NANOTUBES BIOLOGICALLY ACTIVE IN MEDIA WITH HIGH SALT CONCENTRATION

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ABSTRACT

The current work focuses on the effect of silica and titania nanotubes and their synthesis conditions on the potential antibacterial activity. Since most investigations are oriented towards carbon nanotubes, we focused on studying the biological activity of oxide nanotubes obtained under different synthesis and different thermal treatment conditions. The antibacterial activity against Bacillus sphericus, Bacillus subtilis, Escherichia coli and Virgibacillus halodenitrificans were examined by the plate counting method. The biological activity was evaluated as dehydrogenase activity. The results revealed that titania nanotubes exhibit a higher antibacterial activity as compared with silica. This activity could be correlated with differences in the structure and morphology of these two types of nanotubes investigated.

Keywords: Environment depollution, Extreme environments, Nanobiotechnology, Nanotubes

Introduction

The unique properties of nanoparticles, together with the various methods available for the preparation of nanostructures of controlled shape and size, provide building blocks for nanoscale structures and devices. A variety of methodologies for the preparation of nanostructures within a narrow size distribution is available (7). The convergence of biotechnology and nanotechnology has led to the development of hybrid nanomaterials that incorporate properties of biomaterials (protein, enzyme, DNA) with nanoparticles' characteristics (4, 5).

Nanoscience represents one of the most important research and development frontiers in modern science, but little is known about the environmental impact of nanoparticles and nanotubes. Moreover, green nanotechnology is expected to bring solutions to current biotopes' challenges. Nanoparticles show some environmental friendly features, including self-cleaning coatings that limit the need for detergents, pollution-control agents that remove nitrogen oxide from the air, heat insulation materials, air and water filters, new-generation photovoltaic cells and much more. Another application of green nanotechnology is to harness the strong catalytic power of metal oxide nanoparticles to treat water or decontaminate soil. A surface coated with a titanium oxide nanofilm can break down pathogenic organic pollutants or microorganisms in water media by photocatalysis (1, 2, 9). For soil remediation, iron nanoparticles could be introduced into polluted strata to break down pollutants like organonitrogen compounds or chlorinated hydrocarbons, such as pesticides, dioxins and polychlorinated biphenyl. Microbial degradation of chlorinated pesticides in saline environments was also recently reported (8).

Nanotubes are tubular structures with an outer diameter of up to 100 nm which represent an alternative to spherical nanoparticles with various applications in bio and nanotechnologies. Nanotubes offer some interesting advantages as compated to spherical nanoparticles in biotechnologies (6). For example, nanotubes have large inner volumes relative to the dimensions of the tube, which can be filled with any desired chemical or biochemical species with dimensions ranging from those of small molecules to those of proteins. In addition, nanotubes have distinct inner and outer surfaces, which can be chemically or biochemically functionalized (6). This creates the possibility, for example, of loading the inside of a nanotube with a particular biochemical payload, but imparting chemical features to the outer surface that rends it biocompatible. Nanotubes have open ends, which make the inner surface accessible and the incorporation of species in the tubes particularly easy (6).

Since most recent investigations were focused on studying carbon nanotubes, the aim of this work was to reveal the presence of biological activity of titania or silica, obtained under different conditions of synthesis and different thermally treatments.

Materials and methods

Cultures and media – The following microorganisms were used: *Escherichia coli* (IBB collection – isolated from effluent of wastewater treatment plant), *Virgibacillus halodenitrificans* (IBB collection – isolated from the surface of subterranean rock salt), *Bacillus subtillis* (IBB collection – isolated from the surface of subterranean rock salt) and *Bacillus sphericus* (DSMZ 369). The culture media consisted of nutrient broth for *E. coli* and *B. sphericus*, and MH medium with the following composition (g/L): yeast extract (10), proteose peptone (5), glucose (1), NaCl (100), $MgCl_2$ x $6H_2O$ (7), $MgSO_4$ x$7H_2O$ (9.6), $CaCl_2$ x $2H_2O$ (0.36), KCl (2), $NaHCO_3$ (0.06), NaBr (0,026) [10] for *B. subtillis* and *V. halodenitrificans*.

Tested nanotubes were prepared following various procedures. Hydrothermal method was used in the case of TiO_2 nanotubes, while in the case of SiO_2 microtubes, we used the sol-gel method in the presence of templates. The experimental conditions and the main characteristics of the nano(micro)tubes are described in **Table 1**.

The synthesized nanotubes were observed by *electronic microscopy* (TEM) following the method described by Hayat (3).

