



The Process of Preparing Microbiological Medium Containing Beneficial Microorganisms Ensuring Product Quality and Stability

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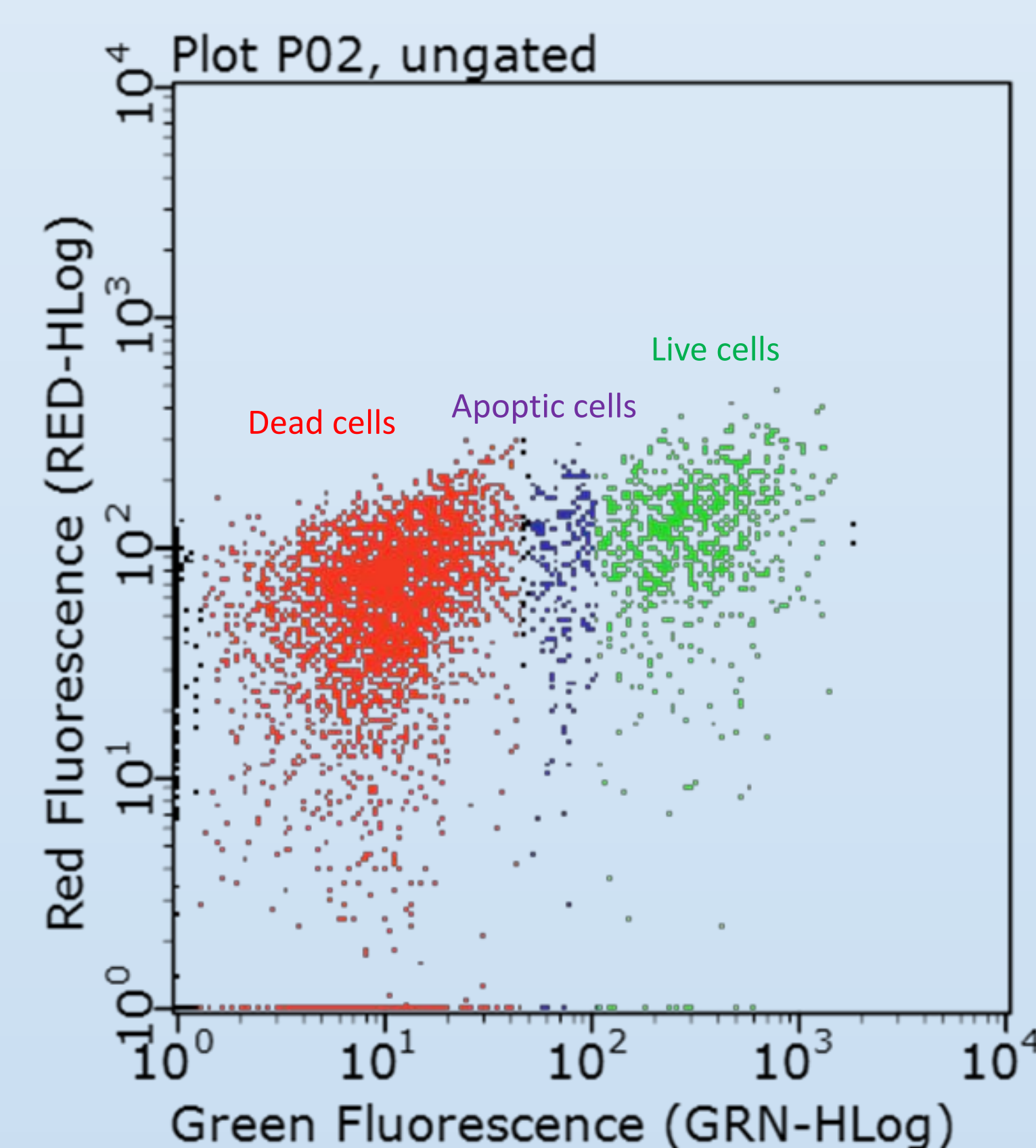
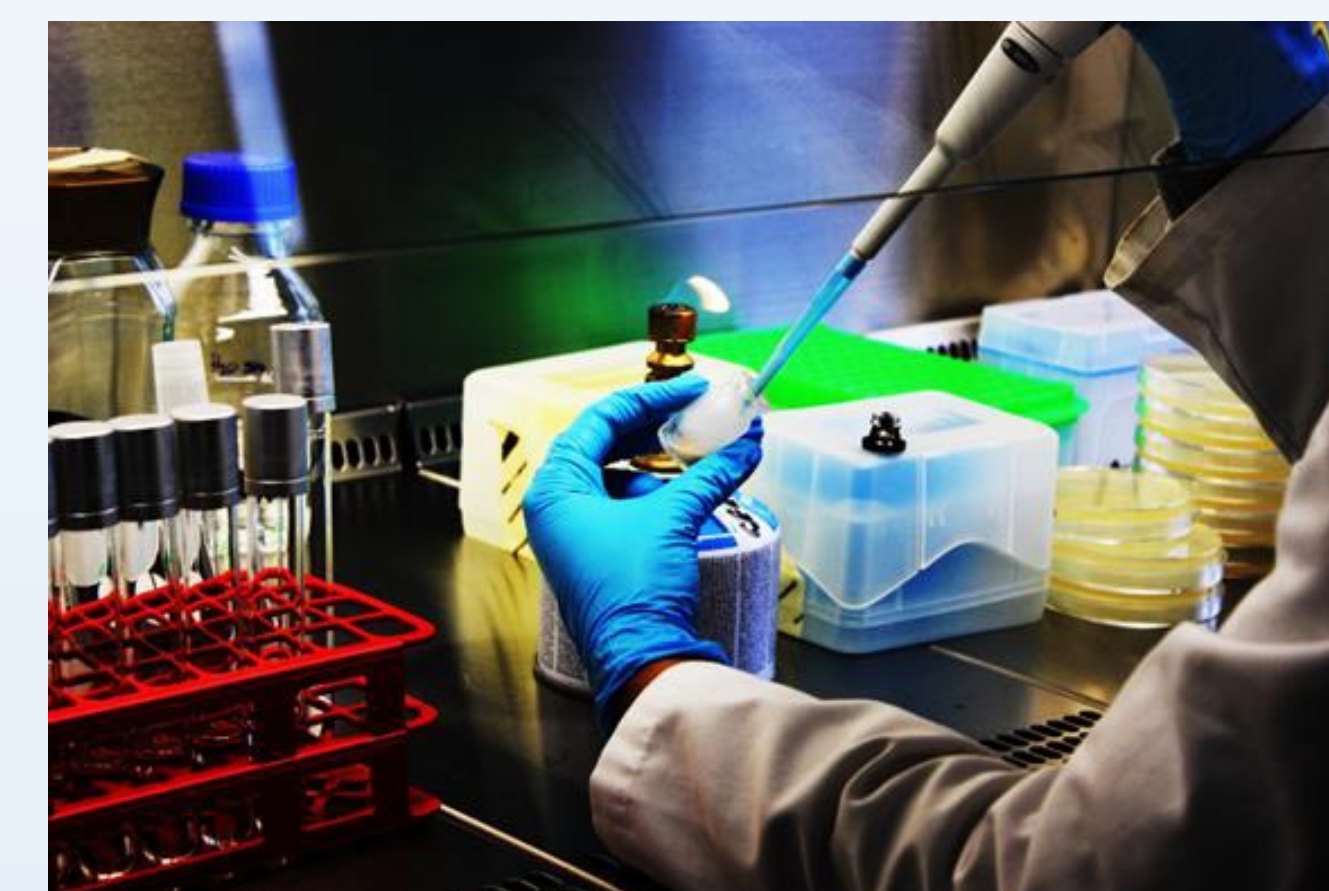
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INTRODUCTION

