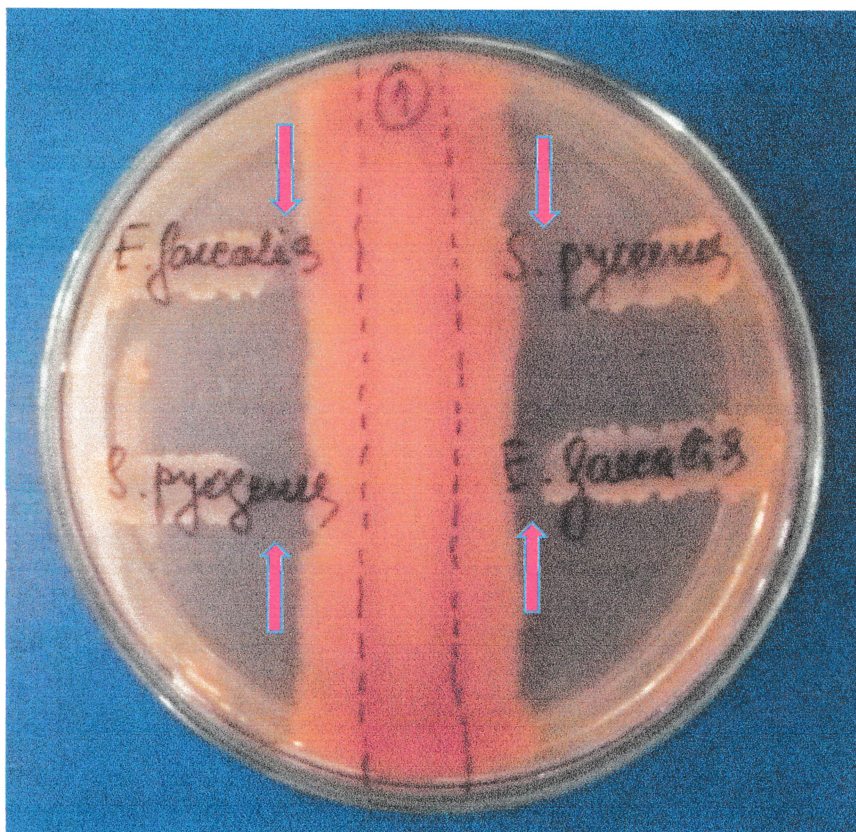
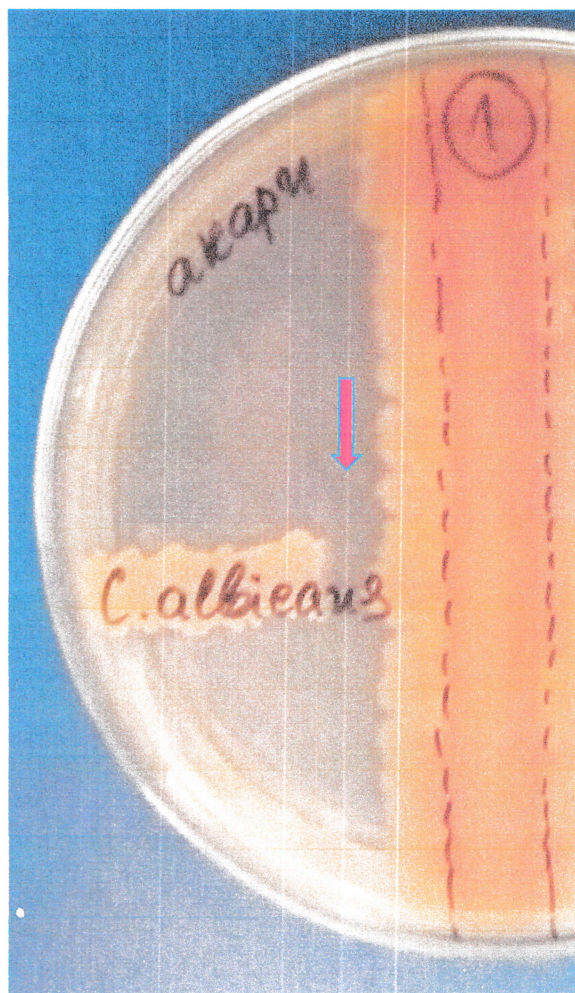


Figure 3B. Product 1 – inhibition of *Streptococcus pyogenes* and *Enterococcus faecalis* evaluated through the method of the parallel streaks.



