Iron complex compounds synthesized by *Pseudomonas aeruginosa* could be of interest as bactericidal agents against other pathogen germs. A possible biotechnological application was studied in an experimental design using magnetite nanoparticles coated with citric acid and dispersed in deionized water. The magnetite concentrations supplied in the culture medium of *Pseudomonas aeruginosa* were equal to: 0.25-0.29-0.34-0.44-0.66-1.1-1.9-3.6-7.0 μg/ml. Stimulatory effect of relative low concentrations of magnetite on the siderophore synthesis was evidenced through fluorescence measurements. The antimicrobial effect of *Pseudomonas* thermyolysate against several strains of *Sarcina lutea* and *Staphylococcus aureus* (clinical isolates from hospital patients) was revealed with higher amplitude in the case of *Pseudomonas* strain supplied with some concentrations of nanosized magnetite—although no specific correlation could be emphasized between size of growth inhibition areas and magnetic nanoparticle concentration.

**Key words:** Iron scavenger, *Pseudomonas* siderophore, pathogen germs.

1. **INTRODUCTION**

*Pseudomonas aeruginosa* is a widely spread microorganism with many ecological nishes including human body. From the viewpoint of medical microbiology *Pseudomonas aeruginosa* is a germ with remarkable resistance against antibiotics. These bacteria are not only human pathogens as they could be found in water, soil, plants and mammals – the adaptability to environment being assigned by some authors [1-3] to the complicated and diverse iron uptake systems discovered in those bacterial species particularly. Most of fluorescent *Pseudomonas* species produces siderophores – organic macromolecules characterized by high affinity complexation sites for the ferric iron. These complexes are iron chelates that act as very efficient iron scavenger systems mainly in iron limiting situations [4–6] this way avoiding the precipitation of iron oxyhydroxides that can not be dissolved in the aqueous cellular media. Various siderophores are described in [7–11] for different other microorganisms, each type of iron complexation molecule being
characterized by different mechanisms of interaction with iron ions. The environmental, nutritional and medical issues related to \textit{Pseudomonas aeruginosa} and its interaction with iron ions [12–13] are not the only points of interest - there is also the biotechnological approach focused on various iron biosensor projects that use \textit{Pseudomonas} strains for iron detection purposes. The most efficient devices based on the bacteria sensitivity to iron sources use bacterial mutants with special features of iron oxidization – transforming ferric iron to ferrous one which is hydrosoluble - when grown in different iron supplied media [14–17].

On another side the challenge of facing various infectious diseases generated by \textit{P. aeruginosa} motivated the scientists to search for new and non-conventional strategies related more and more to nanotechnological approach. Thus, to fight against that bacterium the Trojan Horse strategy was proposed based on antibiotic molecules conjugated with compounds acting as siderophores [18] that could increase the cell wall permeability while the beta-lactamase activity (inactivating certain antibiotic drugs) could be also reduced. This is why production of siderophores is of interest as well as various factors that could help in monitoring siderophore yield and properties.

But it seems that siderophores are not the only metabolites of biomedical interest synthesized by \textit{Pseudomonas} cells. For example, it was found that \textit{Pseudomonas} spp produce pyridine 2,6 dithiocarboxylic acid with antimicrobial properties as shown in [19]. The \textit{Pseudomonas} metabolites utilization as antimicrobial agents for biological protection of plant roots against fungi was justified also due to pyoverdine combination with salicylic acid produced also by \textit{P. aeruginosa} [20].

Taking in account all the above mentioned issues we focused on the influence of nanoparticulate magnetite on the \textit{P. aeruginosa} siderophore synthesis in the frame of an experiment designed to evidence the bactericidal effect of the products released by \textit{Pseudomonas} on other microorganisms.

2. MATERIALS AND METHODS

2.1. BIOLOGICAL MATERIAL

\textit{Pseudomonas aeruginosa} ATCC 17503 and five clinical isolates of \textit{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 inoculums density was adjusted accordingly to standard protocol [21] to about \(10^8\) cell/ml. Incubation was carried out at 35.0±0.5 °C in INCUCCELL thermostatic room. Thermolysed \textit{Pseudomonas} samples for antimicrobial effect testing were prepared by thermal treatment (100 °C) of the \textit{Pseudomonas aeruginosa} ATCC 17503 cell cultures followed by centrifugation (15 minutes at 3,000 cycles/sec in adequate Mettler device).
2.2. MAGNETIC NANOPARTICLES

Magnetite particles dispersed in deionized water were prepared accordingly to Cotae, 1981 [22] by coating magnetite ferrophase (co-precipitated from stoichiometric mixture of ferrous and ferric salts solutions) with citric acid shell – known as the thinnest coating shell for such core/shell system. The magnetite suspension concentrations in the culture medium were taken equal to: 0.0075–0.015–0.03–0.12–0.25–1.0–2.0 μl/ml that correspond to concentrations of: 0.26–0.29–0.34–0.44–0.66–1.1–1.9–3.6–7.0 μg/ml of magnetite. For each concentration and each bacterial strain four tubes of 3 ml volume were used.

