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Research Article

EFFECT OF ZNO NANO PARTICLES AGAINST STRAINS OF *ESCHERICHIA COLI*

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ABSTRACT

The antimicrobial activity of zinc oxide nano particles against *E. coli* strains was investigated as a model for gram-negative bacteria. Zinc oxide nanoparticles were prepared and characterized by scanning electron microscopy (SEM), X-ray diffraction (XRD) and ultraviolet (UV) analysis. The antimicrobial study was performed by well diffusion assays and growth rate studies, with different concentrations of zinc oxide nano particles. The results showed zinc oxide nanoparticles to be an effective bactericide. Scanning electron microscopy was used to study the bactericidal action of these nano scale materials. The treated *E. coli* cells showed damages by formation of "pits" in the cell wall of the bacteria, while the zinc oxide nanoparticles was found to be accumulated in the bacterial membrane. A membrane with such morphology exhibits a significant increase in permeability, resulting in death of the cell. Atomic force microscopy analysis of nanoparticle treated bacterial DNA showed changes in DNA. The results suggest that these nano materials, which can be prepared in a simple and cost-effective manner, may be suitable for the formulation of new types of bactericidal materials

Keywords: Zinc oxide nano particles, Antimicrobial activity, Scanning electron microscopy.

INTRODUCTION

Emergence of antibiotic resistance strain of both Gram-positive and Gram-negative bacteria is a major public health concern. In past 40 years, only two antibiotics (namely linezolid and daptomycin) representing new chemical classes have reached market to treat multi drug resistant infections. Current advancements in nanotechnology have led to the development of new techniques to synthesize nano particles of different size and shape, and physical and chemical properties, which can be a source of development of new antibacterial agents [1-3].

It is found that nearly all the nano particles have toxicological and antibacterial effect on a wide range of microorganisms [4].

The metals and metal oxides such as ZnO are known to be toxic to host human cells at relatively high concentrations; but they are not expected to be toxic at very low concentrations. In fact, it has been shown that ZnO protects against intestinal diseases by protecting intestinal cells from entertoxigenic *Escherichia coli* infection by inhibiting the adhesion and internalization of bacteria [5].

Although the *in vitro* antibacterial activity and efficacy of regular zinc oxides have been investigated, little is known about the antibacterial activity of nanoparticles of ZnO. Preliminary growth analysis data suggest that nano particles of ZnO have significantly higher antibacterial effects on *Staphylococcus aureus* than do five other metal oxide nano particles [6].

Here an attempt was made to study antibacterial activity of ZnO nanoparticles on Gram-negative bacteria, *E. coli*.

METHODS

Materials

E. coli strains (were MTCC 443 and MTCC 1687) from MTCC, Chandigarh. Zinc acetate dihydrate, NaOH, polyvinylpyrrolidone (PVP) from Himedia.

Zinc nanoparticles synthesis

The precursor chemicals used here for the production of nano particles was zinc acetate dihydrate. Zinc nano particles were produced from its precursors by the wet chemical method by metal displacement theory.

$$Zn(CH3COO)_2 + 2NaOH \rightarrow 2Na(CH3COO) + Zn(OH)_2$$

NaOH (0.8 g) was dissolved in 30 ml of deionized waster in a beaker and stirred well until it dissolves. Zinc acetate (0.249 g) was dissolved in 30 ml of de-ionised water in another beaker and stirred well. Into this PVP (0.3 g) was added and stirred for 20 minutes till the solution becomes clear. PVP acts as a surfactant and minimizes the chance of agglomeration of $Zn(OH)_2$ particles to form crystal. NaOH solution was now poured into the burette. With continuous stirring of zinc acetate solution in a beaker, the NaOH solution was allowed to fall very slowly into the zinc acetate solution. As the reaction proceeds a milky solution appeared to be formed. The reaction was allowed to continue till the pH reaches 10 and then it is stopped to get a milky solution. This solution was then centrifuged till particles were observed. Particles were washed again twice and centrifuged again. After the supernatant was discarded the powdery substance was dried in an oven for 24 hrs at 60°C. The dried nano particles were then crushed and then were characterized by scanning electron microscopy (SEM), X-ray diffraction (XRD) and ultraviolet (UV) analysis [7].

