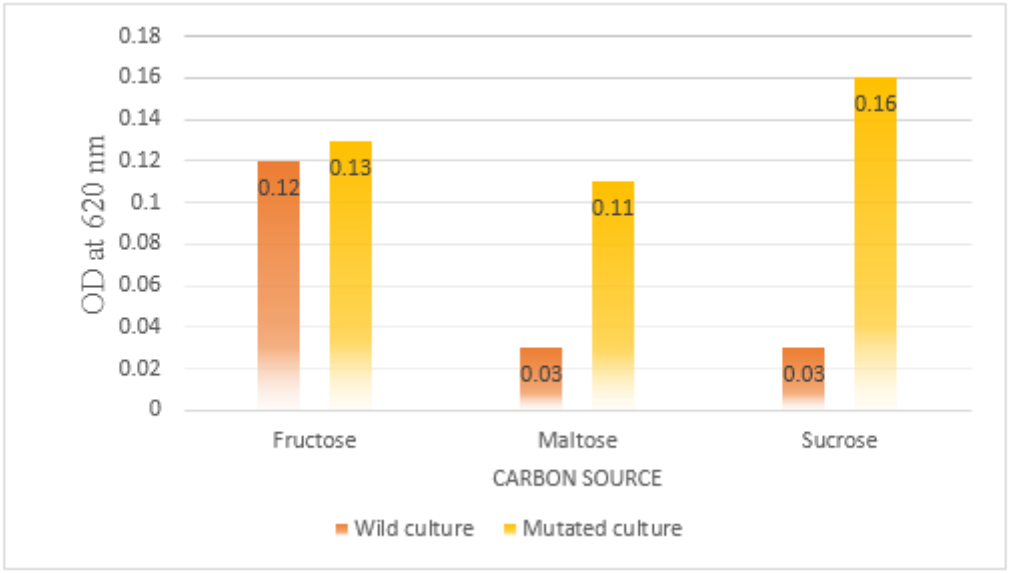

Fig. 6 : Effect of carbon source was seen. Sucrose showed good carbon source for mutated and fructose showed good carbon source for wild type bacteria CPR01903.

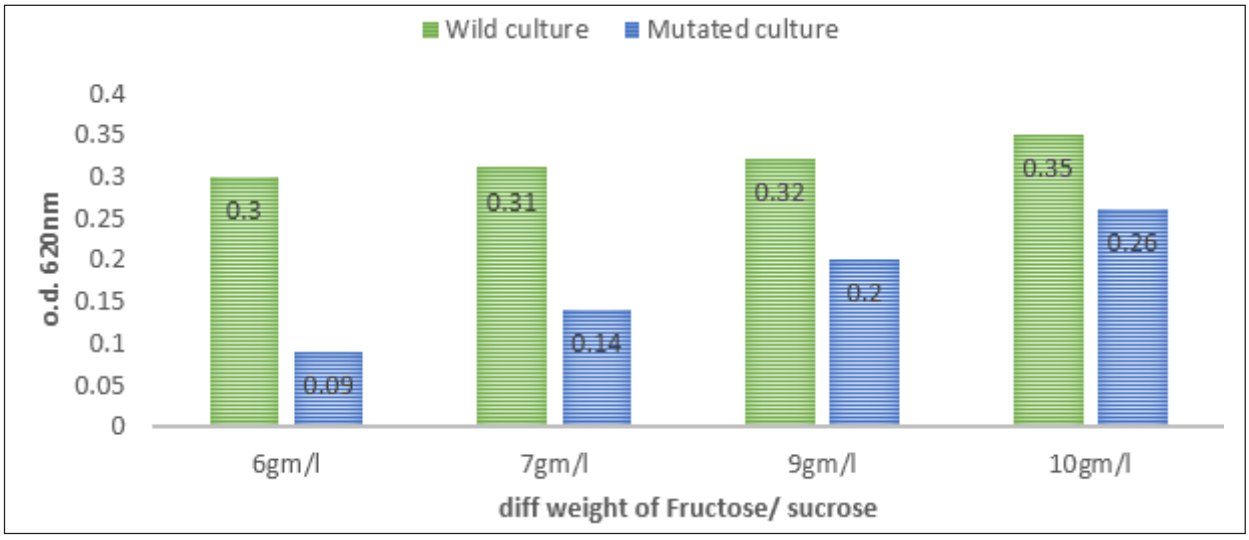

Fig. 7 : Different concentration of fructose for wild and sucrose for mutated type bacteria, data elaborated that 10gm/l fructose and sucrose showed optimum concentration for wild and mutated bacteria.

