Hochschule Ostwestfalen-Lippe
University of Applied Sciences

Microbiology

FACHBEREICH LIFE SCIENCE TECHNOLOGIES
AN DER HOCHSCHULE OSTWESTFALEN-LIPPE

Food Micro 2014, September, 01st to 04th 2014, Nantes, France

Abstract
In the present study, microbial counts and the production of CO_2 was compared in five culture media published for osmotolerant yeasts in a parallel-fermenter-system. The best medium in view of fast growth and maximum gas production was discovered and served as basic medium for optimization experiments. Especially the addition of malt extract, peptone and ammonium salts lead to the development of an optimized culture medium for osmotolerant yeasts. The developed culture medium showed faster beginning and an important gain of fermentation activity in all tested yeast strains compared to the reference medium. For example for *T. delbrueckii*, reduced times of 47 h to the beginning of fermentation were achieved and the maximum fermentation activity was raised threefold.

Development of an optimized culture medium for osmotolerant yeasts by use of a parallel-fermenter-system

Pfannebecker, J., Schiffer-Hetz, C., Becker, B., University of Applied Sciences, Institute for Food Technology.NRW, Microbiology, D-32657 Lemgo, Germany

Introduction and Purpose

Contaminations by osmotolerant yeasts can lead to significant carbon dioxide (CO_2) production in sugar rich products. Spoilage reactions like cracking of marchpane, package swellings and organoleptic changes of products can be observed (Fig. 1). Mainly yeasts of the genera *Zygosaccharomyces*, *Schizosaccharomyces*, *Torulaspora*, *Candida*, *Pichia* and *Debaryomyces* occur in products containing high sugar amounts. Commonly used culture media are not optimized and time to results varies between 10 and 21 days. In the present study, microbial counts and the production of CO_2 were compared in culture media published for osmotolerant yeasts by use of an online parallel-fermenter-system. In further experiments an optimized culture medium was developed.

Fig.1: Package swelling of a poppy seed filling by growth acitivity of osmotolerant yeasts.

Material and Methods

Microbial counts and the production of excess gas of strains from culture collections and yeast isolates were compared in five different culture media (Tab. 1) in a RAMOS® parallel-fermenter-system (HiTec Zang GmbH, Herzogenrath, Germany) (Fig. 2).

Tab.1: Examined culture media for growth of osmotolerant yeasts

Medium	Ingredients	a_w-value	pH-value	Reference
GB 50	Glucose, yeast extract	0.91	4.5	Jermini et al. (1987)
MYG 50	Glucose, malt extract, yeast extract	0.89	5.6	Beuchat (1993)
SY	Saccharose, yeast extract	0.78	6.6	Von Drachenfels (2009)
FSGY	Fructose, saccharose, glucose, yeast extract	0.72	6.0	Häcker (1988)
DG 18	Glucose, peptone, dichloran, chlor-amphenicol, KH_2PO_4, $MgSO_4$, glycerol	0.95	5.6	ISO/FDIS 21527-2 (2008)

Yeasts of the species *Z. bailii*, *Z. rouxii*, *Z. mellis*, *S. pombe*, *T. delbrueckii*, *C. parapsilosis* and *D. hansenii* were examined in low (10 cfu / mL) and higher (100 – 1.000 cfu / mL) microbial counts. The incubation was performed by soft shaking (60 rpm) at 30 °C. The most suitable medium regarding fast growth and maximum CO_2 transfer rate (CTR) was discovered and served as a basic culture medium for subsequent optimization. Optimization experiments were carried out by adding supplements to the basic culture medium and measurement of CTR in the parallel-fermenter-system.

Fig.2: RAMOS®-system equipped with 8 fermentation flasks. Picture: HiTec Zang GmbH.

The influences of yeast extracts, malt extract, sugars, vitamins, ammonium and sulfur compounds on fermentation activity were analysed.

Results

The cultivation of osmotolerant yeasts in GB 50 showed fastest growth and highest CO_2 transfer compared to all analysed culture media and was therefore used as basic medium for optimization (data not shown). The optimization experiments demonstrated that especially the addition of malt extract, peptone and ammonium salts lead to an increased CO_2 production. An optimized culture medium (OM) was developed. It showed faster beginning of fermentation in all tested yeast strains compared to the reference medium (Fig. 3).

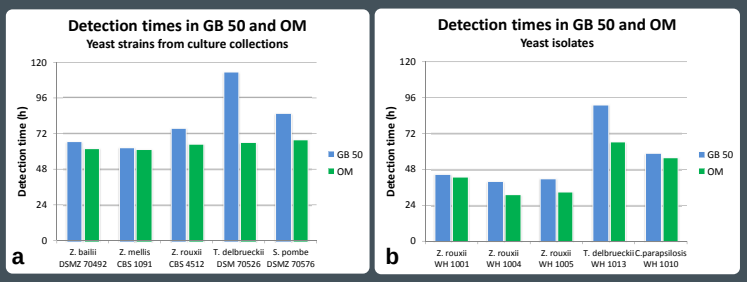

Fig.3: Comparison of detection times (CTR >0.004 mol / L · h) in GB 50 and OM for yeasts from culture collections (a) and yeast isolates (b).

By using the developed culture medium, an important gain of fermentation activity was achieved which is convenient for a fast and sensitive identification of spoilage yeasts in routine analysis. For example for *Z. rouxii* and *T. delbrueckii* the analysis of the maximum CO_2 transfer rate showed a 3 fold raised CO_2 transfer rate in OM compared to GB 50 (Fig. 4).

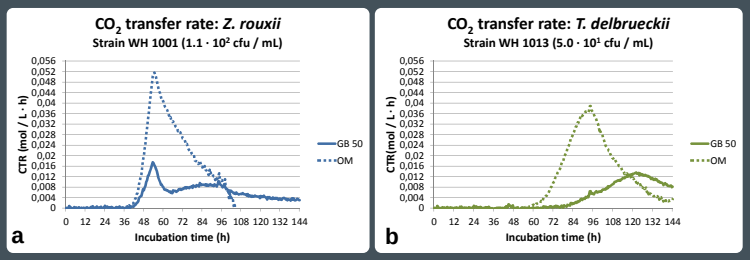

Fig.4: Comparison of CO_2 transfer rate (CTR) by growth of *Z. rouxii* (a) and *T. delbrueckii* (b) in GB 50 bouillon and optimized medium (OM).

Conclusion and Outlook

An optimized medium was developed in regard of fast growth and in particular the CO_2 transfer rate. Detection times of 48 - 96 h were significant lower and accompanied by an up to 3 fold increased CO_2 production. In further analyses, the influence of different product matrices on fermentation activity of osmotolerant yeasts in the developed culture medium will be tested.

This research project was supported by the German Ministry of Economics and Technology (via AiF) and the FEI (Forschungskreis der Ernährungsindustrie e.V., Bonn, Germany). Project AiF 16650 N. We thank HiTec Zang GmbH supporting our study.