JIChEC06
The Sixth Jordan International Chemical Engineering Conference
12-14 March 2012, Amman, Jordan

Viability tests of granulated microorganisms

Weinholz S[1], Wassermann M[2], Cordes C[1]

[1]Anhalt University of Applied Sciences, Strenzfelder Allee 28, 06406 Bernburg, Germany
s.weinholz@loel.hs-anhalt.de, c.cordes@bwp.hs-anhalt.de
[2] VTA GmbH, Wilfried-Pergande-Platz 1, 06369 Weißandt-Gölzau, Germany
wassermann@pergande-group.com

ABSTRACT

The present study is part of the research project WIGRATEC focusing on fluidized bed technologies. The aim of the present study is to develop new methods for testing the viability of granulated microbial starter cultures.

For granulated bacteria it would be beneficial to have a fast method for detecting bacterial viability during production and as a quality control tool. Viability of microorganisms is traditionally tested by methods like colony forming units (CFU) and the LIVE/DEAD® BacLight Assay (Invitrogen).Within the framework of this project alternative assays for viability are developed by means of mass spectrometry and flow cytometric analysis. These assays will be used to test different batches of granulated microorganisms to determine viability. Furthermore the aim is to develop an assay that is able to fulfill the requirements of an assay used in routine process control.

Key words: granulation, microorganisms, viability, biomarker, 2-D gel electrophoresis, mass spectrometry, flow cytometry

INTRODUCTION

In food industry the storage of starter cultures needs to be economic. Therefore new methods are developed to preserve starter cultures. Bacteria are sprayed on carrier materials using fluidized bed technology and are analyzed during storage by testing viability. Aim of the present study is to develop new methods to test the viability of these granulated microorganisms fulfilling the criteria to be applied in routine process control. Biomarkers that might be useful are detected using protein analytical methods like 2-D poly acrylamide gel electrophoresis (PAGE) and MALDI-TOF mass spectrometry (Matrix assisted laser desorption/ionization time of flight). Currently these methods are established for model organism *Lactobacillus plantarum* (DSM 20174^T).Multicolor flow cytometry is established to investigate viability and membrane integrity. In addition to viability whole cell proteome of the model strain is analyzed by 2-D PAGE.

MATERIAL AND METHODS

Bacteria

According to De Man et al. (1960) *Lactobacillus plantarum* is cultured in MRS bouillon pH=5.7 at the temperature of 30°C. After batch cultivation cells are harvested and lyzed with FastPrep®-24 (MP Biomedicals) to purify whole cell proteome.

Viability

The viability of granulated microorganisms is tested by colony forming units (CFU) and by fluorescence spectrometry using the LIVE/DEAD® BacLight™ bacterial viability kit.

Furthermore, flow cytometric analysis of the model strain *Lactobacillus plantarum* is established for viability testing during cultivation and granulation processes. Discrimination between dead cells exhibiting permeabilized membranes and viable cells with an intact membrane is enabled applying SYBR® Green I and propidium iodide.

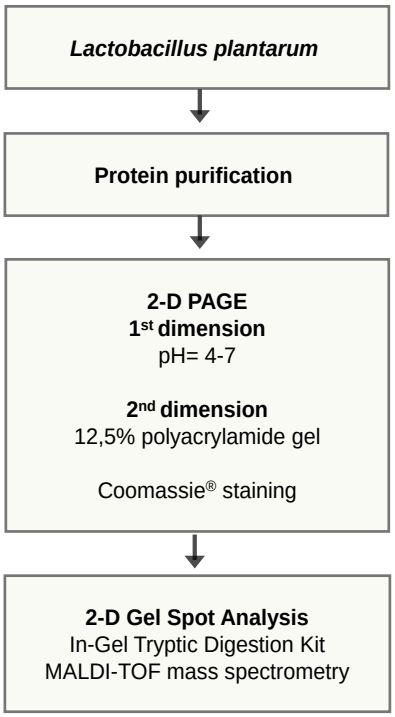

Figure 1.Scheme of experimental schedule.

Protein analysis

Proteins are detected according to Bradford et al. (1976) and loaded to 2-D gel electrophoresis. 150µg of protein sample are purified by ReadyPrep 2-D Cleanup Kit (Bio-Rad Laboratories). Using IPG strips, 7cm, pH=4-7 in isoelectric focusing the proteome of DSM 20174^T is separated. Protein separation in the second dimensionis obtained using 12,5 % acrylamide gel. Protein spots are digested using In-Gel Tryptic Digestion Kit (Thermo) and subsequently are analyzed in mass spectrometry with MALDI-TOF Voyager De PRO (Applied Biosystems) and Mascot software (AB Sciex).