Antibacterial activity

Specific media plates were prepared each with strains of *E. coli*. 200 µl of bacterial culture was uniformly spread on the plates. Wells at equidistant were punched on the solidified agar plates. In each plate different concentrations of nano particles (150 µg/ml, 200 µg/ml, 250 µg/ml, 300 µg/ml and 350 µg/ml). Then the petri plates are sealed and kept at 37°C for incubation for 24 hrs. After 24 hrs zone of inhibition was measured in mm [8].

Antibacterial activity using spectrophotometer

Effect of nano particles on bacterial growth was determined by comparing growth curves of the ZnO treated and untreated bacterial cultures.

The curve was obtained by the following procedure:

The 150 ml of respective broths were taken in four different conical flasks. The minimum inhibitory concentration of nano particles

(150 µg/ml) was transferred into all the flasks. Then 100 µl of the respective overnight cultures were transferred to the flasks. The flasks were kept in orbital shaker. Then OD of the untreated cultures along with the treated cultures was recorded at regular time intervals of 1 hr. The growth curve was prepared and compared.

DNA isolation

The nano particles affect the cellular membrane, by forming pits but their effect on the bacterial DNA can be studied with the genomic DNA isolation.

DNA was isolated from the treated cultures which were incubated at different time intervals (Blount *et al.*, 1987).

The complete effect study will then further be carried out the restriction digestion of the genomic DNA and the band comparative study.

DNA analysis using atomic force microscopy (AFM)

100 µl of the DNA sample was taken and spin coated for AFM analysis.

RESULTS AND DISCUSSION

Zinc nanoparticles synthesis and characterization

ZnO nano particles were synthesized and characterized by XRD, UV-Visible (UV-Vis) and SEM. The result of XRD analysis confirms the particles as hexagonal wurtzite of zinc oxide. The size was calculated by scherrrer formula and found to be range of 20-30 nm. XRD spectra has been reported in Fig. 1.

UV-Vis results were obtained in the form of graph between intensity (a.u) and wavelength (nm). From this graph morphology and size is determined. The absorption was maximum at 367 nm. Fig. 2 graph determines the morphology and size of nano particles.

SEM analysis reveals that these nano particles are rod shaped. Fig. 3 shows the size and shape of nano particles at ×120,000 magnification.

Antibacterial properties of the zinc oxide nanoparticles

Present preliminary studies have demonstrated that ZnO nanoparticles analyzed shows a significant growth inhibition of *E. coli* cells. Toxicological impact studies of characterized ZnO nano particles on *E. coli* were performed by well diffusion assays and growth rate studies. The effect on growth and antibacterial activity of zinc oxide nanoparticles on *E. coli* strain (MTCC 443) is given in Figs. 4 and 5. The effect on growth and antibacterial activity of zinc oxide nanoparticles on *E. coli* strain (MTCC 1687) is given in Figs. 6 and 7. The inhibition zones yielded by different concentration of ZnO nano particles against *E. coli* strains are shown in Tables 1 and 2. The effect of zinc oxide nano particles on growth of *E. coli* strains by spectrophotometer study are given in Tables 3-6. The bacterial growth curve study shows the effect of nano particles in degrading

