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THE EVIDENCE OF THE CHANGES IN THE ANTIMICROBIAL PROPERTIES OF PYOVERDINE AFTER SUPPLYING WITH MAGNETIC NANOPARTICLES

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Pseudomonas aeruginosa is a widely spread microorganism with many ecological nishes including human body. Magnetite nanoparticles coated with sodium oleate and dispersed in deionized water were prepared. The magnetite suspension concentrations in the culture medium of Pseudomonas aeruginosa were equal to: 0.0075–0.015-0.03-0.06-0.12-0.25-1.0-2.0 μL/mL. Fluorescence measurements, possible due to the fluorescent properties of pyoverdine, the siderophore synthesized by this bacterium, evidenced stimulatory effect of magnetite low concentrations. Further test was based on the response of other two bacteria to the pyoverdine obtained by thermolisis of Pseudomonas cultures. The antimicrobial effect of pyoverdine against several strains of Sarcina lutea and Staphylococcus aureus (clinical isolates from hospital patients) was amplified following the treatment with magnetic nanoparticles – though no distinct correlation with the magnetic nanoparticle concentration could be emphasized.

INTRODUCTION

The wide spreading of Pseudomonas aeruginosa in various ecological nishes with implications on human nutrition has raised the research interest for the multidisciplinary approach of this microorganism behavior. Various infectious diseases generated by P. aeruginosa could be related to food bacterial loading as revealed by many hospital patients with health troubles localized at the level of their digestive tube. Due to their sophisticated and diverse iron uptake systems Pseudomonas bacteria have adapted to various environments [1-3]. Most of fluorescent Pseudomonas species produce organic macromolecules with high affinity complexation sites for the ferric iron which acts as very efficient iron scavenger systems mainly in iron limiting situations [4-6] preventing the precipitation of insoluble iron oxyhydroxides. The iron complexes can be found in the form of various siderophore compounds among which those described in [7-11] where some essential properties are presented: structure, binding constants, uptake mechanisms, redox chemistry, interface interaction phenomena. For instance, the ferrichrome siderophores of E. coli (Fig. 1) act differently compared to the pseudobactin of P. aeruginosa though similar six oxygen atom systems are designed to couple an iron ion. Some biochemical and biophysical studies have shown that the behavior of the new identified iron-uptake mechanism in P. aeruginosa is different in comparison to those identified in other Gram-negative bacteria (well represented by E. coli), although it involves all the protein aggregates already known to be related to the uptake of ferric iron [12].

At the same time the environmental issues related to magnetic nanoparticle contamination – from both natural sources and industrial technology became of particular interest for food resources management.

Recently published reports concern the mechanism of iron uptake via pyoverdine (PaA) in P. aeruginosa by means of the interactions between PaA and its outer membrane receptor [8, 10-11] which is able to adopt two different conformations [13]. Based on these findings, iron biosensors using Pseudomonas strains have been designed for iron detection purposes; the best-controlled sensitive devices based on the bacteria ability to respond to iron stimuli have been obtained by using adequate mutants specialized in iron oxidization from different substrates [14 – 17].

From the viewpoint of medical bacteriology P. aeruginosa colonizing the human beings is known as a resistant germ against many bactericide or bacteriostatic agents. The low permeability of the outer membrane and an active export mechanism

for low molecular weight substances are the main findings related to its antibiotic resistance. More beta-lactamase activity diminishes the efficiency of pharmaceutical agents derived from beta-lactam antibiotics. A possible way of taking advantage on the P. aeruginosa resistance lies in the synthesis of antibiotics conjugated with compounds active as siderophores – reference [18] shows that the Trojan Horse strategy against resistant strains of Pseudomonas aeruginosa can be successful. The use of a siderophore pathway increases the permeability of the cell wall, and large substituents seem to reduce the beta-lactamase activity.

