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Research Paper

Illustration of higher Vegetative growth of Acinetobacter sp. (MTCC 10497) in presence of MWCNT-COOH

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ABSTRACT

Nanotoxicity of carbon nanotubes is a rising problem with increasing use of carbon nanotubes in various fields. Understanding the ecological transformation of MWCNTs is vital for their life cycle evaluation and potential environmental footprints.In this project, we have demonstrated that the *Acinetobacter sp*. (MTCC 10497) grows many folds in presence of carboxylated MWCNT. This finding will help engineers to prepare a protocol to reduce nontoxicity and accumulation of carbon nanotubes.

Key words: Multi Walled Carbon Nanotube; Nanotoxicity, Biodegradation of CNTs, Acinetobacter sp. (MTCC 10497).

INTRODUCTION

Carbon nanotubes (CNTs) are long cylinder-shaped allotropic manifestations of carbon. They are most broadly synthesised by Chemical Vapor Deposition (CVD). They have remarkable chemical, electronic, mechanical and optical properties. Being among the most guaranteeing materials in nanotechnology, they are expected to revolutionize medicine [1].

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Considering the increasing use of CNTs in commercial products, many studies emphasizes further needs to study ecotoxicity and highlights that assessing the risks of the CNTs requires a better understanding of their toxicity, bioavailability and behavior into the environment. [2-4]

Alexander Star et. al. reported the catalytic biodegradation of carbon nanotubes in vitro byoxidative activity of horse radish peroxidase (HRP) and low concentrations of hydrogen peroxide (□40 μM). This indicates possible biotechnological and natural (plant peroxidases) ways for degradation of carbon nanotubes in the environment. They provided compelling evidence of the biodegradation of carbon nanotubes by HRP/H_2O_2 overthe period of several weeks. This marks a promising

possibility for nanotubes to be degraded by HRP in environmentally relevant settings. It is tempting to speculate that other peroxidases in plants and animals (e.g., myloperoxidase) may be effective in oxidative degradation of carbon nanotubes. If so, enhancement of these catalytic biodegradation pathways may be instrumental in avoiding their cytotoxicity in drug delivery, gene silencing and tumor imaging [5]. With further insight into this type of biodegradation process, it will be possible to engineer better, more efficient drug delivery platforms, where the patient need not worry about the injection of materials that could possibly accumulate causing cytotoxic effects [5].

On the other hand so far *Burkholderiakururiensis*, *Delftiaacidovorans* and *Stenotrophomonas maltophilia* are reported to degrade acid treated carbon nanotubes through unknown mechanism [6]. Along with further reports into this kind of microbial biodegradation phenomena, it will be possible to access true impact of carbon nanotubes toxicity on environment.

MATERIAL AND METHOD

MWCNT

MWCNTs (TNMC3, -COOH, 10-20nm, >95%) were purchased from Chengdu Organic Chemicals Co. Ltd., Chinese Academy of Sciences. No.16, South section 2, the first Circle road, Chengdu, P.R.China, 610041.

Acinatobacter sp. (MTCC 10497)

Acinatobacter sp. (MTCC 10497) was brought from Microbial Type Culture Collection (MTCC) Institute of Microbial Technology, Sector 39-A, Chandigarh – 160036, India.

- Soyabean Casein Digest Medium (Tryptone Soya Broth) by HiMedia M011-500G

Effect of MWCNT-COOH against *acinatobacter sp.* (MTCC 10497)

1. *Acinatobacter sp.* (MTCC 10497) were cultured to a mid-log phase at 37ºC with shaking (Inoculated at 9:30am, incubated upto 3:30pm) in Soyabean Casein Digest Medium (Tryptone Soya Broth).
2. 1mL of the MWCNT–COOH suspension (100µg/ml & 500µg/ml suspended in PBS) were mixed with 10µl of *Acinatobacter sp.* (MTCC 10497) in Soyabean Casein Digest Medium (Tryptone Soya Broth) and incubated at 37ºC for 1 hour.
3. After Incubation, 10µl of incubated sample was diluted by 10ml of PBS.
4. 100µl of diluted sample was spread on sterile Tryptone Soya agar plates.
5. After overnight incubation at 37ºC, number of colonies on each plate was measured.
6. 1 mL of pure PBS was used as a negative control (7).