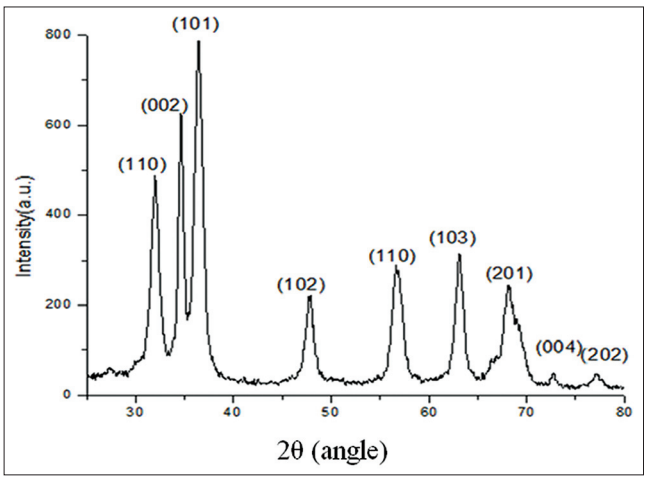

Fig. 1: X-ray diffraction graph determines shape of nanoparticle

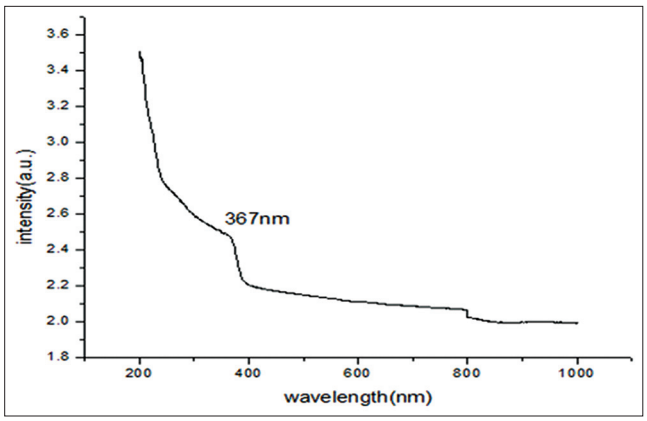

Fig. 2: Ultra violet spectroscopy graph determines morphology and size of nano particles

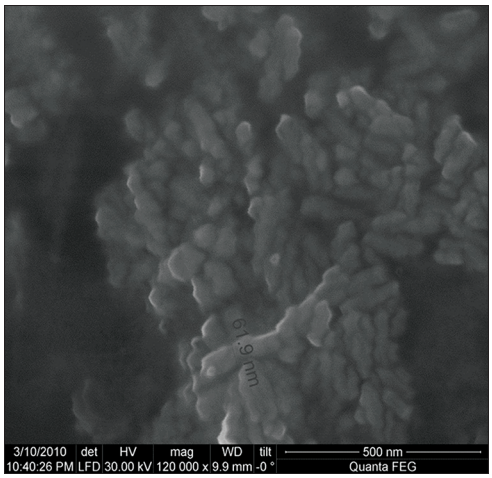

Fig. 3: Scanning electron microscopy images showing size of nano particles at ×120,000 magnification

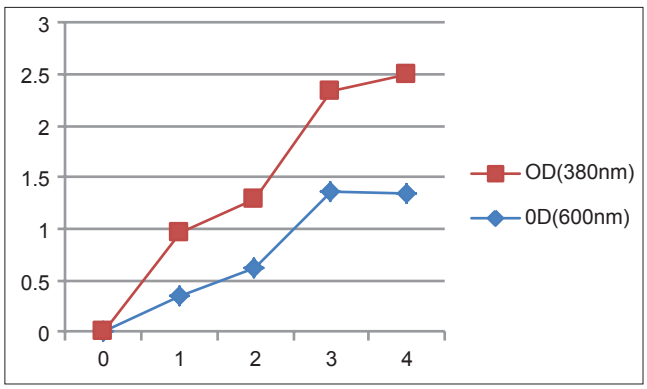

Fig. 4: The effect of zinc oxide nano particles on growth of *Escherichia coli* strain (MTCC 443) at 380 nm and 600 nm

the bacterial cells as the exponential phase was seen to be less than a normal bacterial growth curve.

The further study was done to see any effects on bacterial DNA. Differences were observed in DNA, treated and untreated when run on an agarose gel (Figs. 8 and 9). AFM study also revealed changes in DNA structure (Figs. 10-13). The preliminary findings suggest that ZnO nano particles are more effective at eliminating *E. coli* cells by damaging the cell wall and also affecting the genetic material of the microorganism.

