# Study of the sensitivity of bacteria of the species Pseudomonas stutzeri and their associates to various inhibitors

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**Abstract.** Currently, the development of methods for isolation, indication and identification of Pseudomonas stutzeri bacteria from environmental objects and pathological material is an urgent problem. At the same time, there are no data in the scientific literature on the sensitivity of Ps.stutzeri bacteria to various inhibitors, which are necessary for the development of a selective and differential nutrient medium for them. The article presents the results of studying the sensitivity of Ps. stutzeri and their associates to inhibitors such as sodium benzoate, SDS, nalidixic acid, potassium tellurite and sodium azide, which will be used to develop a selective and differential culture medium for Ps.stutzeri bacteria.

## 1 Introduction

Ps.stutzeri bacteria live in many natural habitats. They are isolated from sewage, ground and sea waters, treatment facilities, soil, manure, objects of the external hospital environment, and much less often from clinical materials of humans and animals [1, 2]. They are biodestructors and are capable of manifesting a pathogenicity factor with the ability to cause disease. Scientific literature describes cases of registration of infections caused by the bacteria Ps.stutzeri, as well as their role in the pathogenesis of domestic and farm animals.

At the moment, it is necessary to develop a fast and highly efficient method for the indication and differentiation of Ps.stutzeri bacteria from other microorganisms isolated from environmental objects and pathological material. In this regard, it requires devepoping a selective and differentiated nutrient medium for Ps.stutzeri bacteria that meets all the requirements [3].

There is no required data for their development in the scientific literature, in particular the data on sensitivity to various inhibitors. Based on this, the aim of the work was to study the sensitivity of Ps.stutzeri bacteria and their associates to various inhibitors. For this, sodium benzoate, SDS, nalidixic acid, potassium tellurite, and sodium azide were taken based on their antimicrobial properties [4-11].

Sodium benzoate has the property of inhibiting the growth of mold and yeast microorganisms, bacteria, fungi.

Sodium dodecyl sulfate (SDS) inhibits the growth of microflora, especially gram-positive bacteria.

Nalidixic acid has pronounced antibacterial activity against gram-negative bacteria, including Proteus mirabilis, P. morganii, P. vulgaris, and P.rettgeri; Escherichia coli, Enterobacter (Aerobacter) and Klebsiella, and Pseudomonas strains are usually resistant.

Potassium tellurite solution has a bactericidal effect on most types of gram-positive bacteria. Potassium tellurite-resistant bacteria form black colonies by reducing the tellurite anion with tellurite reductase.

Sodium azide inhibits the growth of many gram-negative microorganisms. Although proteas can grow in this environment, their swarming being suppressed.

## 2 Materials and equipment

The work was performed on the reference bacterial strains Ps.stutzeri, Yersinia enterocolitica, Klebsiella pneumoniae, Escherihia coli, Proteus mirabilis, Aeromonas hydrophila, Citobacterfreundii, Pseudomonas putida, Pseudomonas aeruginosa 128, Pseudomonas aeruginosa 128 non-type, Pseudomonas aeruginosa type. Staphylococcus aureus, Listeria monocytogenes from Scientific Research Innovation Center for Microbiology and Biotechnology LLC.

We used GRM bouillon (FBUN GNTSPMiB Russia, Obolensk), GRM agar (FBUN GNTsPMiB Russia, Obolensk), sodium benzoate (ZAO Khimreaktiv, Russia, Nizhny Novgorod), SDS BioChemica (PanReac AppliChem), nalidixic acid (PanReac AppliChem)

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tellurite (1% Potassium tellurite solution Sigma-ALDRICH) Sodium azide (JSC LenReaktiv Russia, St. Petersburg), distilled water, laboratory scales, distiller (Liston), electric stove, autoclave (GK-100-3), thermostat (TS-80M-2), thermometer, ultraviolet lamp of the Phillips brand with a wavelength of 253 nm, spirit lamp, bacteriological loop, laboratory sterile glassware.

## 3 Research results

According to the recipe, GRM bouillon was prepared to obtain daily cultures on it, GRM agar to control the growth of bacterial cultures in GRM bouillon and GRM agar for each inhibitor.

