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# THE INFLUENCE OF ORGANIC AND INORGANIC NITROGEN SOURCES TO THE GROWTH OF *PEDICOCCUS* GENUS BACTERIA

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**Abstract.** The 16 strains of lactic acid bacteria *Pedicoccus acidilactici*, *P. cerevisiae*, *P. halophilus*, and *P. pentosaceus* isolated from the phyllosphere of plants in Azerbaijan. The effects of inorganic and organic nitrogen sources to the growth of these bacteria have been studied. It was found that as a nitrogen sources peptone is used very well and urea is poorly absorbed. The growth coefficient of bacteria on peptone was 1.3 and 3.0 times, respectively, higher than on asparagine and urea. As inorganic nitrogen sources $(NH_4)_4SO_4$ and $NH_4\ H_2PO_4$ are used good, $NH_4\ NO_3$ – poor and $NaNO_3$ poorly. The growth coefficient of the first ones were 2.0 and 3.0 times higher, respectively, than second and third ones.

***Keywords:*** *lactic acid bacteria, Pedicoccus, organic and inorganic nitrogen sources, growth coefficient.*

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## 1. Introduction

Many probiotic bacteria, including lactic acid bacteria are used in the industry for production of cultured milk foods, silage for animals, sauerkraut in the household, in medicine, in the treatment of digestive system. At present special attention is attended to the study of properties, including antimicrobial properties of lactic acid bacteria (Ganbarov & Jafarov, 2013; Kvasnikov & Nesterenko, 1975).

The antimicrobial activity of lactic acid bacteria genera *Lactobacillus*, *Streptococcus*, *Enterococcus* spreaded in Azerbaijan is sufficient have been studied (Ganbarov & Dzhafarov, 2006; Gyulakhmedov, 2008).

In our previous studies lactic acid bacteria genus *Pedicoccus* isolated from the surface of plants in Azerbaijan, identified and studied antimicrobial activity against gram negative and gram positive bacteria (Hosseinecad *et al.*, 2015; Hosseinecad *et al*., 2016), and its relation to sugars (Hosseinecad *et al*., 2018).

The purpose of the presented work is to study the influence of organic and inorganic nitrogen sources on the development of lactic acid bacteria genus *Pedicoccus*.

## 2. Materials and methods

As research object *Pedicoccus acidilactici* BDU3, BDU7, BDU10 and BDU21, *P. cerevisiae* BDU8, BDU11, BDU24 and BDU 26, *P. halophilus* BDU1,

BDU28, BDU 29 and BDU42, *P. pentosaceus* BDU15, BDU32, BDU35 and BDU106 lactic acid bacteria strains were used.

The following nutritional medium for cultivation of bacteria was used (%): glucose - 2.0; yeast extract - 0.5; acetic acid sodium salt - 0.5; twin 80 - 0.1; $KH_2PO_4$ -

0.2; $MgSO_4 \cdot 7H_2O$ - 0.01; $MnSO_4$ $4H_2O$ - 0.05 (Molskness, 2003). The amount of nitrogen sources was calculated by nitrogen and was taken 0.03%. Pepton added 0.1% in to nutritional medium.

The growth of bacteria defined on the basis of growth coefficient on solid nutrient medium and calculated by formula:

$$\mathbf{I = B_1 - B_k}$$

I - growth of bacteria
$B_1$ - growth coefficient in the experimental version
$B_k$- growth coefficient on the control (non-nitrogen source)

The growth coefficient of bacteria was calculated by the following formula:

$$\boldsymbol{B = \frac{d \cdot h \cdot s}{t} \cdot 100}$$

B- growth coefficient
d - diameter of the colony (mm)
h - height of colony (mm)
s - density of the colony (on a 5-point scale)
t - time of cultivation (day)

All experiments were repeated 4 times and the actual figures obtained are statistical processed (Plokhinsky, 1998).

## 3. Results and discussion

The effects of organic and inorganic nitrogen sources to growth of 16 strains of lactic acid bacteria *Pedicoccus acidilactici, P. cerevisiae, P. halophilus, P. pentosaceus* isolated from the plant phyllosphere in Azerbaijan have been studied. I has been defined that all bacterial strains used peptone good, asparagine - poor and urea - very bad. So, growth coefficient of *Pedicoccus acidilactici* on the peptone was 1.3 and 3.0 times, respectively, more than growth coefficients on asparagine and urea. Growth coefficient of asparagine was 2.4 times higher than urea one. The ability of bacteria to use organic nitrogen sources was differed. For example, the maximum growth coefficient on peptone was observed by strain *P.acidilactici* BDU7 and minimum – by strain BDU21. The growth coefficient of first strain was 2.0 times more than the growth coefficient of second strain (Table 1).