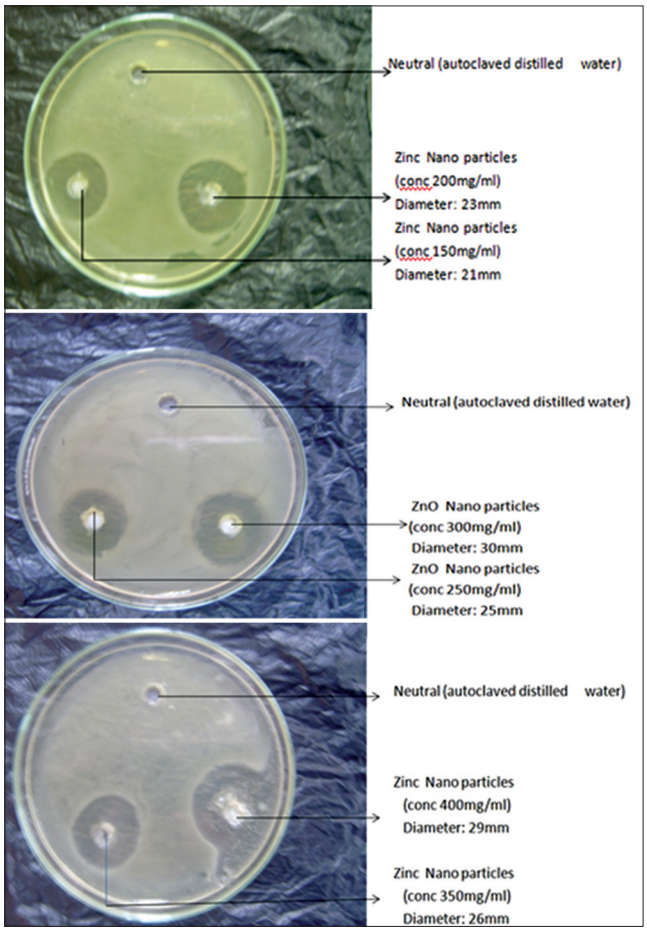

Fig. 5: Antimicrobial activity of ZnO nano particles by well diffusion method of *Escherichia coli* (MTCC 443) at different diameters (a) 21 nm (b) 25 nm (c) 26 nm

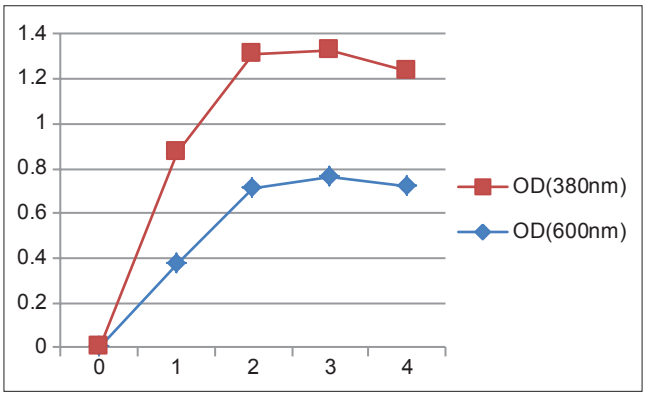

Fig. 6: The effect of zinc oxide nano particles on growth of *Escherichia coli* strain (MTCC 1687) at 380 nm and 600 nm

CONCLUSION

A simple and elegant method is adopted to prepare the nano particles

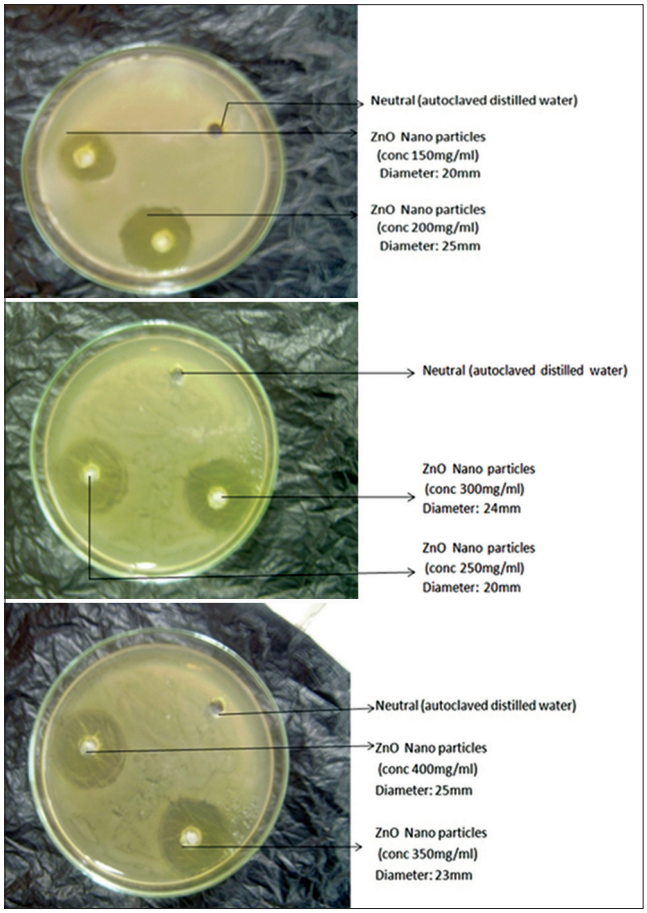

Fig. 7: Antimicrobial activity of ZnO nano particles by well diffusion method of *Escherichia coli* (MTCC 443) at different diameters (a) 20 nm (b) 23 nm (c) 25 nm