Sodium benzoate at the rate of 1g / L, 1.5g / L, 2.0g / L, 2.5g / L, SDS at the rate of 1.5g / L, 2.0g / L, 2.5g / L, 3.0g / l, nalidixic acid at the rate of 0.007g / l, 0.015g / l, 0.033g / l, and sodium azide at the rate of 0.007g / l, 0.013g / l, 0.1g / l were separately added to the timing agars to autoclaving them. Potassium tellurite at the rate of 0.1 g / l, 0.2 g / l, and 0.32 g / l was added to the timing agars after autoclaving.

All these prepared media were autoclaved at a temperature of 112˚C and after 10 minutes of cooling, under sterile conditions, were poured into sterile laboratory glassware. Timing bouillon in test tubes, and timing agar in Petri dishes. GRM agar and GRM agars with the addition of sodium benzoate, SDS, nalidixic acid, potassium tellurite and sodium azide in various concentrations, were left to solidify and dry for a day at room temperature.

All studied reference strains were inoculated in the GRM, and daily bacterial cultures of Ps.stutzeri, Yersinia enterocolitica, Klebsiella pneumoniae, Escherihia coli, Proteus mirabilis, Aeromonas hydrophila, Citobacterfreundii, Pseudomonas putida type, Pseudomonas putida, Pseudomonse type, as well as Pseudomonas fluorescens, Streptococcus pyogenes, Staphylococcus aureus, Listeria monocytogenes. It was confirmed by the turbidity of the GRM bouillons in these tubes.

Then these day old cultures were inoculated on GRM agars, as a control and on GRM agars containing sodium benzoate, SDS, nalidixic acid, potassium tellurite and sodium azide in various concentrations, using the depletion streak method, in order to obtain isolated colonies, to study their growth under the action of inhibiting components.

The inoculations were cultured at 37 °C for 72 hours, with the results being recorded every 24 hours.

As a control, uninoculated Petri dishes with GRM agar and GRM agar containing sodium benzoate, SDS, nalidixic acid, potassium tellurite and sodium azide in all studied concentrations were cultured in parallel under the same conditions.

The growth of bacteria of all the studied reference strains was observed on GRM broths and GRM agars on the first day of cultivation at 37 ° C.

On control, not inoculated Petri dishes with GRM agar and GRM agar containing sodium benzoate, SDS, nalidixic acid, potassium tellurite and sodium azide, the growth of bacterial colonies was not observed during 72 hours of cultivation at 37 °C.

At selected concentrations of sodium benzoate of 1.5 g / l and 2.0 g / l in timing agar, at which the growth of bacteria of the studied reference strains Ps. stutzeri 4792, 3503, 3506, 4904, we observed the growth of bacteria of almost all of the studied associates. At a sodium benzoate concentration of 2.5 g / l, the growth of bacteria Ps. Stutzeri 3503 and 4904 were absent, while practically all of their associates were growing.

On GRM agar containing SDS (1.5 g / l, 2.0 g / l, 2.5 g / l, 3.0 g / l, respectively), there was no growth of all the studied reference Ps. stutzeri strains 4792, 3503, 3506, 4904. At the same time, we observed on the same GRM agar containing SDS at the same concentrations, the growth of bacteria of some of their associates. There was no growth of bacteria of other associates.

At concentrations of nalidixic acid 0.007 g / l and 0.015 g / l in GRM agar, the bacteria of the studied reference strains Ps. stutzeri 4792, 3503, 3506, 4904 grew, as well as almost all of their studied associates.

An increase in the concentration of nalidixic acid to 0.033 g / L entailed not only suppression of the growth of associate bacteria, including Yersinia enterocolitica, Citrobacter freundii, Listeria monocytogenes, but also the reference Ps. Stutzeri 4782 and 4904.

The concentration of 0.033 g / l of Pseudomonas was resistant to nalidixic acid, especially against the background of the gram-positive studied microflora, but not to all the studied reference Ps. stutzeri strains.

At the used concentrations of potassium tellurite of 0.1 g / l, 0.2 g / l, 0.32 g / l in GRM agar, we observed suppression of most of the studied gram-positive microflora and a small part of gram-negative microflora.

Sensitivity of bacteria of the studied reference Ps. stutzeri strains varied a lot. At all these potassium tellurite concentrations, bacterial growth of the reference Ps. stutzeri strains 4792, 4904 and 3503.3506 was absent.