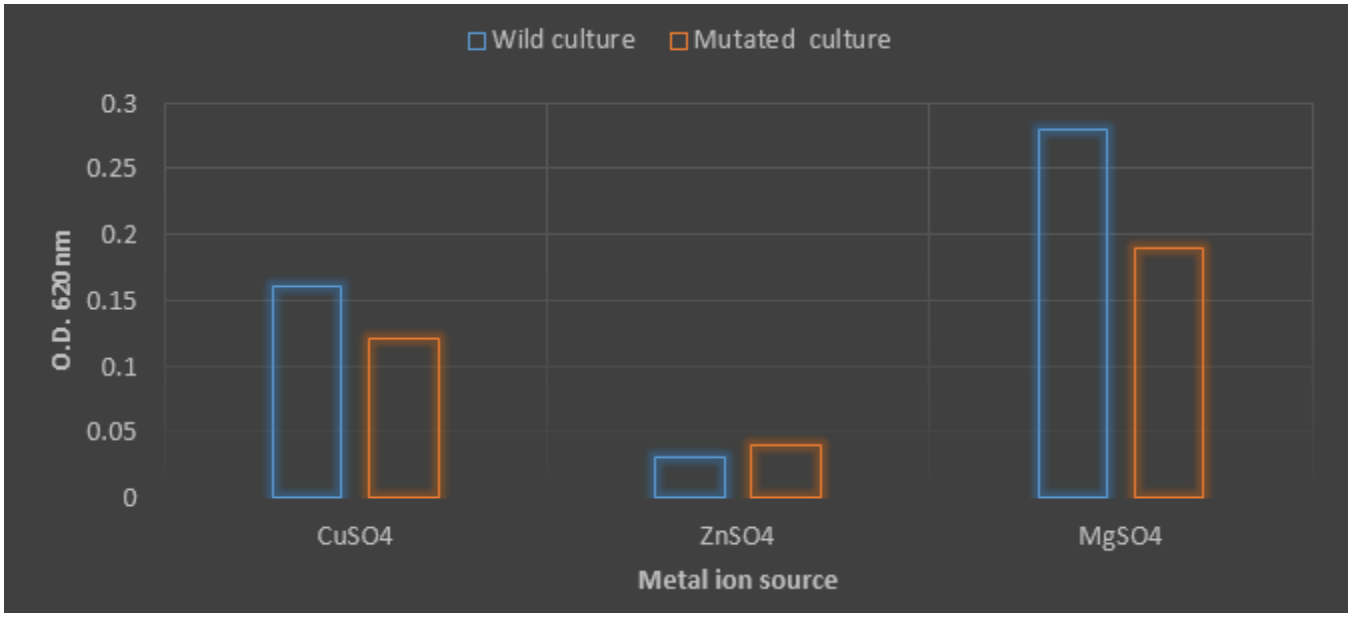

Fig. 8 : Effect of metal ions on wild and mutated bacteria CPR01903. Data indicated that $MgSO_4$ displayed good metal ion for growth of wild and mutated bacteria.