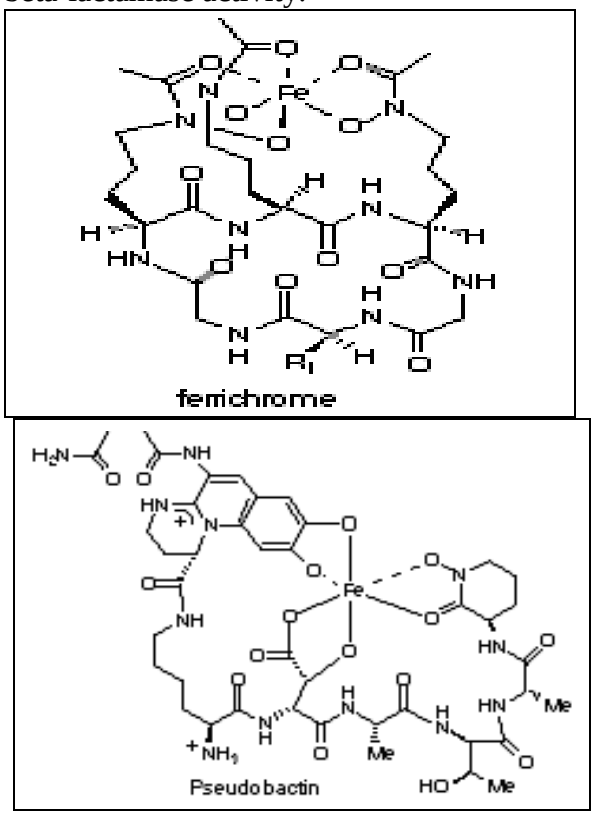

Fig. 1. Structures of E .coli ferrichrome and P aeruginosa pseudobactin (or pyoverdine).

Further the interaction of nano-particulate magnetite from the culture medium with the bacterial siderophore synthesized by P. aeruginosa is studied by mean of fluorescence data.

MATERIALS AND METHODS

Biological material. Pseudomonas aeruginosa ATCC 17503 and five clinical isolates of Pseudomonas spp. were cultivated in glass tubes with standard liquid culture medium (nutritive broth from Oxoid) supplemented with aliquots of magnetic nanoparticle suspension. The initial innoculum density was adjusted accordingly to standard protocol [19] to about 10^8 cell/mL Incubation was carried out at 35.0±0.5 °C in INCUCELL thermostatic room. Pyoverdine samples preparation was accomplished by thermal treatment (100 °C) of the Pseudomonas aeruginosa ATCC 17503 cell cultures followed by centrifugation (15 minutes at 3000 cycles/sec in adequate Mettler device).

Magnetic nanoparticle suspension. Magnetite particles dispersed in deionized water were prepared according to Cotae, 1981 [20] by coating magnetite ferrophase (co-precipitated from stoichiometric mixture of ferrous and ferric salts solutions) with sodium oleate shell. Ferrophase volume percentage was equal to 1.5 %, the physical diameter was ranging between 6.0 and 20.0 nm with maximum frequency of 10.74 nm. The magnetite suspension concentrations in the culture medium were equal to: 0.0075–0.015-0.03 - 0.06 - 0.12 - 0.25 - 1.0 - 2.0 μl/mL while corresponding magnetite concentrations were: 0.26 - 0.29 - 0.34 - 0.44 - 0.66 -1.1 - 1.9 - 3.6 - 7.0 μg/mL. For each concentration and each bacterial strain four tubes of 3 mL volume were used.

Fluorescence measurement. Laboratory assembled installation with convenient versatility was adjusted for florescence excitation in UV light at the wavelength of 300 nm and fluorescence intensity recording all over the visible range. Fluorescence quenching avoiding in the slight turbid samples was arranged by 1:10 dilution in distilled water.

Antimicrobial test. Five S. aureus clinical isolates and five clinical isolates of S. lutea were used to test the antimicrobial action of pyoverdine siderophore released by P. aeruginosa (ATCC strain); equal aliquots (of 0.1 μL from the tubes containing the cultures of P. aeruginosa on nutritive broth supplemented with magnetite nanoparticles were applied on the surface of agarized culture medium of each of the ten clinical isolates. Four repetitions on the same Petri dish were carried out for each bacterial strain and each concentration of magnetic particle

(added in the initial culture medium of P. aeruginosa). After incubation at 35.0±0.5 °C for 18 hours the growth inhibition areas were assessed with millimeter precision.

Statistic analysis. Average values and standard deviations were used to draw graphical plots. Student t test was applied to assess the statistical significance of the differences between the control samples and the experimental variants.

RESULTS AND DISCUSSION

Following the fluorescence spectra recording a large band with the maximum intensity at 410 nm was revealed – no significant shift or band width change being visible for different magnetite concentrations neither for different bacterial strains. The influence of magnetite nanoparticles on the fluorescence of the studied bacterial samples was presented in Fig. 2.

It is visible that the highest fluorescent emissions were recorded for P. aeruginosa ATCC strain –compared to the strains consistent with clinical isolates. In most cases the fluorescence intensity was increased ($p<0.05$) by the nanoparticle concentrations ranging from 0.26 μg/mL to about 1.1 μg/mL while for higher concentrations the fluorescence intensity was diminished back; for two of the clinical isolates (P.a.2 and P.a.3) the nanoparticle supply induced non-significant variations of fluorescence emission for the concentrations smaller than 1.1 μg/mL but the diminutions recorded for higher nanoparticle concentrations were statistically significant ($p<0.05$).