At concentrations of sodium azide 0.007g / l, 0.013g / l, 0.1g / l. in GRM agar, we observed growth of all studied bacteria of the reference Ps. stutzeri strains.

4792, 3503, 3506, 4904 and almost all of their investigated associates.

Sodium azide at these concentrations had an inhibitory effect only on most of the Gram-positive studied microflora, namely on Staphylococcus aureus, Streptococcus pyogenes.

**Table 1.** Results of the studied reference strains Ps. Stutzeri growth and their associates on timing agar containing sodium benzoate, SDS, nalidixic acid, potassium tellurite, sodium azide during cultivation for 72 h at 37 ° C and control.

| No. | Reference strains | GRM agar containing | | | | | | | | | | | | | | | | | | GRM boulene (37°C, 24h) | GRM agar (37°C, 24h) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Sodium benzoate | | | | SDS | | | | Nalidixic acid | | | Potassiumt ellurite | | | Sodium azide | | | | | |
| | | 1,0 g/l | 1,5 g/l | 2,0 g/l | 2,5 g/l | 1,5 g/l | 2,0g/l | 2,5 g/l | 3,0 g/l | 0,001 g/l | 0,015 g/l | 0,033 g/l | 0,1 g/l | 0,2 g/l | 0,32 g/l | 0,007 g/l | 0,013 g/l | 0,1 g/l | | | |
| 1 | Ps. stutzeri 3503 | X | V | V | X | X | X | X | X | V | V | V | V | V | V | V | V | V | | V | V |
| 2 | Ps. stutzeri 4792 | X | V | V | V | X | X | X | X | V | V | X | X | X | X | V | V | V | | V | V |
| 3 | Ps. stutzeri 3506 | X | V | V | V | X | X | X | X | V | V | V | V | V | V | V | V | V | | V | V |
| 4 | Ps. stutzeri 4904 | X | V | V | X | X | X | X | X | V | V | X | X | X | X | V | V | V | | V | V |
| 5 | Pseudomonas aeruginosa 128 not a pigment. | V | V | V | V | X | X | X | X | V | V | V | V | V | V | V | V | V | | V | V |
| 6 | Pseudomonas fluorescens | V | V | V | V | X | X | X | X | V | V | V | V | V | V | V | V | V | | V | V |
| 7 | Pseudomonas putida | V | V | V | V | V | V | V | V | V | V | V | V | V | V | V | V | V | | V | V |
| 8 | Pseudomonas ae ruginosa typical | V | V | V | V | V | V | V | V | V | V | V | V | V | V | V | V | V | | V | V |
| 9 | Klebsiella pneumoniae | V | X | V | V | X | X | X | X | V | V | V | V | V | V | V | V | V | | V | V |
| 10 | Yersinia enterocolitica | V | X | V | X | X | X | X | X | V | V | X | V | V | V | V | V | V | | V | V |
| 11 | Proteus mirabilis | V | X | V | V | V | V | V | X | V | V | V | V | V | V | V | V | V | | V | V |
| 12 | Escherihia coli | V | V | V | V | V | V | V | X | V | V | V | V | V | V | V | V | V | | V | V |
| 13 | Citrobacter freundii | V | V | V | V | V | V | V | V | V | V | X | V | X | X | V | V | V | | V | V |
| 14 | Aeromonas hydrophila | V | V | V | V | V | V | V | V | X | X | X | X | X | X | V | V | V | | V | V |
| 15 | Streptococcus pyogenes | V | V | V | V | V | V | V | V | X | X | X | X | X | X | V | X | X | | V | V |
| 16 | Staphylococcus aureus | V | V | V | V | X | X | X | X | X | X | X | X | X | X | V | X | X | | V | V |
| 17 | Listeria monocytogenes | X | V | X | V | X | X | X | X | V | V | X | V | V | X | V | V | V | | V | V |
| 18 | control | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | | X | X |