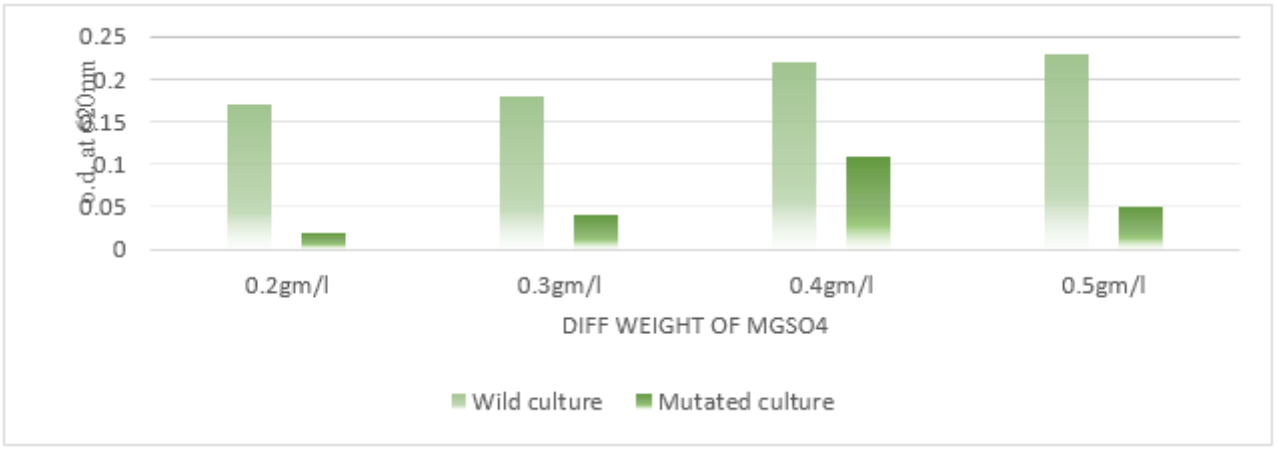

Fig. 9 : Effect of different concentration of $MgSO_4$ on wild and mutated bacteria, 0.4gm/l showed optimum concentration for their growth.

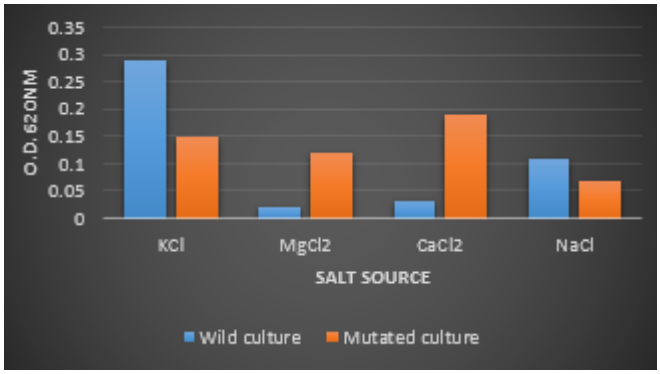

Fig. 10 : Effect of salt was seen, data illustrated that KCl inferred good source for wild and mutated bacteria.

Effect of metal ions on wild and mutated CPR01903 (Fig. 8)

Effect of different concentration of $MgSO_4$ (Fig. 9).

Effect of salt on bacteria (Fig. 10).

Table 3 : Degradation of plastic in optimized media by wild type bacteria.

S. no.	Sample	Original weight (gm)	Weight of plastic after inoculation (gm)			
			24hrs	48hrs	96hrs	120hrs
1.	Plastic 1	0.030	0.030	0.029	0.029	0.028
2.	Plastic 2	0.028	0.028	0.026	0.026	0.024
3.	Plastic 3	0.035	0.034	0.034	0.032	0.029
4.	Plastic 4	0.026	0.026	0.024	0.022	0.020

Table 4 : Biochemical test of isolated bacterial strain.

S.no.	Biochemical test	Results
1	Gram staining	+Ve (Coccus)
2	Catalase	+ve
3	Hemolysis â	+ve
4	PYR	+ve

