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## AMOUNT OF ENDOPHYTIC MICROBIOTA IN GRAPEVINE SHOOTS

The **aim** of the investigation was to evaluate the amount of grapevine endophytic microbiota using different nutritional media. **Materials and methods.** Endophytic microbiota from wooden shoots of grapevine *Vitis vinifera* L. cv Arkadia was isolated on the eight nutritional media with different compositions. **Results.** The highest amounts of bacteria were isolated on YEM, YMA and TY nutritional media. The distinctive features of YMA and YEM media was the presence of mannitol, and of TY – the increased concentration of yeast extract. **Conclusion.** Amount of microbiota representatives from grapevine shoots reached from  $(6.4 \pm 0.3) \times 10^4$  to  $(2.0 \pm 0.4) \times 10^7$  CFU/cm<sup>3</sup> depending on the nutritional medium and decreased from October to December at least in one range.

*Key words:* endophytic microbiota, grapevine, nutritional media.

Grapevine endophytic microbiota includes bacteria inhabiting internal plant tissues – more commonly xylem vessels, where bacteria can freely move and firmly attach [10]. The normal microbiota of plants are usually represented by saprophytic bacteria. If pathogens penetrate into a plant, the survived in xylem harmful bacteria (*Xylella fastidiosa*, *Clavibacter xyli*, *Pseudomonas syzygii*, *Rhizobium vitis*, *R. radiobacter* etc.) can cause a disease [7].

Endophytic microbiota may also be a source of antagonistic strains for the control of infectious plant diseases [8].

The **aim** of investigation was to evaluate the amount of grapevine endophytic microbiota using different nutritional media.

### Materials and methods

The wooden shoots of grapevine *Vitis vinifera* L. were collected from the cv Arkadia plants. Microorganisms in grapevine were detected by the method of J. Lehoczky [10]. Wooden shoots of grapevine were selected from the lateral trunk branches in October-December. The shoots were cut close to the branching. The shoots were washed with detergent under running water, thoroughly rinsed, flambéed and then fragments from the proximal ends of the shoots with 0.6–0.7 cm length and 10 cm in diameter were cut to 0.5 cm discs. The disks were placed in sterile boxes and poured with sterile saline to completely cover the cut material. The disks were shaken for one hour at room temperature and later placed at 4 °C. After 24 hr of exposition, 100 µl of the obtained suspensions were plated on nutrient media and incubated for



2 days at 28 °C. The amount of bacteria was calculated as the number of colony forming units per cm<sup>3</sup> volume of the tested grapevine shoot fragments (CFU/cm<sup>3</sup>). Mean values of seven repeats of each variants with 95% confidential interval were calculating using “Microsoft Excel”.

To isolate the wide range of endophytic grapevine microbiota and to estimate the amount of bacteria in grapevine shoots, eight nutritional media of different compositions were used (Table 1).

Table 1

### Composition of the nutritional media for endophytic grapevine microbiota

Medium	Medium composition, g/l
PSA	300 g of potatoes (extract), Ca(NO <sub>3</sub> ) <sub>2</sub> ·x4H <sub>2</sub> O - 0,5 g, Na <sub>2</sub> HPO <sub>4</sub> ·x12H <sub>2</sub> O – 2 g, sucrose - 20 g, agar-agar – 15 g [9]
YEM	Mannitol – 10 g, yeast extract - 0,4 g, K <sub>2</sub> HPO <sub>4</sub> - 0,5 g, MgSO <sub>4</sub> – 0,2 g, agar-agar – 15 g [14]
PYGA	Peptone – 3 g, yeast extract – 5 g, glycerine – 10 ml, agar-agar – 20 g [7]
YMA	Yeast extract – 1 g, mannitol – 10 g, K <sub>2</sub> HPO <sub>4</sub> x 3H <sub>2</sub> O - 0,65 g, MgSO <sub>4</sub> x 12H <sub>2</sub> O – 0,2 g, NaCl – 0,12 g, agar-agar – 15 g [6; 8]
LB	Peptone – 10 g, yeast extract – 5 g, NaCl - 10 g, agar-agar – 15 g [5]
TY	Tryptone – 5 g, yeast extract - 3 g, CaCl <sub>2</sub> ·x6H <sub>2</sub> O - 1,3 g, agar-agar – 15 g [12]
YPGA	Yeast extract - 5 g, glucose - 10 g, peptone – 5 g, agar-agar - 15 g [11; 13]
PA	Potato extract (200 g in 1 liter of tap water), agar-agar - 1,5 g [2]

### Results and discussion

The results of isolation of microorganisms from 27 grapevines have shown that the amount of wooden shoot microbiota in different plants varied significantly during the investigation period. On the media with the best compositions for endophytic grapevine microbiota from  $(6.4 \pm 0.3) \times 10^4$  to  $(2.0 \pm 0.4) \times 10^7$  CFU/cm<sup>3</sup> were revealed (Table 2).