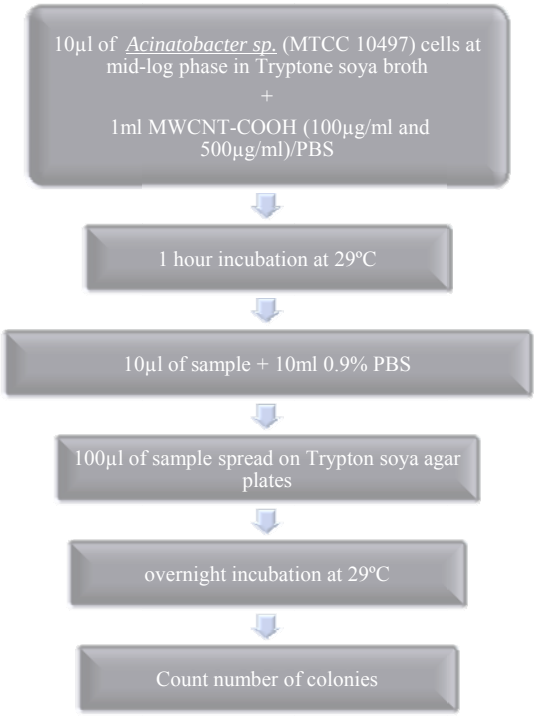

Figure 1: Flow chart of procedure for detection of effectof MWCNT-COOH on *Acinatobacter sp.*(MTCC 10497).

RESULT AND DISCUSSION

Acinetobacter sp. (MTCC 10497) showed 1200% higher growth in suspension of 100 µg/ml carboxylated MWCNT's, whereas 237% higher insuspension of 500 µg/ml carboxylated MWCNT's. *Acinetobacter sp.* (MTCC 10497) is specialized in bioremediation meaning ability of growing in presence of high aromatic groups and high organic content. Carbon nanotubes provide both things to *Acinetobacter sp.(*MTCC 10497).Further study need to be carried out in order to prove this theory/ hypothesis. No. of colonies measured after overnight incubation at 29ºC (Table1 and 2).

Bacterial growth percentage (% growth) was defined as

$$(N_{MWCNT\text{-}COOH} - N_{Control})/N_{MWCNT\text{-}COOH} \text{ X } 100$$

Where,

$N_{Control}$ and $N_{MWCNT\text{-}COOH}$ represent the number of bacteria inNaCl and the MWCNT-COOH suspension, respectively. Increase in CFU of *Acinatobacter sp.*is represented in figure2.

Robustness CONCLUSION

In this project, we have demonstrated that the *Acinetobacter sp*. (MTCC 10497) grows many folds in vicinity of carboxylated MWCNT. This finding will help engineers to prepare a protocol to abatenano toxicity and accumulation of carbon nanotubes.

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ANNEXURE

Table 1: Number of colonies of *Acinatobacter sp.* (MTCC 10497) measured

	No. of Colonies					
	100µg/ml			500µg/ml		
	Set-I	Set-II	Set-III	Set-I	Set-II	Set-III
Control	124	70	91	81	28	20
MWCNT	1159	1191	1356	126	166	142

Table 2: Average number of colonies of*Acinatobacter sp.*(MTCC 10497)measured

	Average no. of Colonies	
	100µg/ml	500µg/ml
Control	95	43
MWCNT	1235	145

Table 3: kill percentage of*Acinatobacter sp.* (MTCC 10497)measured

	Percent increase in Colony number	
	100µg/ml	500µg/ml
MWCNT	1200%	237%

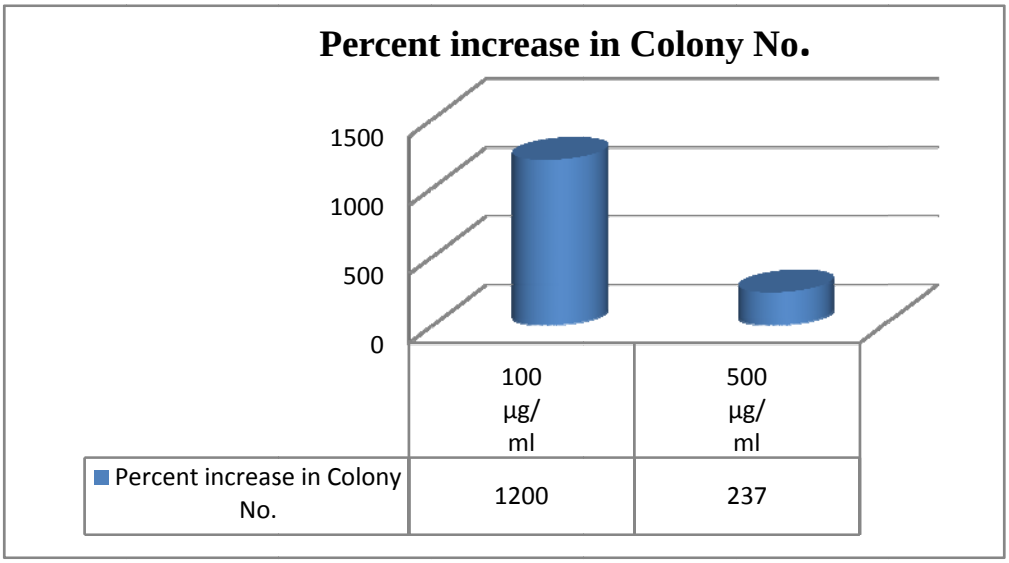

Figure 2: Percent increase in growth of *Acinetobacter sp.* (MTCC 10497)