**Note:**
V - there is a growth of bacterial colonies
X – there is no growth of bacterial colonies

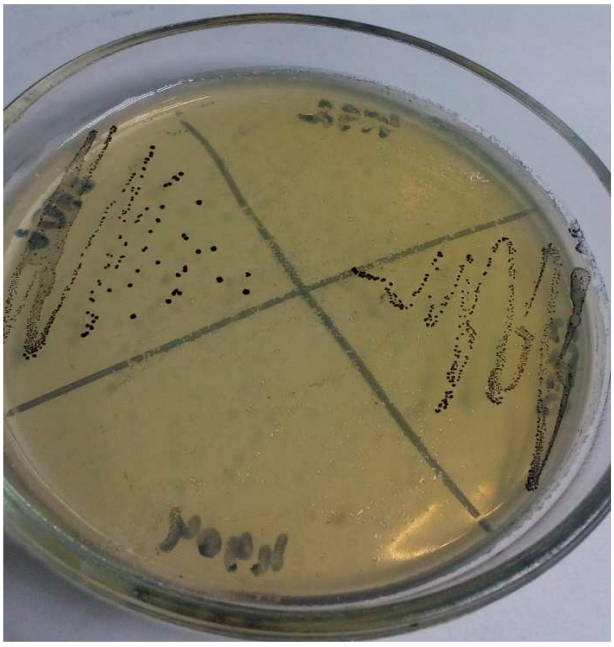

**Fig. 1.** Lack of growth of bacterial colonies of the reference Ps. stutzeri 4792, 4904 and the rise of Ps. stutzeri 3503, 3506 on GRM agar containing 0.2 g / l potassium tellurite during cultivation for 96 h at 37 °C.

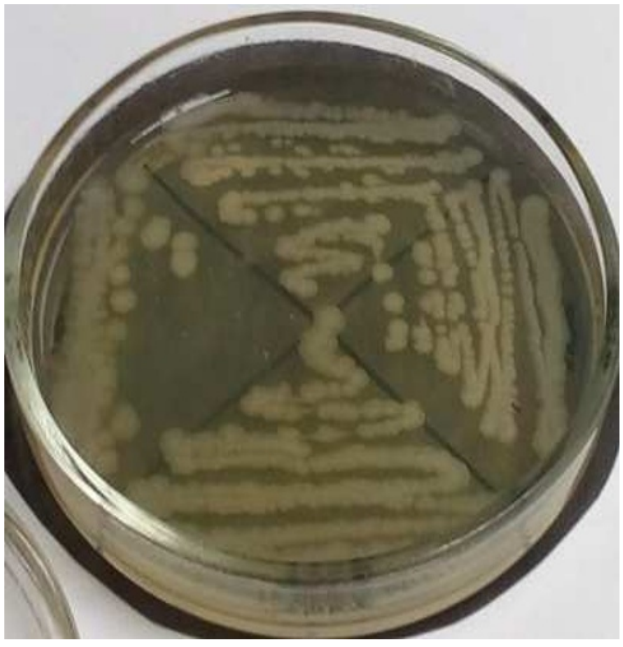

**Fig. 2.** Growth of bacterial colonies of reference strains Ps. stutzeri 3503, 4792, 3506, 4904 on timing agar containing 1.5 g / l sodium benzoate when cultured for 24 hours at 37 °C.

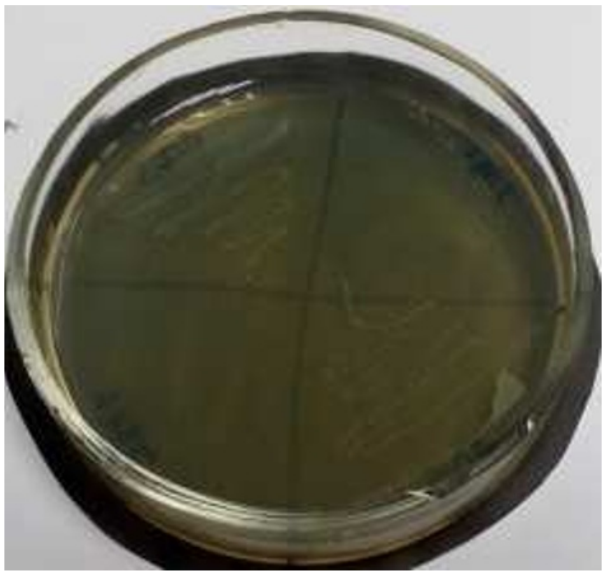

**Fig. 3.** Growth of bacterial colonies of reference strains Ps. stutzeri 4792, 3503, 4904, 3506 on GRM agar containing 0.015 g / l of nalidixic acid when cultured for 24 hours at 37 °C.