Test of antibacterial properties – The antibacterial test was performed by the plate counting method, as follows: approximately 0.009 g of tested nanotubes were added into 40 ml of a phosphate buffer solution (pH 7) containing bacterial strains, and the mixture was incubated at 28^0C or 37^0C, with agitation for 24 hours. One ml of the above suspension was cultured on agar plate and incubated at 28^0C or 37^0C for 24/48 hours. After this period, the exact number of colonies and the number of remaining bacteria were counted.

Dehydrogenase activity – 3 ml of bacterial suspension were mixed with 0.5 ml of 3% triphenyltetrazolium chloride (TTC) and incubated at 37^0C for 24 hours. The reaction was stopped by adding acetone. The resulted mixture was filtered, and the absorbance at 490 nm was then measured. The dehydrogenase activity was assessed as mg formazan %.

TABLE 1

Experimental conditions for TiO_2 and SiO_2 nantubes synthesis

Sample	Experimental conditions				Samples characteristics
	Reaction mixture	Reaction time (h) and temperature (°C)	Post reaction thermal treatment (h)		
			110°C	400°C	
I.a	TiO_2+NaOH	24/140	12	-	Nanotubes, L≈ 50 nm, Ø ≈ 8-9 nm and different amount of amorphous TiO_2 powder
I.b	TiO_2+NaOH	24/140	12	1	
II.a	TiO_2+NaOH	48/140	12	-	
II.b	TiO_2+NaOH	48/140	12	1	
III	TEOS*+ TA	4/20	5	-	Micrometric tubes L≈2-5 µm Ø≈0.1-0.2 µm

* TEOS = tetraethoxysilan, as SiO_2 source; TA = tartaric acid

Results and Discussion

The TEM images of the synthesized nano(micro)tubes used for antibacterial tests are presented in **Fig. 1**. One may notice that the size and the morphology of the synthesized nano(micro)tubes is very different. Nanometric tubes are obtained in the case of TiO_2, while in the case of SiO_2 the tubes have micormetric size, being more than 10 times larger then the TiO_2 tubes. In this way, different antibacterial activity could be expected from the two types of tubes.

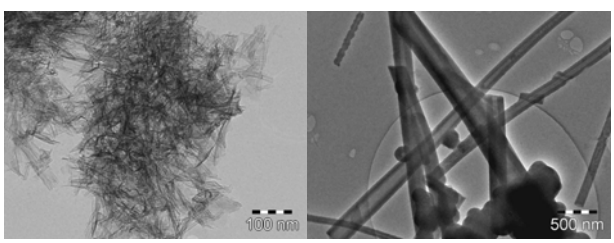

Fig. 1. TEM micrographs on silica micromertric tubes (left) and titanate-based nanotubes (right)

The investigated bacterial strains showed various resistances in the presence of tested nanotubes in their culture

media. The TiO_2 nanotubes presented significant inhibitory effect in the case of *B. subtillis* (**Fig. 2b**) and *V. halodenitrificans* (**Fig. 2c**) strains isolated from the surface of a salt rock formed in Neogen period and located at 200 meters in subterranean salt deposits in Slanic, Romania. On the other hand some of the investigated nanotubes (samples I.b, II.a, and III) do not show antibacterial activity towards *E. coli* (**Fig. 2d**) and when the effect is present, they have a lower intensity as compared to *V. halodenitrificans*. All the investigated nanotubes showed antibacterial activity in the presence of *B. sphericus* (**Fig. 2a**) but this is lower as compared with *V. halodenitrificans* and *B. subtilis* strains.

In the case of TiO_2 nanotubes could be observed that biological activity is present for *B. sphericus*, *B. subtilis* and *V. halodenitrificans* but not for *E. coli*. By increasing either reaction time for nanotubes synthesis (sample II.a and II.b) or thermal treatment after reaction (samples I.b and II.b) the antibacterial properties became more evident (**Fig. 2. a-d**) and could be associated with improving of the nantoube/powder ratio in the resulted material in the first case or with better ordering of the structures of the nanotubes, in the second one. This behavior could be observed for samples I.a and II.a towards *B. sphericus*, *B. subtilis* and *V. halodenitrificans* most probably as a consequence of increasing of the reaction time from 24 to 48 hours (**Table. 1**). The thermal treatment after synthesis (samples I.b and II.b) led, probably, to a better consolidation of the nanotubes and consequently conducted to a better antibacterial activity towards all bacterial strains tested.

The antibacterial activity of silica nanotubes was higher towards *B. sphericus* very low towards *V. halodenitrificans* and absent against of *B. subtilis* and *E. coli*. In the presence of *B. sphericus,* the antibacterial activity of the silica nanotubes was higher as compared with titania nanotubes. In the presence of *V. halodenitrificans* the antibacterial activity was lower as compared with that of titania tubes. In the cases of *B. subtilis* and *E. coli* that was no antibacterial activity registered.