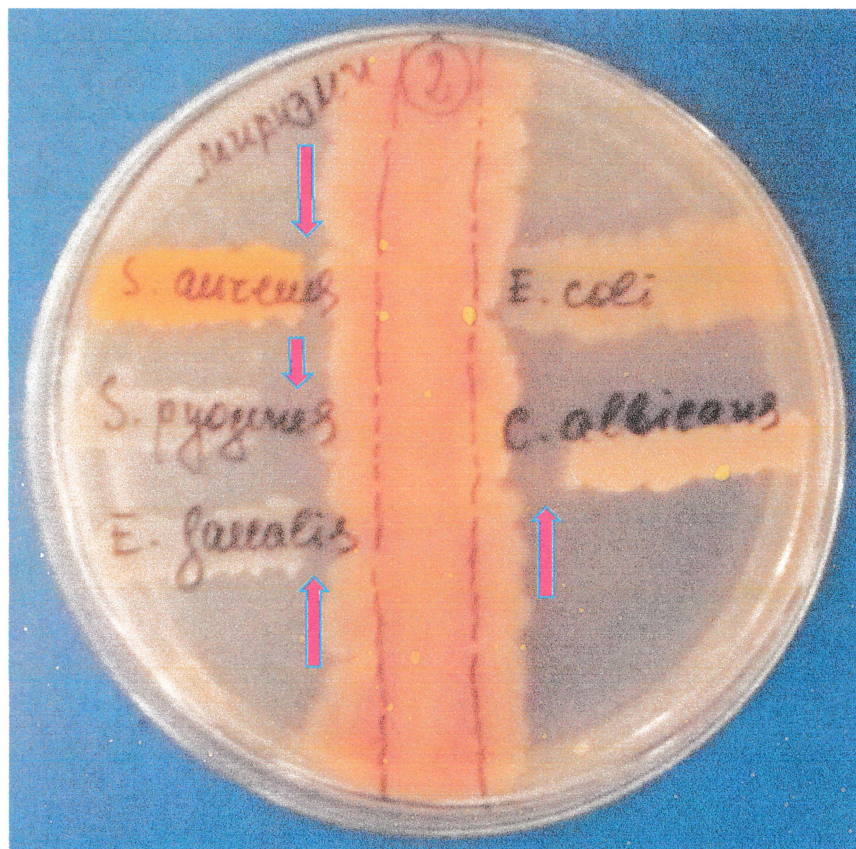
The inhibitory zones between the pathogenic strains and the probiotic strains from product 1 are indicative for the antimicrobial potential of the product.

Figure 3C. Product 1 – inhibition of *Candida albicans* evaluated through the method of the parallel streaks.



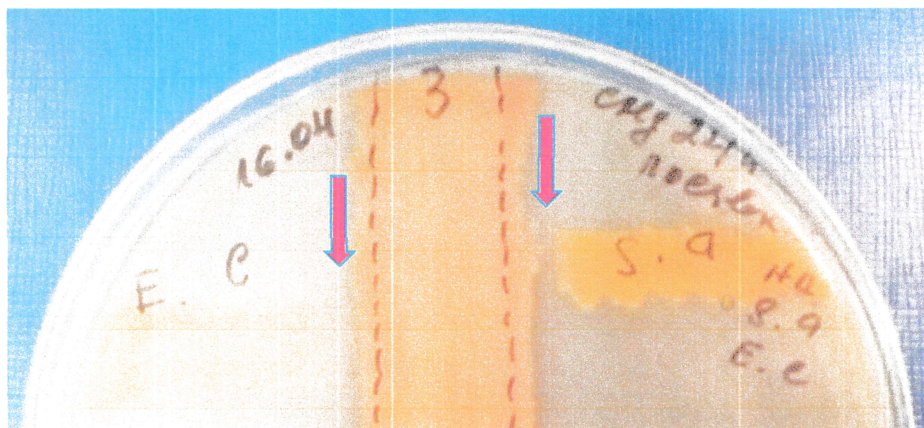
Product 1 inhibited the growth of *C. albicans* as evidenced by the inhibitory zone between the probiotic strains and the pathogenic fungi.