**Fig. 4.** Growth of bacterial colonies of reference strains Ps. stutzeri 3503, 4792, 3506, 4904 on timing agar containing 0.007 g / l sodium azide when cultured for 24 h at 37 °C.

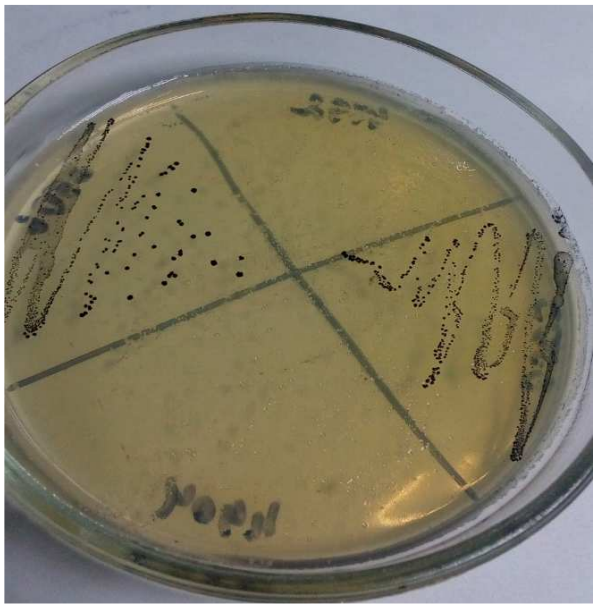

**Fig. 5.** Lack of growth of bacterial colonies of reference Ps. stutzeri 4792, 4904 and the rise of Ps. stutzeri 3503, 3506 on GRM agar containing 0.2 g / l potassium tellurite during cultivation for 96 h at 37 °C.

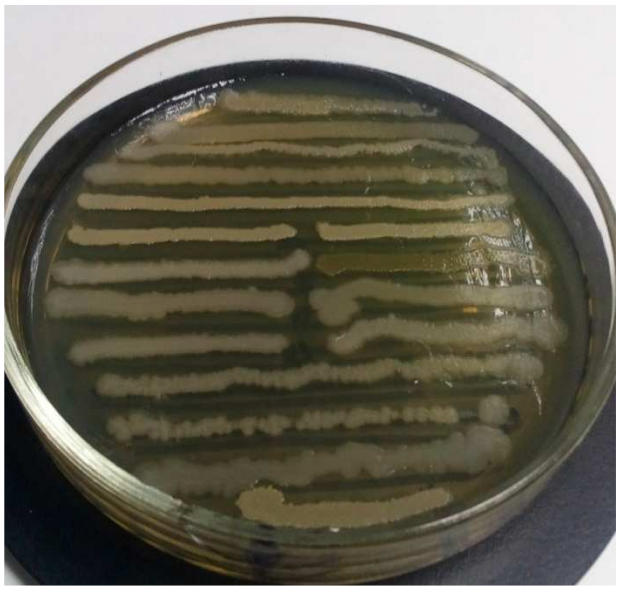

**Fig. 6.** Growth of bacterial colonies of reference strains Ps. stutzeri 3503, 3506, 4792, 4904, Pseudomonas putida, Pseudomonas aeruginosa 128 non-type, Pseudomonas fluorescens, Yersinia enterocolitica, Pseudomonas aeruginosa type, Escherihia coli, Proteus mirabilcus, Stisteriamonocytogenus, Kleomonas pneumonia, Citlebs. hydrophila on GRM agar as a control during cultivation for 24 h at 37 °C.

## 4 Conclusion

As a result of the studies, the sensitivity of bacteria of the Pseudomonas stutzeri species and their associates to sodium benzoate, SDS, nalidixic acid, potassium tellurite, and sodium azide, is considered as possible inhibitory components of the constructed selective medium for Ps. stutzeri.

On the basis of the obtained research results, it was concluded that sodium and potassium benzoate tellurite should be used as an inhibiting component of the constructed selective medium for Ps.stutzeri is impractical, as is the study of the sensitivity of the studied bacteria to their lower or higher concentrations.

Further consideration and study for this purpose is possible at a concentration of sodium azide higher than 0.1 g / l and of sodium dodecyl sulfate (SDS) at a concentration lower than 15 g / l.

Nalidixic acid at a concentration of 0.007 to 0.015 g / l can be used as an inhibitory component of gram-positive microflora, a selective medium for Ps. stutzeri.

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