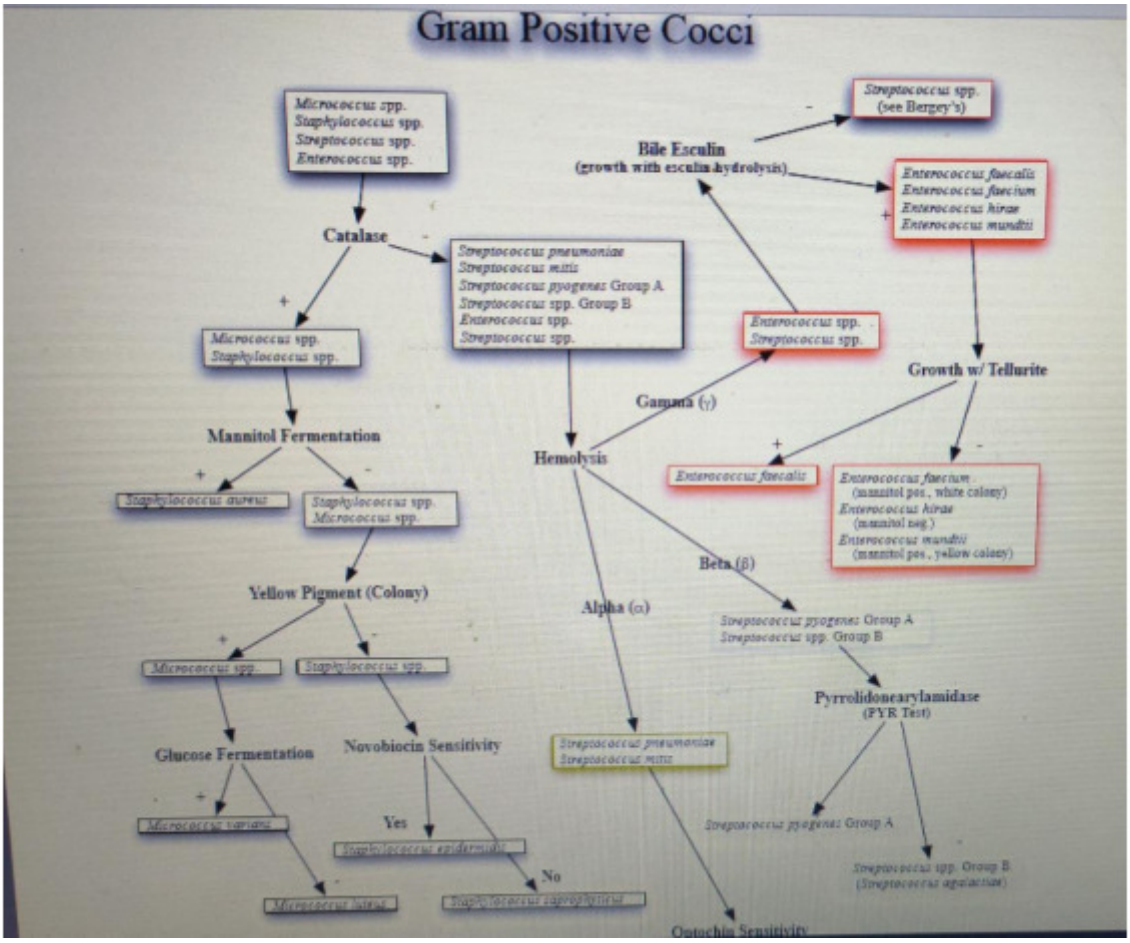

Fig. 11 : Bergey's manual of determinative bacteriology (Young, 1926).

Biochemical test

Biochemical test is given in Table 4.

DISCUSSION

Existing research was completed for isolation of polythene degrading bacterial strain form various waste. One isolate was selected after screening to find suitable bacterial isolates that has potential to degrade polythene. The strain was mutated with EtBr to increase the potential of polythene degradation. CPR01903 culture was characterized from their colony morphology, shape and biochemical test and scrutinized as *Streptococcus pyrogen*. The media optimization of various physiochemical factors like nitrogen source, carbon source, metal ions, temperature and pH was evaluated. Mutated *Streptococcus pyogenes* showed high tendency to degrade the plastic. The comparative study was completed between mutated and wild type *Streptococcus pyogenes* for degrading the plastics.

CONCLUSION

Throughout the research the strain S*treptococcus pyogenes* was found best polythene degrading bacteria after mutation through the EtBr ploythene degrading activity was enhanced relative to wild type *Streptococcus pyrogen*. Enhancement of ploythene degrading capacity was also enhanced by media optimization of mutated bacteria.

