Isolation of plastic degrading micro-organisms from soil.

Contestant : Asmita Deepak Kamble . **Mentor** – Dr. Tejashree Shanbhag.

AIM OF THE PROJECT

1. To isolate Polyethylene terephthalate(PET) and Polystyrene(PS) degrading microorganisms from 5 different soil sample: Garden soil, Mangrove soil, Forest soil, Soil near petrol pump, Garbage soil.
2. To find out the percent of weight loss of Polystyrene and Polyethylene terephthalate by 4 bacterial species *Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, Streptococcus pyogenes* and and 1 fungal species *Aspergillus niger*.

PROCEDURAL DETAILS .

Material and methods

Soil sample collection : 5 soil samples were collected from 5 different locations. All soil samples were collected by digging few meters depth.

Soil sample	Garden	Mangrove	Forest	Soil near petrol pump	Garbage
Place	Sidhivinayak temple garden	Versova beach	Sanjay Gandhi national park	Worli	Prabhadevi

Preparation of Winogradsky's column.

- Place the soil samples in respective bottles (bottle – 1.5 litre thumbs up bottle. Cut the top off).
- Weigh and cut Equal strips of PET (bottle sample) and PS (disposable plate) and place in respective soil sample.
- Seal the top off again and incubate it for 4 months.

Isolation of probable plastic degrading microorganisms from soil.

- Remove plastic samples from columns with the help of sterile gloves.
- Place them in labelled sterile Bushnell Hass Broth tube and wash those samples in aspectic condition in it
- Remove it and Place them on filter paper to dry them completely.
- Weigh it accurately and calculate %weight loss.
- By standard streaking technique isolate these probable plastic degrading microorganisms on Nutrient (NA) and Sabourauds (SAB) agar medium
- Study colony characteristics of isolated microorganisms

Isolation of exact plastic degrading microorganisms:

- Few strips of PET and PS was cut and weigh it accurately.
- Place it in test tubes containing Bushnell Hass broth and sterilze this setup in autoclave.
- Inoculate sterile broth containing sterile plastic sample with isolated probable plastic degrading microorganisms.
- Incubate it for 1 month at room temperature .
- In aspectic condition Remove plastic sample and dry , weigh it accurately and calculate weight loss
- Colonies which degrade plastic will show the weight loss of plastic sample subjected to it.
- Study colony characteristics and Gram staining of plastic degrading microorganisms.

Calculation :

$$\%Total\ weight\ loss = \frac{W_i - W_f}{W_i} \times 100$$

Where, W_i = Initial weight (Weight of plastic before incubation)

W_f = Final weight (Weight of plastic after incubation).

Biodegradation of PET and PS by different microorganisms.

Names of microorganisms use:

- *Aspergillus niger.(A.niger)*
- *Bacillus subtilis(B.subtilis)*
- *Pseudomonas aeruginosa.(P.aeruginosa)*
- *Staphylococcus aureus.(S.aureus)*
- *Streptococcus pyogenes.(S.pyogenes)*

Method

- Sterile piece of PET and PS plastic samples in sterile 50 ml NA, Bushnell Hass, Rose Bengal broth
- Inoculate with selected micro-organisms . Incubate it for 1 month
- Remove plastic samples in aspectic condition and dry it completely .
- Weigh it and calculate percent weight loss
- Microorganisms capable of degrading plastic sample will be indicated by weight loss of plastic sample subjected to it.

A.  B. 

Figure 1 :Winogradsky's column before (A) and after (B) incubation for 4 months

Biodegradation in Winogradsky's column

Sr. no	Soil sample	PET		Total loss in weight (gm)	% loss in weight
		Initial weight (gm)	Final weight (gm)		
1	**Garden**	1.387	1.315	0.072	**5.191**
	Forest	1.416	1.383	0.033	2.330
3	Mangrove	1.437	1.387	0.05	3.479
4	Soil near petrol pump	1.435	1.380	0.055	3.832
5	Garbage	1.171	1.142	0.029	2.553

Sr. no	Soil sample	PS		Total loss in weight (gm)	% loss in weight
		Initial weight (gm)	Final weight (gm)		
1	Garden	0.129	0.099	0.033	23.256
2	Forest	0.074	0.053	0.021	28.378
3	mangrove	0.079	0.077	0.002	2.531
4	Soil near petrol pump	0.098	0.092	0.006	6.122
5	**Garbage**	0.153	0.108	0.045	**29.411**

Probable and exact PET degrading microorganisms from Garden soil.

Isolation on Nutrient Agar Medium.

No . of colonies	1	2
Colour	Yellow	**White**
Margin	Circular	Filamentous
Elevation	Concave	Flat
Opaque / translucent	Opaque	Opaque
Shape	Round	Irregular
Size(cm)	0.1	2.5
Consistency	Butyrous	Butyrous
Gram nature	Gram positive coccobacillus	**Gram positive cocci in cluster**
Initial weight (gm)	0.252	0.287
Final weight (gm)	0.252	0.250
Total loss in weight (gm)	0.252	0.037
% loss in weight	0	**12.891**

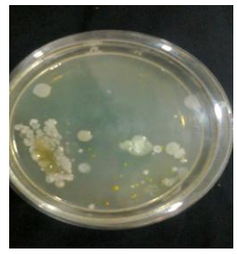
A.  B. 

Figure 1: Isolation of probable PET degrading organisms from Garden soil on Nutrient agar medium (A) & Sabouraud's agar medium (B).