2.3. FLUORESCENCE MEASUREMENT

Fluorescent pyoverdine level in the bacterial cells was indirectly assessed by fluorescence assay. Laboratory assembled installation with convenient versatility was adjusted for fluorescence excitation in UV light at the wavelength of 300 nm and fluorescence intensity recording allover the visible range as mentioned in [23]. Fluorescence quenching avoiding in the slight turbid samples was ensured by 1:10 dilution in distilled water.

2.4. ANTIMICROBIAL TEST

Five *S. aureus* clinical isolates and five clinical isolates of *S. lutea* were used as test germs for the antimicrobial action of thermolysate prepared from *P. aeruginosa* (ATCC strain); equal aliquots (of 0.1 ml each) from the *P. aeruginosa* thermolysates were dropped on the surface of agarized culture medium plates inoculated with clinical isolates. Four repetitions on the same Petri plate were carried out for each bacterial strain and each concentration of magnetic particles (added in the initial culture medium of *P. aeruginosa*). After incubation at 35.0±0.5 °C for 18 hours the diameters of the growth inhibition areas were measured 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.

3. RESULTS AND DISCUSSION

From the recorded fluorescence spectra a large band with the maximum intensity at 410 nm was revealed – no significant band shift or band width change being detected for none of the magnetite concentrations neither for different bacterial strains. The influence of magnetite nanoparticles on the fluorescence intensity of the studied bacterial samples was presented in Fig. 1. *Pseudomonas*
samples grown in the presence of only citric acid equivalent to the ratio present in coated magnetite nanoparticles resulted in no change compared to the control *Pseudomonas* culture grown on standard medium with no other ingredient.

It was found considerable highest fluorescent emissions for ATCC strain of *P. aeruginosa* – compared to the strains provided by clinical isolates. In most cases the fluorescence intensity was increased \((p < 0.05)\) by nanoparticle concentrations ranging from 0.26 \(\mu\)g/ml to about 1.1 \(\mu\)g/ml while for highest magnetite concentrations the fluorescence intensity was diminished back toward the control level.

![Graph showing bacterial sample fluorescence for different concentrations of magnetic nanoparticles – linear logarithmic graph.](image)

For two of the clinical isolates (*P.a.2* and *P.a.3*) the nanoparticle supply induced non-significant variations of fluorescence intensity for the concentrations smaller than 1.1 \(\mu\)g/ml but the diminutions recorded for higher nanoparticle concentrations had statistical significance \((p < 0.05)\). The standard deviation ranged from 5.1 to 6.3%. It seems that the biosynthesis of pyoverdine was generally stimulated by iron oxides (composing the nanosized magnetite) supplied in the culture medium in relatively small concentrations.
In Figs. 2-3 the effect of *Pseudomonas* thermolysate on several strains of *Sarcina lutea* and *Staphylococcus aureus* is presented.

In all tested clinical isolates bacteria growth inhibition was recorded for all *Pseudomonas* thermolysates cultivated on standard medium or supplied with magnetic nanoparticles. In the case of *S. aureus* clinical isolates (Fig. 2) the growth inhibition areas have diameter ranging from 14.5 to 20.0 mm (standard deviation of about 8.5%) and certain supplementary antimicrobial effect up to 20% was revealed meaning generally increased growth inhibition diameter compared to the control thermolysate (in the lack of magnetic nanoparticles influence).

![Graphs showing the antimicrobial effect of *P. aeruginosa* thermolysate on *S. aureus*](image)

Fig. 2 – The antimicrobial effect of *P. aeruginosa* thermolysate on *S. aureus* (C- magnetite nanoparticle concentration; D- diameter of growth inhibition area).
In some strains (S. aureus1, S. aureus4) the data trend suggests similar variation as for fluorescence intensity – meaning that the antimicrobial effect of P. aeruginosa metabolites, basically pyoverdine but possibly also other molecules, led to significant antimicrobial effect (p < 0.05) especially for small concentrations of magnetic nanoparticles (up to 1.1 µl/ml).