The standard deviation ranged from 5.1 to 8.3 %. It seems that the biosynthesis of pyoverdine was generally stimulated by iron oxides (composing the magnetite) supplied in the culture medium in small concentrations. In case of human body feeding with food affected by magnetic environmental contamination the medical issue of magnetic particles interference with potential non-critical microbial loading could be of general concern. In Figs. 3-4 the antimicrobial effect of pyoverdine on several strains of Sarcina lutea and Staphylococcus aureus is presented. In the case of S. aureus clinical isolates (Fig. 3) the growth inhibition areas have diameter values ranging from 14.5 to 20.0 mm; in some strains (S. aureus1, S. aureus2, S. aureus5) the response shape suggests the same variation as the fluorescence intensity – meaning that the antimicrobial effect of P. aeruginosa pyoverdine is correlated to the nanoparticle concentration that have induced the pyoverdine synthesis stimulation.

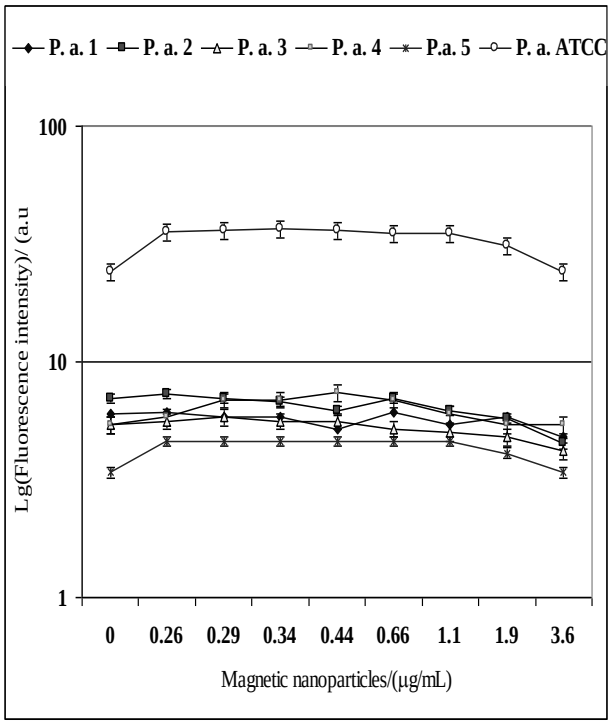

Fig. 2. The logarithmic representation of bacterial sample fluorescence for different concentrations of magnetic nanoparticles.

For S. lutea strains (Fig. 4) less amplified fluorescent emission was recorded – with growth inhibition diameters from 6.5 to 16.5 mm. No distinct correlation with the nanoparticle concentration was shaped. The study of P. aeruginosa behavior in magnetite supplied culture medium could be useful since it offers the possibility to extract new data regarding the mission of the pyoverdine molecules to sequester traces of Fe^{3+} and to transport them through the cell membrane into the periplasmatic space Bose et al, 2009 [27] and Bosh et al, 2010, [28] also found that iron oxide colloidal nanoparticles could be processed by iron reducing bacteria. Mainly the chemical component of iron oxides interference with bacteria cell was discussed. We believe that beside the role of the chemical agent one might consider also that the colloidal iron oxide supplied in the culture

medium could interfere with the microorganisms by other mechanisms able to influence the cell proliferation. Intrinsic magnetism of mono-domain ferrophase particles could also represent a cause of ion transport perturbation at the level of the bacterial cell membrane.

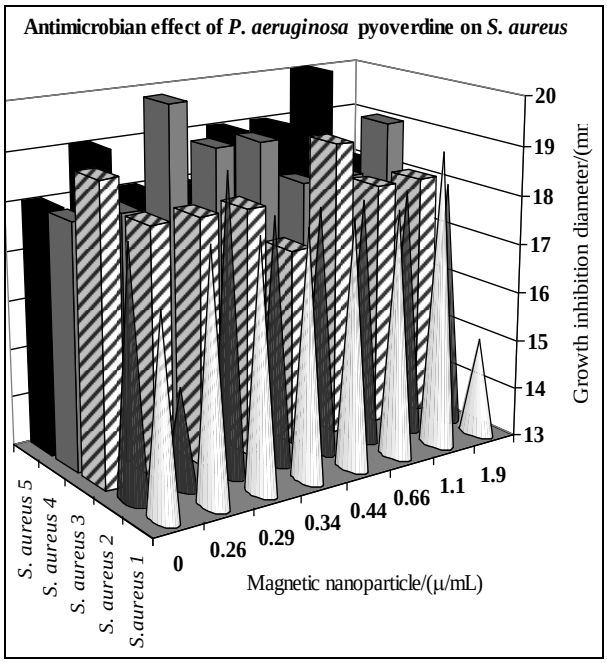

Fig. 3. The antimicrobial effect of *P. aeruginosa* pyoverdine on *S. aureus* clinical isolates.