The antibacterial activity of titania nanotubes appears to be correlated with their chemical composition and synthesis pathway. The best activity has been observed towards *B. subtilis* for the samples I.a and II.b (**Fig. 2b**) and *V. halodenitrificans* (**Fig. 2c**) for the samples I.b and II.a. The cells of *B. sphericus* were also inhibited by the titania nanotubes mainly in the case of sample II.a (**Fig. 2a**). A different behavior was observed towards *E. coli* cells, in particular for the samples I.b, II.a, III. In these cases, titania tubes were inactive as antibacterial agents (**Fig. 2d**).

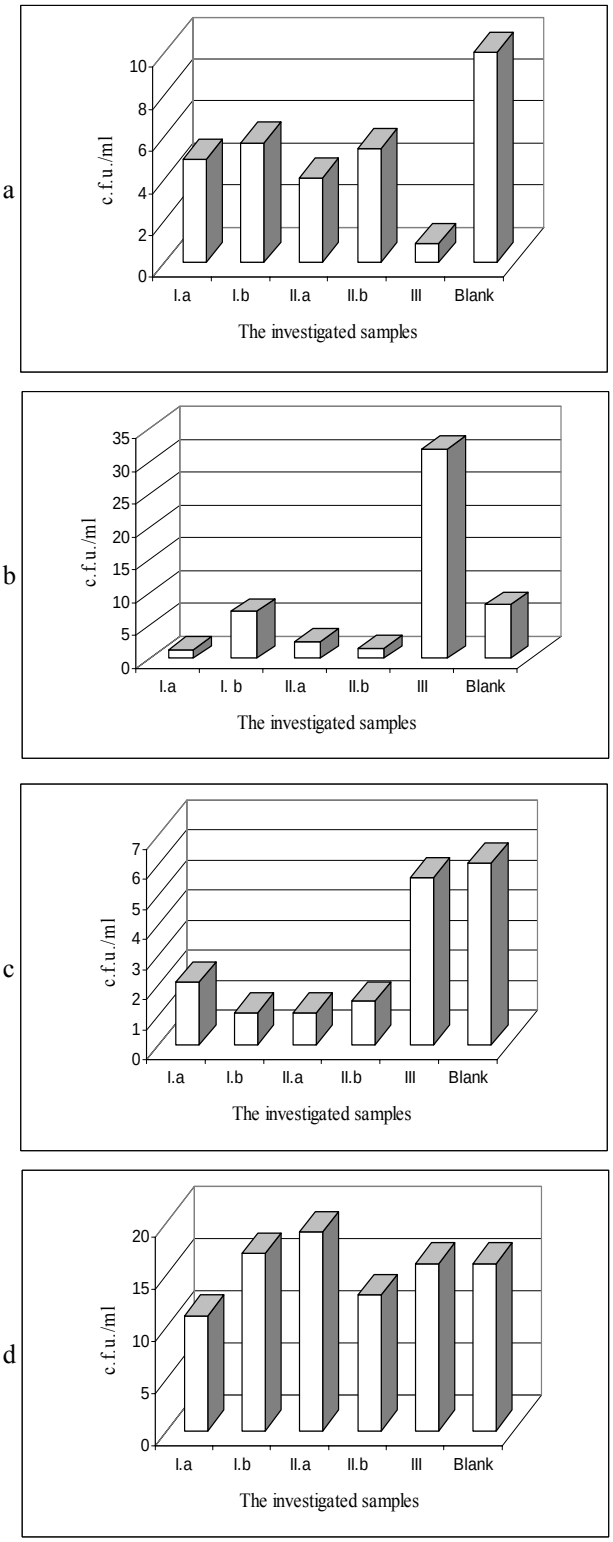

Fig. 2. Total u.f.c. number quantified after 24 hours of growth of *B. sphericus* ($x10^7$ **a**), *B. subtilis* ($x10^5$, **b**), *V. halodenitrificans* ($x10^5$, **c**), *E. coli* ($x10^7$ **d**) at 28^0C or 37^0C. For description of samples see Table 1.

The dehydrogenase activity, as indicator of total biological activity, was also influenced by the investigated nanotubes for all tested strains (**Table 2**). Similarly with the results of the plate counting method, the best inhibitory activity was observed in the case of *B. subtillis*. The various effects of the nanotubes on the bacterial strains and their metabolic processes could be associated also with the structure and composition of the samples. The results obtained in the case of titania nanotubes showed that the antibacterial activity is reduced after thermal treatment at 400^0C (**Fig. 2a,b,d**; samples Ia. Ib.). When the reaction time for nanotube synthesis was 48 hours (sample II), the antibacterial activity after thermal treatment is lower in the case of Gram-negative strains as compared to Gram-positive ones. The silica nanotubes showed activity only towards Gram-positive tested bacteria. These results suggested that the methods of nanotubes synthesis have an intensive effect on the antibacterial properties of nanotubes.