For other strains namely S. aureus2, S. aureus3 and S. aureus5 the variations of up to 10% in the growth inhibition diameter – measured relatively to the control thermolysate – are rather non-significant, either the positive or negative ones.

Fig. 3 – The antimicrobial effect of P. aeruginosa thermolysate on S. lutea (C-magnetite nanoparticle concentration; D- diameter of growth inhibition area).
It could be supposed that not only the fluorescent pyoverdine but also other non-fluorescent components of the *Pseudomonas* thermolysate influence the growth of *S. aureus* which resulted in a graph different from that of the fluorescence intensity - because different *S. aureus* strains could differ in their sensitivity to non-fluorescent metabolites (either salicylic acid or others).

In the case of *S. lutea* strains (Fig. 3) smaller diameters of the growth inhibition area were recorded – with diameters ranging from 6.5 to 16.5 mm (standard deviation of about 6.5%) which suggests smaller sensitivity to *Pseudomonas* thermolysate. In the same time the range of growth inhibition diameter is higher with either positive or negative variations – up to 40%.

The thermolysates provided by *Pseudomonas* grown under relatively small magnetite concentrations induced increased antimicrobial effect on *S. lutea*2, (0.34 µg/ml), *S. lutea*3 (0.66 µg/ml) and *S. lutea*5 (0.34 µg/ml) where the growth inhibition diameter was increased with more than 40% compared to the control. The graphs corresponding to *S. lutea*1 and *S. lutea*4 evidenced rather diminished antimicrobial effect for the thermolysates provided by *Pseudomonas* strains cultivated with magnetic nanoparticles.

So the antimicrobial effect of *Pseudomonas* metabolite products was demonstrated with generally higher amplitude in *S. aureus* where all the growth inhibition diameters were larger than for *S. lutea*; however no evident correlation could be evidenced with the nanoparticle concentration influencing *Pseudomonas* metabolism.

One may consider also that the contributions of pyoverdine and/or other antimicrobial metabolites to the growth inhibition of *S. lutea* and *S. aureus* are different from a thermolysate to another – as consequence of possible different origins of bacterial clinical isolates extracted from different human bodies.

The study of *P. aeruginosa* behavior under the influence of magnetite supplied in the culture medium could provide a useful data pool regarding the property of the pyoverdine molecules to sequester traces of Fe$^{3+}$ and to transport them through the cell membrane into the periplasmatic space. Some authors [24–25] hypothesized that iron oxide colloidal nanoparticles could be processed by iron reducing ability of bacterial cells.

We believe that beside the role of chemical agent the colloidal iron oxide could interfere with the microorganisms by other mechanisms able to influence the cell metabolism. In this respect the intrinsic magnetism of ferrophase particles could represent also a cause of perturbation of various ions transport at the level of the bacterial cell membrane. The main structural and functional features of *Pseudomonas* siderophore evidenced in the last years concern the high value of binding constant for the chelation of ferric iron and the nature of the chromophore - which makes the siderophore colored and fluorescent [26–29]. In [30] 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
Magnetic nanoparticle influence on *Pseudomonas* metabolites

appear to be capable of penetrating the outer cell wall, offering at least one possible pathway for iron 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.

It would be of high interest to design further experimental investigations focused on the local magnetic effects of magnetic nanoparticle interaction with the bacterial cells, especially in those hosted by the human body (considering also medical applications) – an actual challenge generated by remarkable environmental pollution with nanoparticulate matter of nowadays world. Nevertheless the deeper insight in the composition of *Pseudomonas* thermolysates is needed to get information on possible presence of metabolites like acetyl salicylic acid or pyridine 2,6 dithiocarboxylic acid.

4. CONCLUSION

Fluorescence measurements revealed the sensitivity of *Pseudomonas* bacterial strains to the iron oxide supplied in the form of magnetite colloidal nanoparticles. The iron metabolism of *P. aeruginosa* cells known for their iron scavenger feature seems to result in the stimulation of pyoverdine synthesis for relatively low magnetite nanoparticle concentration (from 0.26 μg/ml) both in ATCC *P. aeruginosa* and clinical isolates of *Pseudomonas* spp. The growth of *S. aureus* clinical isolates was inhibited by metabolites synthesized by *P. aeruginosa* supplied with some concentrations of magnetic nanoparticles. The study of pyoverdine production monitoring using magnetic nanoparticles could have practical applications in yielding of pharmaceutical forms designed by association of pyoverdine with antibiotics to prevent bacteria resistance phenomena.

REFERENCES