ACKNOWLEDGEMENT

I wish to show my immense gratitude to Mr. Manoj Verma, Director, MRD LifeSciences Pvt. Ltd., Lucknow. I am very grateful and my heartiest thanks to Ms. Shraddha Prakash (Research Scientist), Raj Shekhar Mishra (Research Assistant) and Pragya Srivastava (Research Assistant) MRDLS, Lucknow, for there kind support throughout the research work, I am also thankful to the almighty without whose blessings nothing was possible.

REFERENCES

Alexander M (1981) Biodegradation of chemicals of environmental concern. *Science* **211**(4478), 132–138.

Botre S, Jadhav P, Saraf L, Rau K and Wagle A (2015) Screening and Isolation of Polyethylene degrading Bacteria from various sources. *Int. Res. J. Environ. Sci*. **4**(11), 58–61.

Chee J-Y, Yoga S-S, Lau N-S, Ling S-C, Abed R M M and Sudesh K (2010) Bacterially produced polyhydroxyalkanoate (PHA): converting renewable resources into bioplastics. *Curr. Res., Tech.*

and Education Topics in Appl. Microbiol. and Microbial Biotech. **2**, 1395–1404.

Dey U, Mondal N K, Das K and Dutta S (2012) An approach to polymer degradation through microbes. *IOSR J. Pharm*. **2**(3), 385–388.

Jumaah O S (2017) Screening of plastic degrading bacteria from dumped soil area. *IOSR J. Environ. Sci. Toxicol. Food Technol*. **11**, 93–98.

Kathiresan K (2003) Polythene and plastics-degrading microbes from the mangrove soil. *Revista de Biologia Tropical* **51**(3–4), 629–633.

Kim D Y and Rhee Y H (2003) Biodegradation of microbial and synthetic polyesters by fungi. *Appl. Microbiol. Biotech.* **61**(4), 300–308.

Kumar S, Hatha A A M and Christi K S (2007) Diversity and effectiveness of tropical mangrove soil microflora on the degradation of polythene carry bags. *Revista de Biología Tropical* **55**(3–4), 777–786.

Milstein O, Gersonde R, Huttermann A, Frund R, Feine H J, Ludermann H D, Chen M-J and Meister J J (1994) Infrared and nuclear magnetic resonance evidence of degradation in thermoplastics based on forest products. *J. Environ. Polymer Degradation* **2**(2), 137–152.

Pramila R and Ramesh K V (2015) Potential biodegradation of low density polyethylene (LDPE) by *Acinetobacter baumannii*. *Afr. J. Bacteriol. Res.* **7**(3), 24–28.

Seo H-S, Um H-J, Min J, Rhee S-K, Cho T-J, Kim Y-H and Lee J (2007) *Pseudozyma jejuensis* sp. nov., a novel cutinolytic ustilaginomycetous yeast species that is able to degrade plastic waste. *FEMS Yeast Res.* **7**(6), 1035–1045.

Shekhar S and Chitranshu P (2020) Studies on the effects of ethidium brom. *Studies* **9**(1), 7–16.

Shimao M (2001) Biodegradation of plastics. *Curr. Opin. Biotech.* **12**(3), 242–247.

Starnecker A and Menner M (1996) Assessment of biodegradability of plastics under simulated composting conditions in a laboratory test system. *Int. Biodeterioration & Biodegradation* **37**(1–2), 85–92.

Usha R, Sangeetha T and Palaniswamy M (2011) Screening of polyethylene degrading microorganisms from garbage soil. *Libyan Agric. Res. Cent J. Int*. **2**(4), 200–204.

Yang J, Yang Y, Wu W-M, Zhao J and Jiang L (2014) Evidence of polyethylene biodegradation by bacterial strains from the guts of plastic-eating waxworms. *Environ. Sci. Tech.* **48**(23), 13776–13784.

Young C C (1926) *Bergey's Manual of Determinative Bacteriology*. American Public Health Association.