Figure 4. Product 2 – inhibition of bacterial and fungal growth evaluated through the method of the parallel streaks.

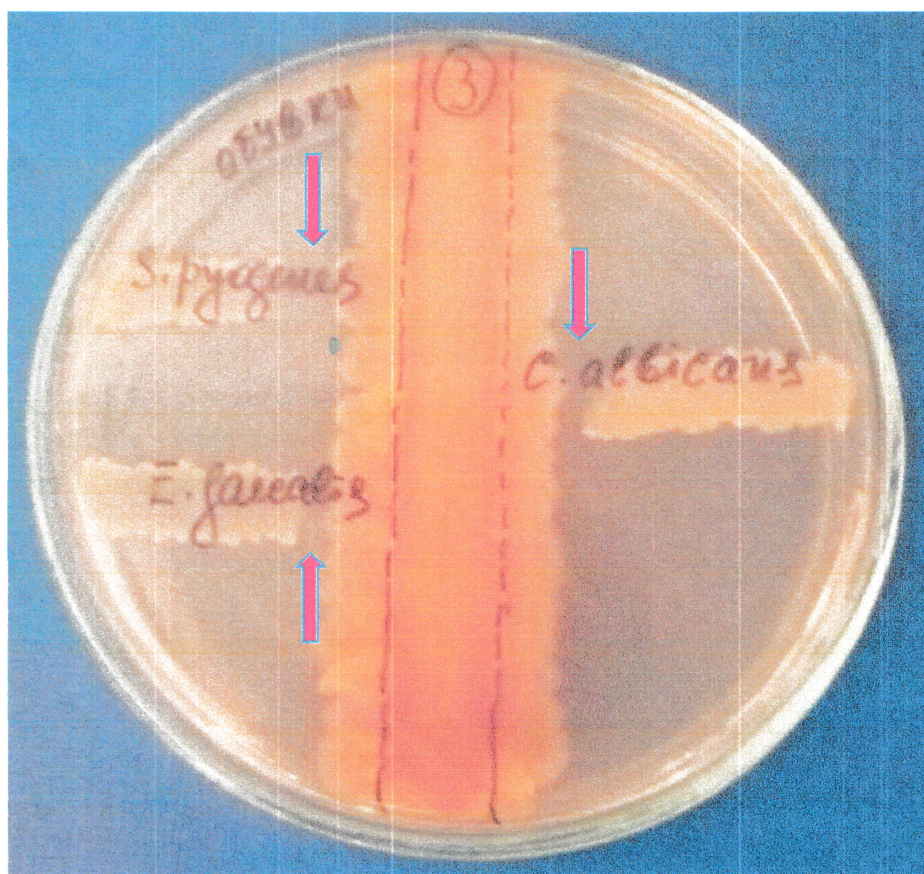


Similar to product 1, product 2 inhibited the growth of Gram-positive bacteria and *C. albicans*.

Figure 4. Product 3 – inhibition of bacterial growth evaluated through the method of the parallel streaks.



The same pattern of activity as by products 1 and 2 was observed by product 3, namely – antimicrobial activity against Gram-positive pathogenic strains and fungi *C. albicans*.



9.2 Results from the agar diffusion test

Figure 5. Antifungal activity of the filtrates from the test samples on pathogenic fungi from the species *Candida albicans*.