Isolation on Sabourauds agar medium

No . of colonies	1	2	3	4
Colour	Yellow	**White**	Red	Creamish white
Margin	Circular	Circular	Circular	Wavy
Elevation	Raised	Flat	Raised	Flat
Opaque / translucent	Opaque	Opaque	Opaque	Opaque
Shape	Round	Round	Round	Irregular
Size(cm)	0.1	0.3	0.1	0.8
Consistency	Butyrous	Butyrous	Butyrous	Butyrous
Gram nature	Gram positive coccobacillus	**Gram negative cocci in single**	Gram negative bacilli rod shape	Gram negative cocci in cluster
Initial weight (gm)	0.268	0.245	0.263	0.260
Final weight (gm)	0.261	0.243	0.262	0.259
Total loss in weight (gm)	0.007	0.02	0.001	0.001
% loss in weight	2.612	**8.163**	0.380	0.384

Isolation of probable and exact PS degrading microorganisms from Garbage soil .

On Sabourauds agar medium

No . of colonies	1	2
Colour	**Yellow**	White
Margin	Circular	Circular
Elevation	Concave	Raised
Opaque / translucent	Opaque	Opaque
Shape	Round	Round
Size(cm)	0.2	00.1
Consistency	Butyrous	Butyrous
Gram nature	**Gram negative cocci**	Gram positive coccobacillus
Initial weight (gm)	0.029	0.027
Final weight (gm)	0.028	0.027
Total loss in weight (gm)	0.001	0
% loss in weight	**3.45**	0

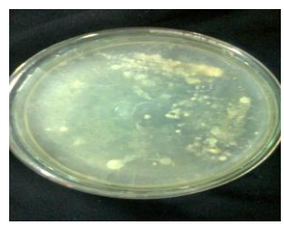
A.  B. 

Figure 2 : Isolation of probable PS degrading organisms from Garbage soil on Nutrient agar medium (A) & Sabouraud's agar medium (B).

Biodegradation of PET & PS by 5 different microorganisms

PET in Nutrient Broth inoculated separately with following microorganisms.

Microorganisms	**Initial weight (gm)**	**Final weight (gm)**	**Total loss in weight (gm)**	**% loss in weight**
P.aeruginosa	0.048	0.048	0	0
B.subtilis	0.244	0.062	0.182	**74.59**
S.aureus	0.08	0.073	0.007	8.75
S.pyogenes	0.052	0.050	0.002	3.846

PET degradation in Bushnell Hass Broth inoculated separately with following microorganisms.

Microorganisms	**Initial weight (gm)**	**Final weight (gm)**	**Total loss in weight (gm)**	**% loss in weight**
P.aeruginosa	0.052	0.050	0.002	3.845
B.subtilis	0.057	0.056	0.001	1.754
S.aureus	0.086	0.086	0	0
S.pyogenes	0.051	0.048	0.002	**3.922**

PS degradation in Nutrient Broth inoculated separately with following microorganisms.

Microorganisms	**Initial weight (gm)**	**Final weight (gm)**	**Total loss in weight (gm)**	**% loss in weight**
P.aeruginosa	0.020	0.019	0.001	5
B.subtilis	0.010	0.008	0.002	**20**
S.aureus	0.021	0.020	0.001	4.762
S.pyogenes	0.012	0.011	0.001	8.33

PS degradation in Bushnell Hass Broth inoculated separately with following microorganisms.

Microorganisms	Initial weight (gm)	Final weight (gm)	Total loss in weight (gm)	% loss in weight
P.aeruginosa	0.008	0.008	0	0
B.subtilis	0.017	0.007	0.010	**58.823**
S.aureus	0.016	0.010	0.006	37.5
S.pyogenes	0.009	0.008	0.001	11.11

PET and PS degradation in Rose Bengal Broth inoculated separately with following micro-organism.

Micro-organism – *A.niger*

Plastic sample	**Initial weight (gm)**	**Final weight (gm)**	**Total loss in weight(gm)**	**% loss in weight**
PET	0.085	0.040	0.045	**52.94**
PS	0.022	0.022	0	0

Overall Result		
Type of plastic	PET	PS
Maximum degradation in different soil sample		
Type of soil	Garden soil	Garbage soil
Exact plastic degrading isolates on NA and SAB agar medium	Gram positive coccobacillus, Gram negative cocci, Gram negative bacilli (rod shape),Gram positive cocci (clusters).	Gram negative cocci in singles
Biodegradation of PET and PS by liquid culture medium.		
Medium and microorganisms	*Streptococcus pyogenes* and *Bacillus subtilis* in Bushnell Hass broth and Nutrient broth respectively. *A.niger* in Rose Bengal Broth	*B.subtilis* In Nutrient broth and Bushnell Hass broth .

Discussion and Conclusion .

- Garden soil exported from Panvel side and it was provided with bio-fertilizer in garden for better growth of plants for many years.
- Use of bio-fertilizer – enhance nutritional quality- flourish growth of micro-organisms.
- Out of these some were able to degrade PET better than PS.
- Garbage soil was rich in nutrients because of the waste dump in it. It was rich in microbial flora .Some found to degrade PS better than PET .
- Biofilms attached to synthetic plastic degrade PS and PET by enzymatic degradation.
- Symbiotic association may had also played an important role in degradation
- Microbial degradation of PET and PS in liquid culture medium indicates that PET and PS can be degraded if provided with suitable medium and microorganisms by liquid culture medium

Social benefit

Material name	**No. of years to decompose**	**Solution**	**Social benefit**
Plastic containers	50- 80	Biodegradation by isolated plastic degrading microorganisms from soil samples Biodegradtion by liquid culture method	Reduces plastic pollution and its adverse effect on human health.
Foamed plastic cups	50		
Plastic bottles	450		
Plastic bags	200-1000		