The growth coefficient of the bacterium *Pedicoccus cerevisae* on the peptone was 1.3 and 3.1 times, respectively, more than growth coefficients of asparagine and urea. The growth coefficient of asparagine was 2.4 times more than the growth coefficient of urea . So bacterial strains used peotone better than others. Maximum growth coefficient in peptone was recorded by *P.cerevisae* BDU24 and minimum growth coefficient was observed by *P.cerevisae* BSU 26. The growth coefficient of first one was 1.6 times higher than the growth coefficient of second one (Table 1).

The growth coefficient of bacterium *Pedicoccus halophilus* on pepton was 1.3 and 2.9 times, respectively, more than growth coefficient of asparagine and urea Growth coefficient on asparagine was 2,3 higher than on urea. So, these bacterial strains also better used peptone. The maximum growth coefficient on pepton was observed by *P.halophilus* BDU20 and BSU28 and the minimum growth coefficient - by *P.halophilus*

BSU42. The growth coefficient of first one was 1.4 times higher than the growth coefficient of second one (Table 1).

The coefficient of growth of the bacterium *Pedicoccus pentosaceus* on pepton was 1.3 and 3.0 times, respectively, higher than growth coefficients on asparagines and urea.

**Table 1.** Impact of organic nitrogen sources on the growth of bacteria genus *Pedicoccus*

| Species and strains of bacteria | Growth coefficient of bacteria | | |
|---|---|---|---|
| | Asparagine | Peptone | Urea |
| *P.acidilactici* | | | |
| BDU3 | 10±0,5 | 12±0,6 | 3±0,06 |
| BDU7 | 12±0,5 | 14±0,6 | 5±0,2 |
| BDU10 | 8±0,3 | 11±0,4 | 3±0,1 |
| BDU21 | 6±0,3 | 8±0,2 | 4±0,2 |
| *P.cerevisae* | | | |
| BDU8 | 8±0,2 | 11±0,5 | 4±0,06 |
| BDU11 | 9±0,3 | 12±0,6 | 5±0,2 |
| BDU24 | 11±0,5 | 14±0,6 | 3±0,07 |
| BDU26 | 8±0,3 | 9±0,4 | 3±0,1 |
| *P.halophilus* | | | |
| BDU4 | 6±0,2 | 8±0,4 | 3±0,04 |
| BDU20 | 8±0,2 | 10±0,4 | 4±0,2 |
| BDU28 | 7±0,3 | 10±0,2 | 2±0,06 |
| BDU42 | 7±0,2 | 7±0,2 | 3±0,1 |
| *P.pentosaceus* | | | |
| BDU15 | 10±0,5 | 14±0,6 | 4±0,2 |
| BDU32 | 12±0,5 | 16±0,7 | 6±0,2 |
| BDU55 | 8±0,3 | 12±0,6 | 3±0,1 |
| BDU106 | 8±0,4 | 9±0,3 | 4±0,08 |

The growth coefficient on pepton was 2,3 time than on urea. The maximum growth coefficient on peptone was observed by *P. pentosaceus* BDU32 and minimum growth coefficient was observed by *P.pentosaceus* BDU106. The growth coefficient of the first one was 1.8 times greater than the growth rate of the second one (Table 1).

In whole, pepton was better used by bacterium *P. pentosaceus*, than bacterium P. bacterophilus. So, the growth coefficient of first one was 1.5 times higher than the growth coefficient of the second one.

The attitude of bacteria genus *Pedicoccus* to inorganic nitrogen sources was very different. The growth coefficients of bacerium *Pedicoccus acidilactici* on $(NH_4)_2SO_4$ and $NH_4H_2PO_4$ were 2.0-2.2 and 2.8 - 3.0 times higher, respectively, than on $NH_4NO_3$ and $NaNO_3$. The growth coefficient on $NH_4NO_3$ was 1,4 time more than on $NaNO_3$. Consequently, $(NH_4)_2SO_4$ and $NH_4H_2PO_4$ as a nitrogen sources are good used by this bacterium. The maximum growth coefficient was observed by *Pedicoccus acidilactici* BDU7 and minimum growth coefficient - by *P. acidilactici* BDU21. The growth coefficient of first one was 1.4 - 1.5 times more than second one (Table 2).

The maximum growth coefficient of bacterium *Pedicoccus cerevisiae* was observed on $(NH_4)_2SO_4$ and minimum growth coefficient - on $NaNO_3$. The growth coefficient on $(NH_4)_2SO_4$ was 1.3, 2.5 and 2.6 times, respectively, more than growth coefficient on $NH_4H_2PO_4$, $NH_4NO_3$ and $NaNO_3$. Growth coefficient on $NH_4H_2PO_4$ was 1.9 and 2.0 times, respectively, higher than growth coefficient on $NH_4NO_3$ and $NaNO_3$. So, $(NH_4)_2SO_4$ is better inorganic nitrogen sources for this bacteria. Maximum growth coefficients were observed by *P.cerevisae* BDU11 and BDU24, and minimum- by

*P.cerevisae* BDU8. The growth coefficient first one was 1.3 time higher than second one (Table 2).