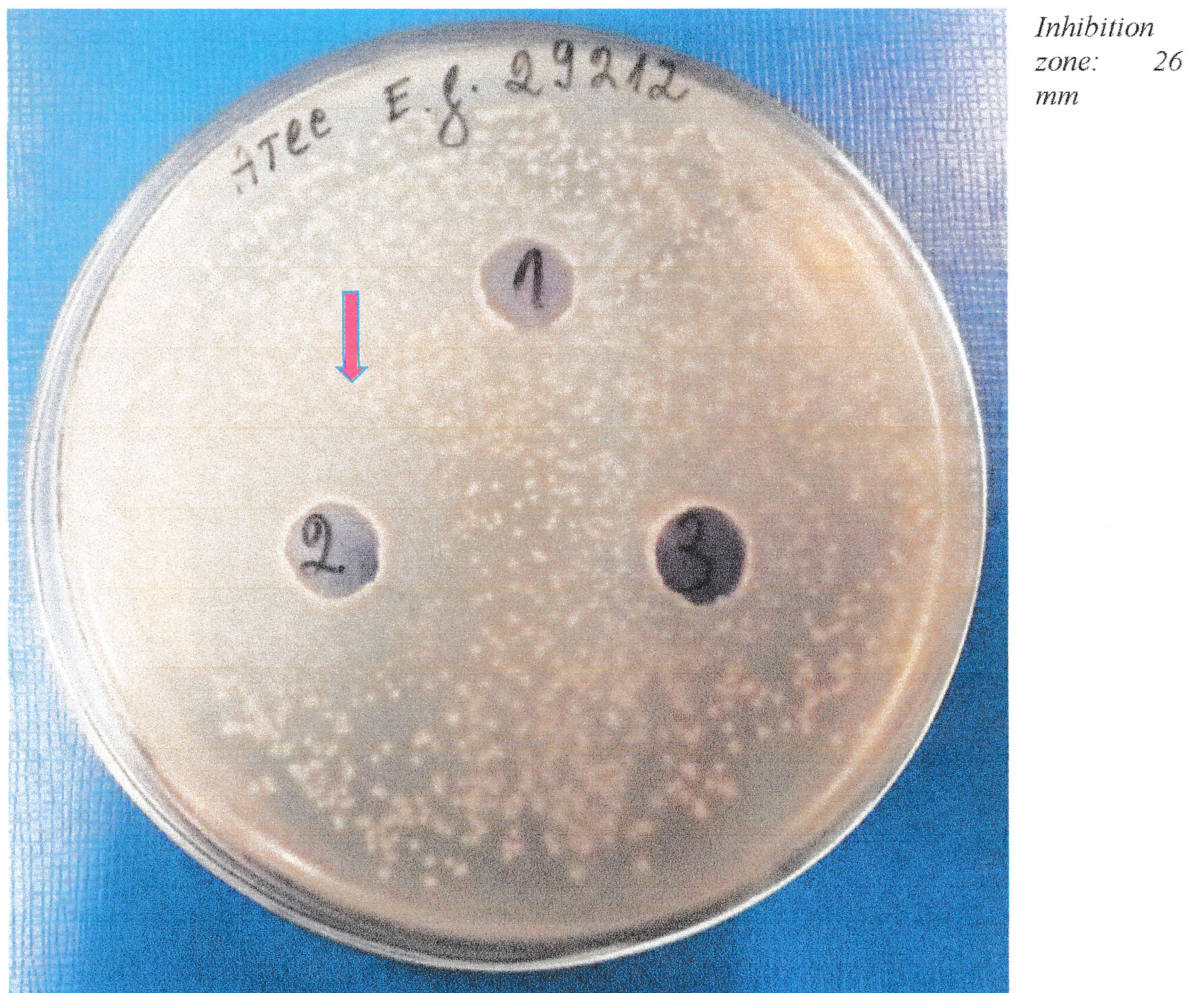
The filtrate of product 2 showed slight inhibitory effect on the growth of *C. albicans* as evidenced by 10 mm inhibitory zone.

Figure 6. Antimicrobial activity of the filtrates from the test samples on the pathogenic bacterial species *Staphylococcus aureus*.