Nowadays more and more attention is paid to natural products, ecological lifestyle and healthy food. Probiotics play an increasingly important role in healthy eating, body care and now in agriculture. Together with growing public awareness one can observe an increasing demand for biologically active products containing beneficial microorganisms. The present study focuses on describing the process of preparing a product containing live microorganisms.

METHODS

In this experiment there have been used three strains of microorganisms (E41, E42, R85), isolated, examined and identified in the project called acronym: Phyto2Energy (call identifier: FP7-PEOPLE-2013-IAPP). Having selected and chosen proper microorganisms, the next step of preparing a inoculum was adjusting appropriate substrate that would enable the growth of all the microorganisms used and ensure their stability. During the experiment the wastes from the agri-food industry, such as: sugar cane molasses, sugar beet molasses, apple extract were used. Also, the combination of the traditional microbiological media were applied. 1-2% inulin as a form of a prebiotic was added to all media used. The following methods were used to evaluate the number of bacteria during the bioreactor: measurements of optical density, cytometry analysis and classical plating cultures. In cytometry analysis, the BacLight™ RedoxSensor™, Green Vitality Kit and propidium iodide were applied for evaluation of bacteria count and their liveness.



Cytometry analysis result

RESULTS

The presence of inulin as a prebiotic affected the better quality and stability of the produced inoculum in all tested media. The use of agri-food industry waste can only be applied in this case after the sterilization process because indigenous microflora tends to displace introduced microorganisms. The research showed that sugar cane molasses can be successfully used as the medium to prepare inoculum. The number of the microorganisms was 1.7×10^6 cfu/ml after 100 days of incubation.

Time (day)	Medium					
	LB (cfu/ml)	LB + 2% inulin (cfu/ml)	LB + 1% inulin (cfu/ml)	pepton +NaCl+2% inulin (cfu/ml)	Yeast extract +NaCl+ 2% inulina (cfu/ml)	50% LB (cfu/ml)
20	2.6×10^7	4.2×10^7	1.8×10^7	2.4×10^7	1.2×10^7	1.5×10^7
27	9.2×10^6	8.0×10^7	1.1×10^7	6.3×10^7	2.8×10^7	1.1×10^7
35	1.5×10^7	2.9×10^7	2.7×10^7	1.3×10^7	6.2×10^6	7.1×10^6
42	1.2×10^7	2.7×10^7	2.5×10^7	1.0×10^7	7.9×10^6	1.3×10^7
49	5.0×10^6	3.2×10^7	2.5×10^7	1.0×10^6	5.8×10^6	4.5×10^6
56	6.3×10^6	3.1×10^7	2.1×10^7	7.4×10^6	5.0×10^6	4.1×10^6
63	7.0×10^6	3.9×10^7	2.9×10^7	8.3×10^6	8.3×10^6	3.3×10^6
71	4.4×10^6	5.7×10^7	2.7×10^7	7.6×10^6	6.8×10^6	3.2×10^6
85	2.1×10^5	2.2×10^7	3.3×10^7	3.1×10^6	6.7×10^7	1.5×10^6

Effect of inulin on the growth and viability of the tested bacteria



The decrease count of bacteria during their displacement caused by sugar cane molasses indigenous microflora . From left: 0h; 24h, 46h incubation time.

CONCLUSIONS

The use of waste from the agri-food industries as the substrates is cost-effective way for the microbiological inocula production. However, the inulin as supplement improves the stability and quality of cultures. Finally, the composition of the inocula is friendly-environment and have a positive effects on human health.

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