**Table 2**. Impact of inorganic nitrogen sources on the growth of bacteria genus *Pedicoccus*

| Species and strains of bacteria | Growth coefficient of bacteria | | | |
|---|---|---|---|---|
| | $NaNO_3$ | $NH_4NO_3$ | $(NH_4)_2SO_4$ | $NH_4H_2PO_4$ |
| *P. acidilactici* | | | | |
| BDU3 | 3±0,1 | 5±0,2 | 9±0,3 | 10±0,4 |
| BDU7 | 4±0,1 | 6±0,2 | 11±0,3 | 12±0,4 |
| BDU10 | 3±0,06 | 3±0,1 | 8±0,2 | 9±0,3 |
| BDU21 | 3±0,07 | 4±0,1 | 8±0,1 | 8±0,2 |
| *P. cerevisae* | | | | |
| BDU8 | 3±0,08 | 4±0,1 | 8±0,2 | 6±0,3 |
| BDU11 | 4±0,2 | 4±0,2 | 10±0,4 | 8±0,3 |
| BDU24 | 3±0,1 | 4±0,06 | 10±0,5 | 7±0,2 |
| BDU26 | 4±0,1 | 3±0,1 | 9±0,3 | 7±0,3 |
| *P. halophilus* | | | | |
| BDU4 | 2±0,04 | 3±0,05 | 7±0,3 | 6±0,1 |
| BDU20 | 3±0,03 | 3±0,06 | 8±0,2 | 7±0,3 |
| BDU28 | 4±0,02 | 4±0,1 | 9±0,4 | 7±0,3 |
| BDU42 | 3±0,1 | 3±0,2 | 7±0,3 | 7±0,2 |
| *P. pentosaceus* | | | | |
| BDU15 | 4±0,2 | 4±0,1 | 10±0,4 | 7±0,2 |
| BDU32 | 3±0,1 | 5±0,2 | 12±0,6 | 8±0,3 |
| BDU55 | 3±0,07 | 5±0,2 | 11±0,3 | 8±0,4 |
| BDU106 | 3±0,05 | 4±0,1 | 10±0,2 | 7±0,1 |

The maximum growth coefficient of bacterium *Pedicoccus cerevisiae* was observed on $(NH_4)_2SO_4$ and minimum growth coefficient - on $NaNO_3$. The growth coefficient on $(NH_4)_2SO_4$ was 1.3, 2.5 and 2.6 times, respectively, more than growth coefficient on $NH_4H_2PO_4$, $NH_4NO_3$ and $NaNO_3$. Growth coefficient on $NH_4H_2PO_4$ was 1.9 and 2.0 times, respectively, higher than growth coefficient on $NH_4NO_3$ and $NaNO_3$. So, $(NH_4)_2SO_4$ is better inorganic nitrogen sources for this bacteria. Maximum growth coefficients were observed by *P.cerevisae* BDU11 and BDU24, and minimum- by *P.cerevisae* BDU8. The growth coefficient first one was 1.3 time higher than second one (Table 2).

The maximum growth coefficients of *Pedicoccus halophilus* were noted on $(NH_4)_2SO_4$ and $NH_4H_2PO_4$ and minimum growth coefficients - on the media containing $NH_4NO_3$ and $NaNO_3$. The growth coefficients of first one were 2.1 - 2.4 and 2.3 -2.6 times, respectively, more than second one. So, $(NH_4)_2SO_4$ and $NH_4H_2PO_4$ are better inorganic nitrogen sources for this bacterium. Maximum growth coefficient on optimal nitrogen sources was observed by *P.halophilus* BDU28 and minimum – by *P.halophilus* BDU4 and BDU42. The growth rate of the first one was 1.3 time more than second one (Table 2).

The maximum growth coefficient of *Pedicoccus pentosaceus* was observed on $(NH_4)_2SO_4$ and minimum- on $NaNO_3$. The growth coefficient on $(NH_4)_2SO_4$ was 1.4, 2.4 and 3.3 times, respectively, more than on $NH_4H_2PO_4$, $NH_4NO_3$ and $NaNO_3$. The growth coefficient of $NH_4H_2PO_4$ was 1.7 and 2.3 times, respectively, more than growth coefficients of $NH_4NO_3$ and $NaNO_3$. The growth coefficient of $NH_4NO_3$ was 1.4 times higher than $NaNO_3$. The highest growth coefficient on $(NH_4)_2SO_4$ was observed by *P. pentosaceus* BDU32 and weak growth coefficient – on *P. pentosaceus* BDU15 and

BDU106. The growth coefficient of the first one was 1.2 time more than second one (Table 2).

Thus, it has been found that lactic acid bacteria genus *Pedicoccus* use better pepton, good asparagine and bad urea as a organic nitrogen sources. From inorganic nitrogen sources $(NH_4)_2SO_4$ and $NH_4H_2PO_4$ are used good $NH_4\ NO_3$ - weak and $NaNO_3$ -very weak.

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