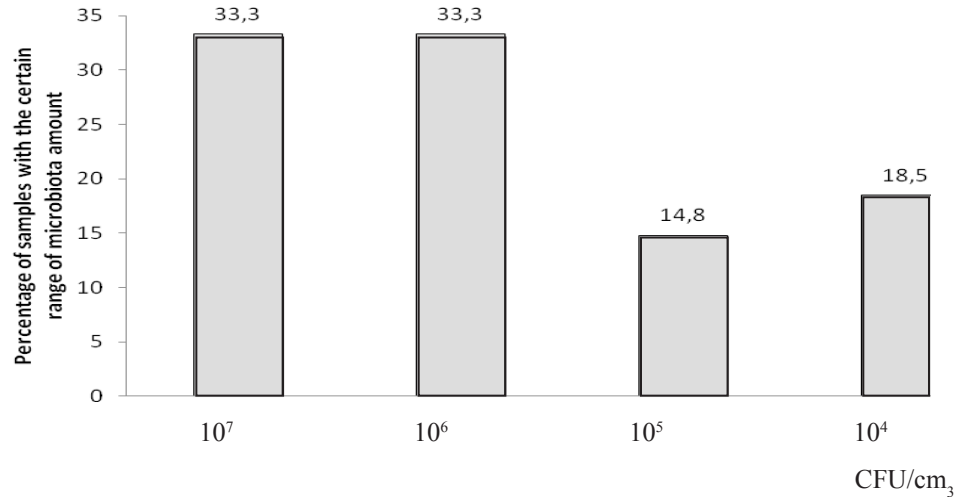
Table 2

### Mean amount of endophytic microbiota in different months of investigations (CFU/m<sup>3</sup>)

Month	Nutritional media							
	PSA	YPGA	YEM	YMA	PA	LB	TY	PYGA
October	$(1,1 \pm 0,2) \times 10^7$	$(1,3 \pm 0,5) \times 10^7$	$(2,0 \pm 0,4) \times 10^7$	$(1,1 \pm 0,2) \times 10^7$	$(8,8 \pm 0,6) \times 10^6$	$(1,4 \pm 0,3) \times 10^7$	$(1,0 \pm 0,7) \times 10^7$	$(1,1 \pm 0,3) \times 10^7$
November	$(1,6 \pm 0,3) \times 10^6$	$(2,7 \pm 0,6) \times 10^6$	$(3,5 \pm 0,5) \times 10^6$	$(1,2 \pm 0,3) \times 10^6$	$(4,2 \pm 0,7) \times 10^6$	$(6,4 \pm 0,5) \times 10^5$	$(8,7 \pm 0,4) \times 10^6$	$(2,5 \pm 0,3) \times 10^5$
December	$(6,9 \pm 0,5) \times 10^5$	$(1,1 \pm 0,3) \times 10^5$	$(6,4 \pm 0,3) \times 10^4$	$(4,5 \pm 0,4) \times 10^6$	$(9,2 \pm 0,5) \times 10^5$	$(3,8 \pm 0,2) \times 10^4$	$(9,7 \pm 0,6) \times 10^4$	$(1,5 \pm 0,2) \times 10^5$



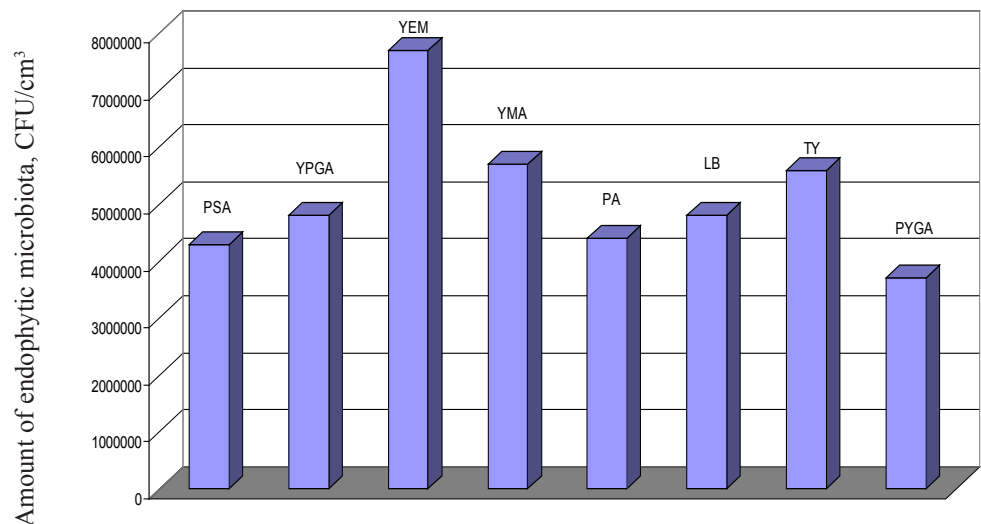
As it can be seen from Figure 1, the amount of grapevine endophytic bacteria in the majority of cases (66.6%) reached up to  $10^6$  and  $10^7$  CFU/cm<sup>3</sup>.



**Fig. 1. Percentage of the certain ranges of microbiota amount in October–December.**

The obtained results coincide with the data of the previous investigators which have shown that grapevine vessels contained from  $10^2$  to  $10^5$ /ml of microorganisms depending on the detection method [4].