The main structural and functional features of Pseudomonas siderophore evidenced in the last years concern the binding constant for the chelation of ferric iron - which is very high - and the nature of the chromophore -which makes the siderophore colored and fluorescent [21-25]. In [26] the effect of non-magnetic iron oxide (haematite) on bacteria growth and siderophore synthesis was studied; the authors showed that particles with less than 10 nm diameter appear to be capable of penetrating in to the outer cell wall, offering at least one possible pathway for Fe acquisition.

Small ferrophase particles could remain attached to the bacterial cell wall masking some membrane receptors so important in microorganism cell inter-communication; or they could remain embedded in the biomembrane altering ion channels - molecular basis of the microtransport, or interfering with macrotransport vesicles.

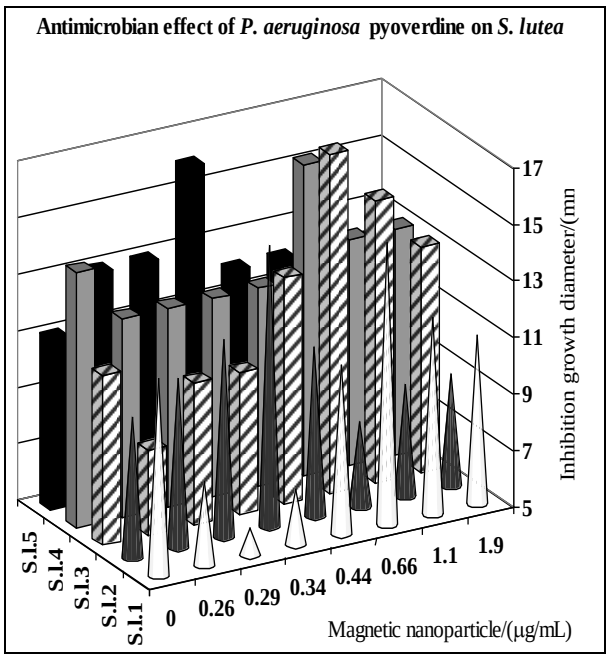

Fig. 4. The antimicrobial effect of *P. aeruginosa* pyoverdine on *S. lutea* clinical isolates.

It will be of high interest to arrange experimental investigations focused on the magnetic effects of magnetic nanoparticle impact on the bacterial cells, especially in those hosted by the human body (also considering medical applications) – an actual challenge generated by the remarkable environmental pollution with nanoparticulate matter of nowadays world.

CONCLUSION

Fluorescence measurements revealed the sensitivity of collection bacterial strain to the colloidal ferric and ferrous iron delivered in the form of magnetite colloidal nanoparticles. The iron metabolism within the P. aeruginosa cells seems to result in the stimulation of pyoverdine synthesis for relatively low magnetite nanoparticle concentration (0. 26 μg /mL) both in ATCC P. aeruginosa and clinical isolates of Pseudomonas spp. The growth of S. aureus clinical isolates could be inhibited by pyoverdine synthesized in P. aeruginosa supplied with magnetic nanoparticles.

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EVIDENŢIEREA MODIFICĂRII ACTIVITĂŢII ANTIMICROBIENE A PIOVERDINEI DUPĂ ADĂUGAREA DE NANOPARTICULE MAGNETICE

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Pseudomonas aeruginosa este un microorganism larg răspîndit, cu multe nişe ecologice, inclusiv organismul uman. Nanoparticule de magnetită învelite cu oleat de sodium şi dispersate în apă deionizată au fost preparate în laborator. Concentraţiile de suspensie de magnetită din mediul de cultură al bacteriei Pseudomonas aeruginosa au fost de 0.0075–0.015-0.03-0.06-0.12-0.25-1.0-2.0 μL/mL. Măsuratorile de fluorescenţă, posibile datorită proprietăţilor fluorescente ale pioverdinei, sideroforul sintetizat de aceasta bacterie, au evidenţiat efectul stimulator al magnetitei în mici concentraţii. Următorul test s-a bazat pe raspunsul altor două bacterii la administrarea de pioverdină obţinută prin termoliza culturilor de Pseudomonas. Efectul antimicrobian al pioverdinei faţă de câteva surse de Sarcina lutea şi Staphylococcus aureus (izolate de la pacienţi din spital) a fost amplificat după tratarea cu nanoparticule magnetice – dar nu s-a putut evidenţia vreo corelaţie matematică cu concentraţia de nanoparticule magnetice.

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