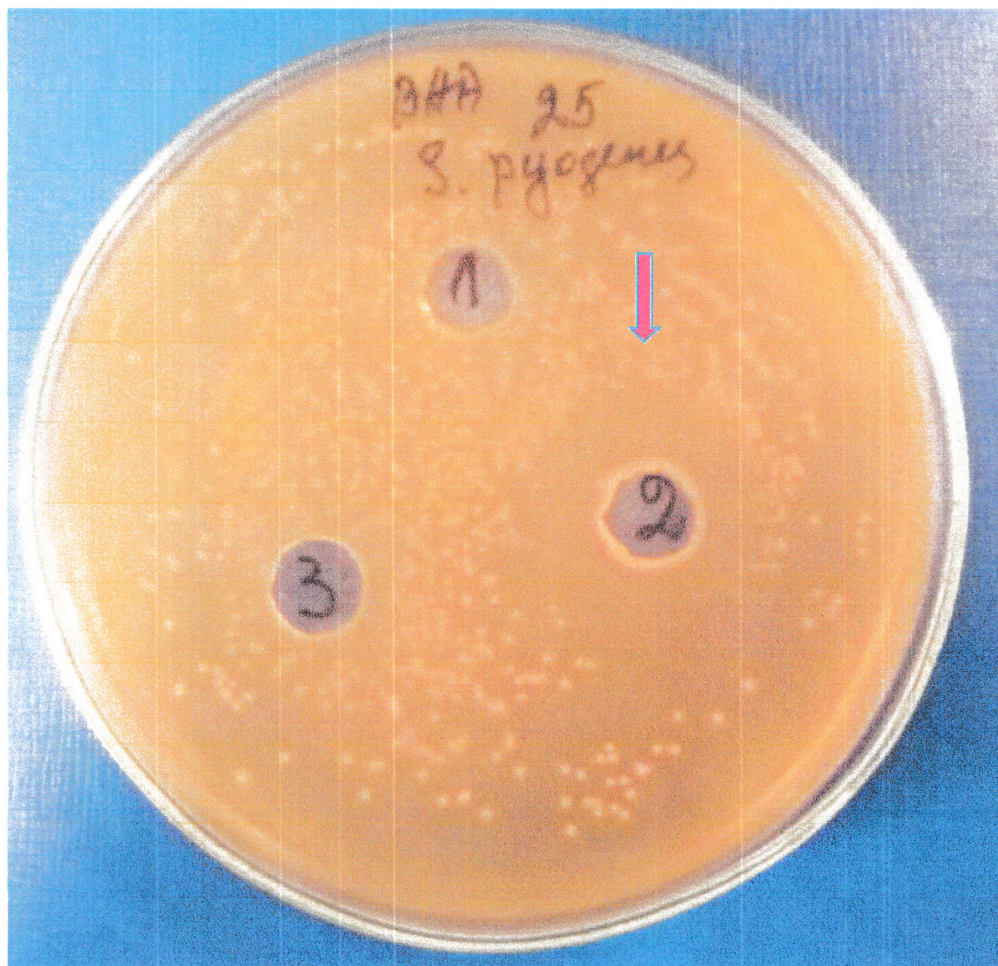
The filtrate of product 2 showed strong antibacterial activity, as evidenced by the inhibitory zone of 24 mm.

Figure 7. Antimicrobial activity of the filtrates from the test samples on the pathogenic bacterial species *Enterococcus faecalis*.



This result confirms the antibacterial activity of filtrate from product 2 against Gram-positive bacterial strains.

Figure 8. Antimicrobial activity of the filtrates from the test samples on the pathogenic bacterial species *Streptococcus pyogenes*.