The resistance of bacterial strains in the presence of various titania or silica nanotubes could be associated with the chemical composition of the cell wall. The best activity has been obtained for the Gram positive strains like *B. sphericus* and *V. halodenitrificans*.

TABLE 2

Dehydrogenase activity of bacterial *strains* in the presence of investigated nanotubes. The strains were cultivated for 24 hours at 28^0C or 37^0C in agitation conditions (150 rpm). For description of the samples see Table 1.

Sample	Dehydrogenase activity (mg formazan %)							
	B. sphericus		*B. subtilis*		*V. halodenitrificans*		*E. coli*	
	Initial	After 24 h	Initial	After 24 h	Initial	After 24 h	Initial	After 24 h
I.a	0.092	0.083	0.257	0.016	0.077	0.079	0.178	0.079
I.b	0.085	0.081	0.173	0.017	0.086	0.079	0.156	0.074
II.a	0.075	0.083	0.305	0.017	0.097	0.104	0.143	0.112
II.b	0.079	0.132	0.20	0.016	0.082	0.086	0.159	0.090
III	0.075	0.072	0.261	0.016	0.075	0.079	0.178	0.084
Blank	0.074	0.110	0.127	0.017	0.082	0.541	0.148	0.086

These results revealed that silica nanotubes present a higher antibacterial activity against *B. sphericus,* while titania nanotubes are more active to *B.subtilis* and *V. halodenitrificans*. This activity could be correlated with differences in the structure and morphology of the two types of studied nanotubes.

The obtained results showed that antibacterial activity of tested nanotubes has been influenced by reaction time for synthesis or thermal treatment after synthesis. The obtained data showed that the interaction between bacterial cells and nanotubes conducted also to different inhibition degree consequently of the composition of the bacterial cells wall. The further investigations will be oriented towards description of the mechanisms of interaction of the nanotubes (silica or titania) with bacterial cell in order to establish the presence of the potential target for the nanotubes at the level of cell wall, or interaction is randomly one. The investigations will help for development of modern technologies for decontamination and sterilization in various fields of industry, agriculture and bionanotechnologies.

REFERENCES

1. **Gartner M., Trapalis C., Todorova N., Giannakopoulou T., Dobrescu G., Anastasescu M., Osiceanu P., Ghita A , Enache M., Dumitru L., Stoica T., Zaharescu M., Bae J.Y., Suh S.H.** (2008) Bull. Korean Chem. Soc., **29**, 1038-1042.
2. **Gartner M., Stoica T.F., Stoica T., Anastasescu C., Zaharescu M., Enache M., Dumitru L., Trapalis C.** (2008) In: Nanostructured Materials and Nanotechnology II, Ceramic Engineering and Science Proceedings (S. Mathur, M. Singh, T. Ohji and A. Wereszczak eds.), Vol. 29, Issue 8, 67 – 77.
3. **Hayat M.A.** (1972) Basic Electron Microscopy Technique, Van Nostrand Reinhold Company, New York, USA.
4. **Katz E., Shipway A.N., Willner I.** (2003) In: Nanoparticles – From Theory to Application, (Ed.: G. Schmidt), Wiley-VCH, Weinheim, 368-421.

5. **Katz E., Willner I.** (2004) Angew. Chem. Int. Ed., **43**, 6042-6108.
6. **Kohli P., Martin C.R.** (2006) Current Pharma. Biotechnol., **6**, 35-47.
7. Metal Nanoparticles: Synthesis, Characterization, and Application (2002) (Eds.: D.L. Feldheim, C.A. Foss Jr), Marcel Dekker, New York .
8. **Oncescu T., Oancea P., Enache M., Popescu G., Dumitru L., Kamekura M.** (2007) Cent. Eur. J. Biol., **2**, 563 – 573.
9. **Văcăroiu C., Enache M., Gartner M., Popescu G., Anastasescu M., Brezeanu A., Todorova N., Giannakopoulou T., Trapalis C**., **Dumitru L.** (2009) World J. Microbiol. Biotechnol., **25**, 27-31.
10. **Ventosa A., Garcia M.T., Kamekura M., Onishi H., Ruiz-Berraquero F.** (1989) Syst. Appl. Microbiol., **12**, 162–166.