Table 1: Antimicrobial activity of different concentration of ZnO nano particles. (*E. coli* 1687)

S.No.	Concentration (µg/ml)	Result	Zone of inhibition (mm)
1	100	--	00
2	150	++	20
3	200	++	25
4	250	++	26
5	300	++	28
6	350	++	30
7	400	++	32

E. coli: Escherichia coli

Table 2: Antimicrobial activity of different concentration of ZnO nano particles (*E. coli* 443)

S.No.	Concentration (µg/ml)	Result	Zone of inhibition (mm)
1	100	--	00
2	150	++	21
3	200	++	23
4	250	++	25
5	300	++	30
6	350	++	32
7	400	++	34

E. coli: Escherichia coli

Table 3: The effect of zinc oxide nano particles on growth of *E. coli* strain (MTCC 443) at 600 nm

S.No.	Time (hrs)	OD at 600 nm
1	0	0
2	1	0.347
3	2	0.616
4	3	1.354
5	4	1.378

OD: Optical density, *E. coli: Escherichia coli*

Table 4: The effect of zinc oxide nano particles on growth of *E. coli* stain (MTCC 443) at 380 nm

S.No.	Time (hrs)	OD at 380 nm
1	0	0
2	1	0.629
3	2	0.682
4	3	0.989
5	4	1.154

OD: Optical density, *E. coli: Escherichia coli*

Table 5: The effect of zinc oxide nano particles on growth of *E. coli* stain (MTCC 1687) at 600 nm

S.No.	Time (hrs)	OD at 600 nm
1	0	0
2	1	0.214
3	2	0.276
4	3	0.292
5	4	0.414

OD: Optical density, *E. coli: Escherichia coli*

Table 6: The effect of zinc oxide nano particles on growth of *E. coli* stain (MTCC 1687) at 380 nm

S.No.	Time (hrs)	OD at 380 nm
1	0	0
2	1	0.495
3	2	0.596
4	3	0.842
5	4	0.910

OD: Optical density, *E. coli: Escherichia coli*

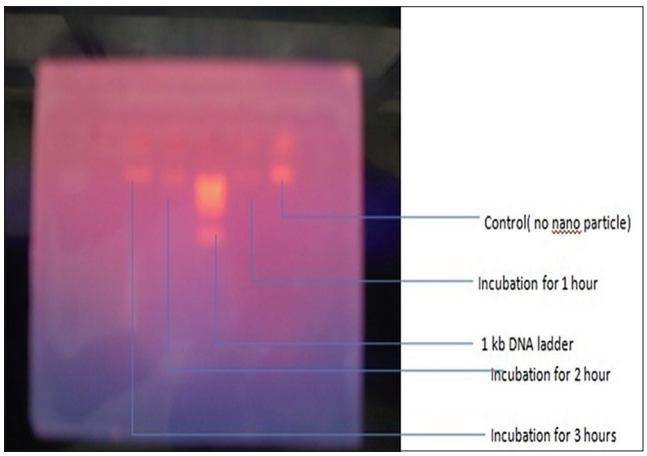

Fig. 8: Isolation of genomic DNA from nanoparticle treated *Escherichia coli* strain (MTCC443) by gel electrophoresis