Inhibition
zone: 28
mm

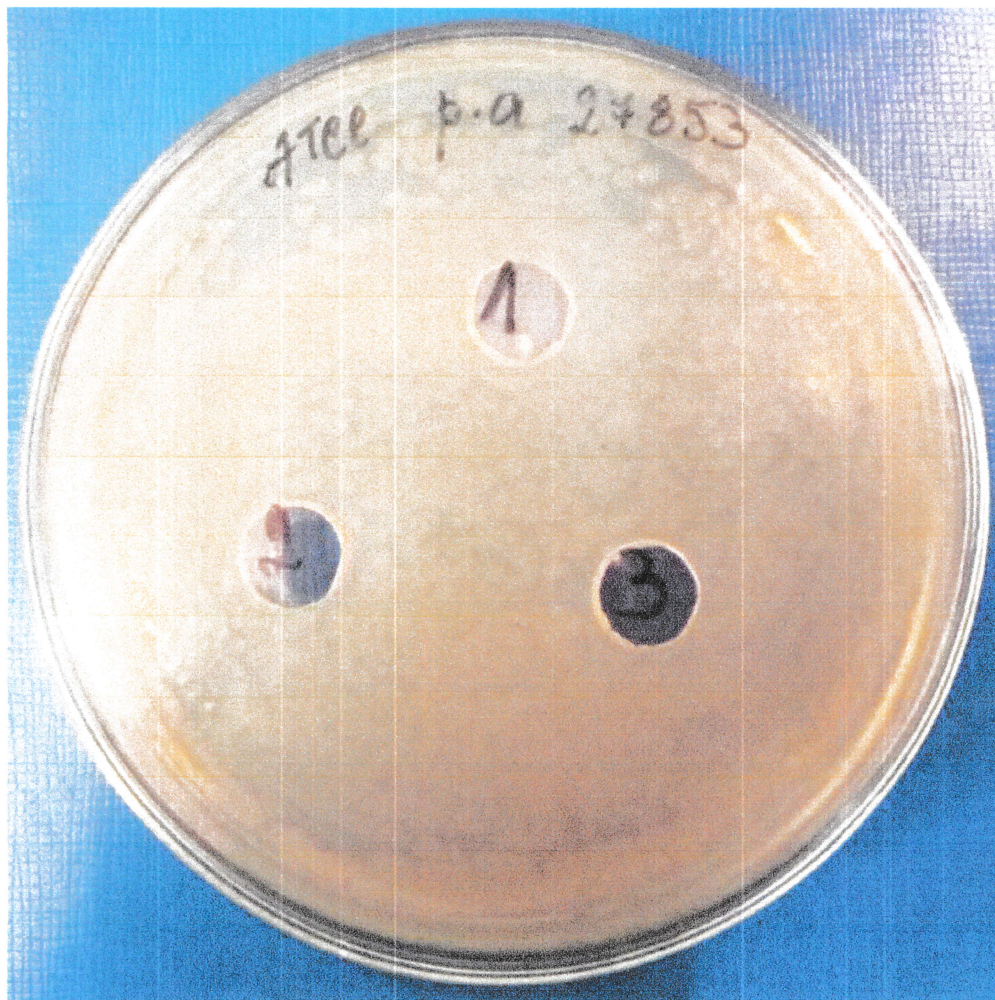
Filtrate of product 2 showed strongest inhibitory activity against *S. pyogenes* as compared to the other Gram-positive strains.

Figure 9. Antimicrobial activity of the filtrates from the test samples on the pathogenic bacterial species *Escherichia coli*.



Escherichia coli, no inhibitory effect

Figure 10. Antimicrobial activity of the filtrates from the test samples on the pathogenic bacterial species *Pseudomonas aeruginosa*.



Pseudomonas aeruginosa,
no inhibitory effect

10 CONCLUSION

In conclusion, all three products showed antimicrobial activity when plated in the same nutrition milieu with the tested Gram-positive pathogenic bacterial and fungal strains. Filtrate obtained from product 2 showed strong antibacterial activity against Gram-positive pathogens and slight antifungal activity against *Candida albicans*.

This result confers only to the tested three products and any extrapolation or transfer to other products is responsibility of Perilis Trading EOOD.

11 REFERENCE LIST

- [1]. Song D, Ibrahim S, Hayek S. Chapter 1: Recent Application of Probiotics in Food and Agricultural Science. In: Rigobelo E, ed. Probiotics: IntechOpen, 2012
- [2]. Sorokulova I. Modern Status and Perspectives of *Bacillus* Bacteria as Probiotics. Journal of Probiotics & Health 1 (4): 1000e106, 2013.
- [3]. Elshagabee FMF, Rokana N, Gulhane RD, Sharma C, Panwar H. *Bacillus* As Potential Probiotics: Status, Concerns, and Future Perspectives. Frontiers in microbiology 8: 1490-1490, 2017.

12 APPENDICES

Appendix 1: laboratory equipment, consumables, media and reagents.

Date of preparation:

Prepared by:

/Assist. Prof. Maya Zaharieva, PhD/

Date of approval:

Approved by:

/Prof. Hristo M. Najdenski, DSc, DVM, Corr.-member of BAS /