RESULTS AND DISCUSSION

The model organism *Lactobacillus plantarum* is cultured to early stationary phase within 24 hours. The bacterial suspension is sprayed on carrier materials using fluidized bed technology. The viability of microbial granulates is analyzed by flow cytometric analysis. The results obtained with *Lactobacillus plantarum* show the discrimination between dead and viable cells.

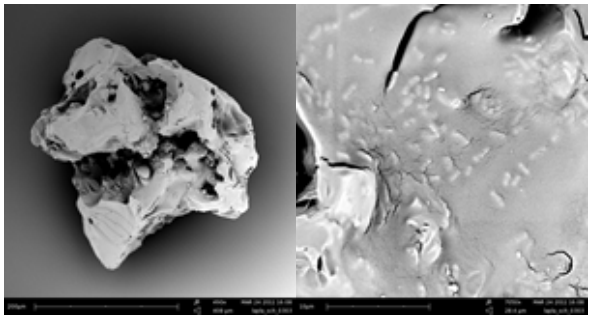

Figure 2. Scanning electron microscopy of microbial granulates (Wassermann, Institute Process Engineering, Otto von Guericke University, Magdeburg, Germany).

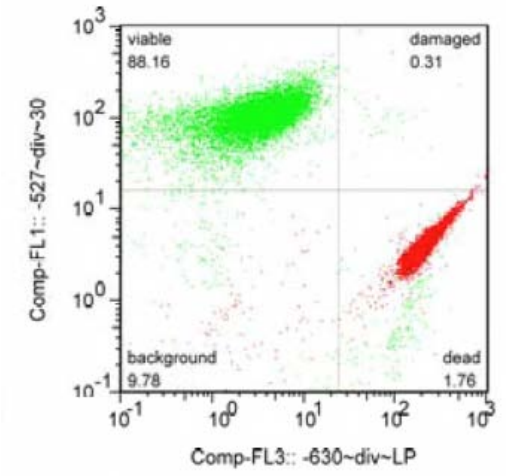

Figure 3. Flow cytometry of DSM 20174^T.Viability measured using SYBR® Green I [green] and propidium iodide [red].

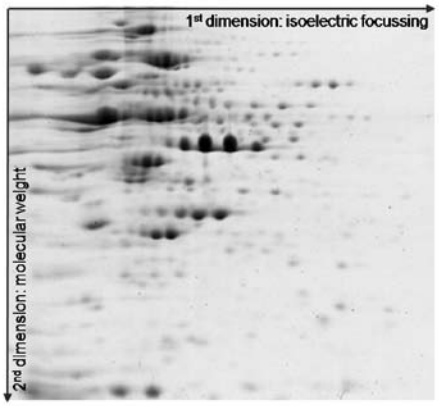

Figure 4. 2-D gel electrophoresis of DSM 20174^T.

CONCLUSION

In summary the granulated microorganisms are stable at room temperature for minimal six months during storage. Our experiments show that flow cytometry can be used for our organism to separate viable and dead cells and is therefore an applicable monitoring tool to analyze different batches of granulated microorganisms. With 2-D gel electrophoresis further investigations will be done to search for other biomarkers which might be useful in quality control assays for granulated microorganisms.

Acknowledgment

WIGRATEC is founded by BMBF (FKZ 03WKBQ04C; www.wigratec.de). Sincere thanks for valuable support to Marc Rüger and Sabine Kluge (Max Planck Institute for Dynamics of Complex Technical Systems and Otto von Guericke University, Bioprocess Engineering, Magdeburg, Germany) and staff members from Anhalt University of Applied Sciences.

REFERENCES

BRADFORD, M. M. 1976. A rapid and sensitive method for the quantitation of micro gram quantities of protein utilizing the principle of protein-dye binding.Analytical biochemistry, 72, 248-54.

De Man, J.D., M.Rogosa and M.E. Sharpe. 1960. A medium for the cultivation of lactobacilli. J. Appl. Bacteriol, 23, 130-35.

Invitrogen and Molecular Probes (2009).“LIVE/DEAD BacLight Bacterial Viability Kits – Product Information.”