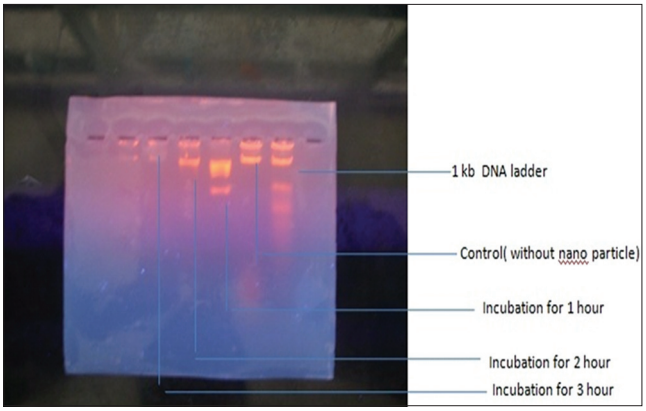

Fig. 9: Isolation of genomic DNA from nanoparticle treated *Escherichia coli* strain (MTCC1687) by gel electrophoresis

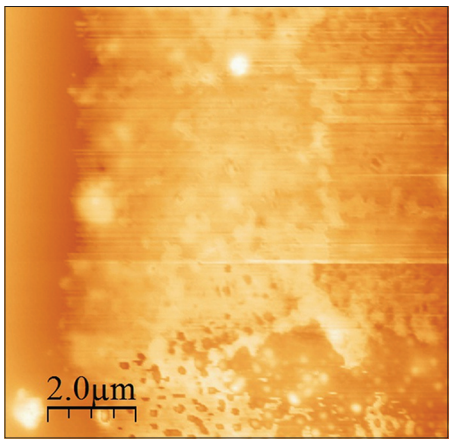

Fig. 10: DNA structure of bacterial strain (MTCC 443) viewed under atomic force microscopy after treatment of nanoparticles

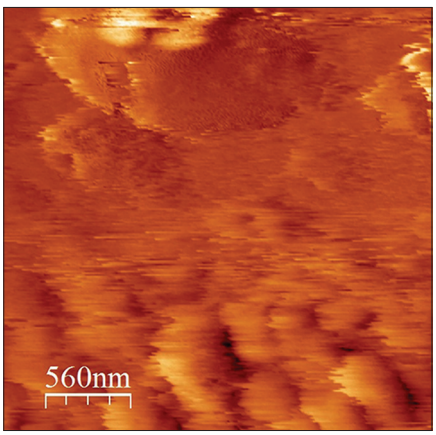

Fig. 11: DNA structure of bacterial strain (MTCC 443) viewed under atomic force microscopy without any treatment with Nano particles

of ZnO. The sizes of the prepared metal oxide nano particles are lesser than the size of 61.9 nm in diameter. The nano particles exhibits

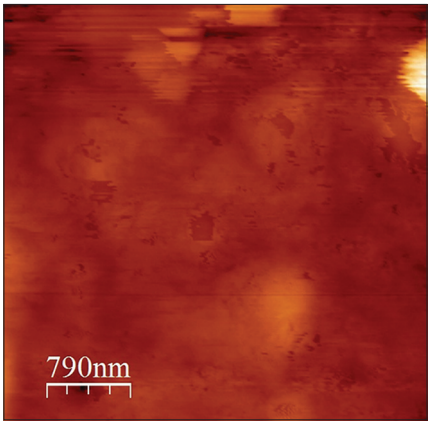

Fig. 12: DNA structure of bacterial strain (MTCC 1687) viewed under atomic force microscopy after treatment of nanoparticles

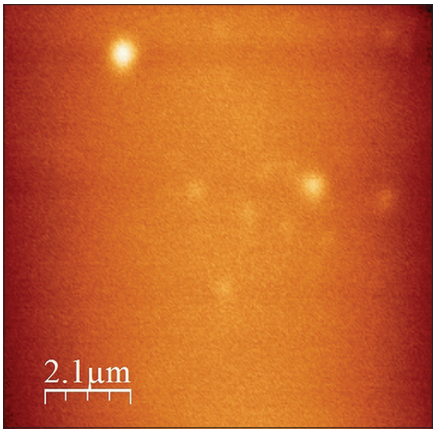

Fig. 13: DNA structure of bacterial strain (MTCC 1687) viewed under atomic force microscopy without any treatment with nano particles

antimicrobial activity against two strains of Gram-negative *E. coli* (MTCC 443 and 1687). This demonstrates that the nano particles can be employed as environmental friendly antimicrobial surface coating. The presented strategy opens up wide applications in various fields like optical switches, shutters, waveguides, and optical filters and in biomedical applications. The adopted synthetic procedure is amenable to fine tune the properties of the particles produced by choosing the suitable constituents at molecular level.

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