Comparison of the mean quantities of bacteria (Figure 2) in all 27 tested grapevine plants by the isolation on each of eight media allowed us to reveal the highest number of bacteria on the media YEM ( $7.7 \pm 0.4$ ) x  $10^6$  CFU / cm<sup>3</sup>), YMA ( $5.7 \pm 0.8$ ) x  $10^6$  CFU/cm<sup>3</sup>) and TY ( $5.6 \pm 1.1$ ) x  $10^6$  CFU/cm<sup>3</sup>).



**Fig. 2. Mean amount of endophytic microbiota as determined by the isolation on different nutritional media in October–December (CFU/cm<sup>3</sup>)**



YEM («yeast-mannitol medium») [14] and YMA («yeast-mannitol agar») contain yeast extract and mannitol (carbon source), but differ by the composition of salts. TY («tryptone yeast») medium besides of yeast extract [12] contains also trypton as an available source of amino acids for microorganisms, and  $\text{CaCl}_2$ . In case of YEM and YMA media showing the best results, we may suppose that the high level of bacterial growth could be explained by the presence of mannitol in their compositions. In case of TY medium a high level of growth could be explained by the high quantity of yeast extract. The results coincide with the data of our previous studies that also showed better growth of endophytic grapevine bacteria on YMA and YEM media [1].

The results of investigations carried out for three months in the end of grapevine vegetation period – in the beginning of the dormancy period (October, November, December) have showed that the mean amount of bacteria grown on nutritional media clearly decreased every month.

In December, the number of bacteria grown on nutritional media decreased in two - three ranges comparing with October (Table 2). Thus, the mean number of endophytic microbiota on YEM decreased from  $(2.0 \pm 0.4) \times 10^7$  CFU/cm<sup>3</sup> in October to  $(6.4 \pm 0.3) \times 10^4$  CFU/cm<sup>3</sup> in December.

With YMA medium it was possible to detect the maximum number of bacteria in December, which was a range higher than the results of isolation on other media  $(4.5 \pm 0.4) \times 10^6$ /cm<sup>3</sup>. Thus, YMA medium can be recommended for the isolation of endophytic grapevine microbiota in winter period.

Our results coincide with the data of Bauer et al. (1994) who described the population amounts of some representatives of endophytic grapevine microbiota with peaks in May and October and the subsequent decrease in November - December.

During our investigations it was found out that in October the number of endophytic bacteria reached approximately  $10^6$  and  $10^7$  CFU/cm<sup>3</sup>. From October to December the amount of endophytic microbiota on the majority of media decreased in 2–3 ranges.

The obtained results have shown that the highest amount of endophytic bacteria could be isolated on YEM medium. With YEM medium it would be possible to detect the number of bacteria in shoots during all seasons and to establish the role of microorganisms in grapevine plants.

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## **ЧИСЕЛЬНІСТЬ ЕНДОФІТНОЇ МІКРОБІОТИ ПАГОНІВ ВИНОГРАДУ**

### **Реферат**

*Метою роботи було визначення чисельності ендоефітної мікробіоти рослин винограду за висівах на середовища різного складу. **Матеріали і методи.***



Ендофітну мікробіоту винограду виділяли на вісім середовищ різного складу. Для досліджень відбирали здерев'янілу лозу винограду *Vitis vinifera L.* сорту Аркадія. **Результати.** Найбільшу кількість бактерій можна було виділити висівами на середовища УМА, УЕМ та ТУ, відмінною особливістю перших двох яких була наявність манітолу, а середовища ТУ – підвищеної кількості дріжджового екстракту. **Висновок.** Чисельність представників мікробіоти судин пагонів винограду становила від  $(6,4 \pm 0,3) \times 10^4$  до  $(2,0 \pm 0,4) \times 10^7$  КУО/см<sup>3</sup> в залежності від середовища культивування і у період з жовтня місяця по грудень зменшувалася щонайменше на один порядок.

Ключові слова: ендофітна мікробіота, виноград, живильні середовища.

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## ЧИСЛЕННОСТЬ ЭНДОФИТНОЙ МИКРОБИОТЫ ПОБЕГОВ ВИНОГРАДА

### Реферат

**Целью** работы было выявление численности эндофитной микробиоты винограда при высевах на среды разного состава. **Материалы и методы.** Эндофитную микробиоту винограда выделяли на восемь сред разного состава. Для исследования отбирали одревесневшую лозу винограда *Vitis vinifera L.* сорта Аркадия. **Результаты.** Наибольшее количество бактерий можно было выделить посевами на среды УМА, УЕМ и ТУ, отличительной особенностью первых двух из которых было наличие маннитола, а среды ТУ – повышенного количества дрожжевого экстракта. **Вывод.** Численность представителей микробиоты сосудов побегов винограда составляла от  $(6,4 \pm 0,3) \times 10^4$  до  $(2,0 \pm 0,4) \times 10^7$  КОЕ/см<sup>3</sup> в зависимости от среды культивирования и в период с октября месяца по декабрь уменьшалась по меньшей мере на один порядок.

Ключевые слова: эндофитная микробиота, виноград, питательные среды.

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