DEVELOPMENT OF NEW YEAST STRAINS FOR LOWERING ETHANOL CONTENT OF WINES AND INCREASE OF GLYCEROL

Juergen FROEHLICH, Hannes WENINGER, Michael SOBE
Erbsloeh Geisenheim AG • Erbsloehstrasse 1 • Geisenheim D-65366 • Germany

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Introduction

As a result of the climate change, rising sugar content in grape must and the concomitant increase in alcohol levels in wine are some of the main challenges affecting winemaking nowadays. Among the several alternative solutions currently applied, the use of special wine yeasts isolated after different selective pressures shows promising results to relieve this problem. Attempts to produce such yeasts comprise intentional genetic modification without application of GM techniques and processes based on selective cultivation. Beside GM technologies, alternative methods can be applied to manipulate yeasts to produce less alcohol and increased amount of glycerol by using their molecular response to osmotic stress. Gene modifications can be introduced by mutagenic substances or UV light as well as different selection pressures which are suited to adapt yeasts. A first selection step is performed after the first round of mutagenesis, then a second selection step is performed after the second mutagenesis, whereby the mutants resulting from the respective preceding mutagenesis are exposed to selection factors like hypertonic medium or alcohol dehydrogenase inhibitor. Finally, these strains were selected by a pipette-robot. The best performing strains are then subjected to RNA microarray tests, showing that genes of the HOG (High Osmolarity Glycerol) pathway were mainly affected by the mutagenesis [1, 2]. The new yeast show less production of alcohol and increased amounts of glycerol along with positive sensorial effects on the wine [3].

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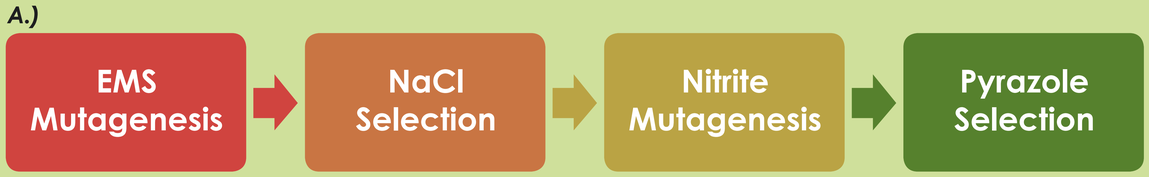
Mutagenesis and Selection of Yeast Clones

Material and Methods

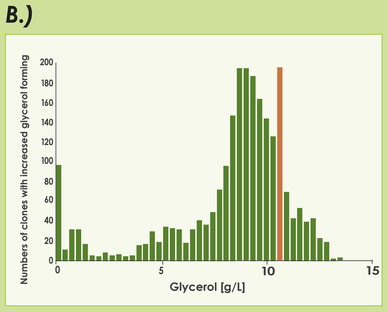
The subsequent mutagenesis cannot be successfully carried out in random order. It rather indicates that a very particular sequence of mutagenic treatments leads to best results in combination with a certain selection pressure. The following mutagens have been applied: ethyl methane sulfonate (EMS; produces alkylated DNA) and sodium nitrite was used as a nucleotide-deaminating agent. Furthermore, it was demonstrated that EMS along with high amounts of sodium chloride produces useful mutants for the second mutagenesis.

A.)

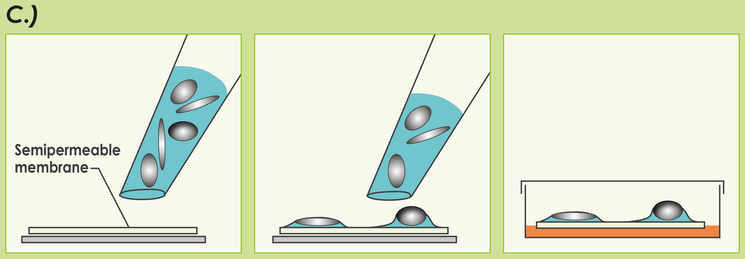


The best glycerol producing mutants were obtained after the application of EMS and the selection under high osmophilic conditions followed by nitrite treatment and the use of pyrazole for the second selection pressure (cf. [1,2], A, B). After mutagenesis and selection, individual cells were isolated by micromanipulation to get pure cultures (C). For the identification of these pure cultures (n) SAPD PCR was applied (data not shown cf. [1,2]).

B.)



C.)

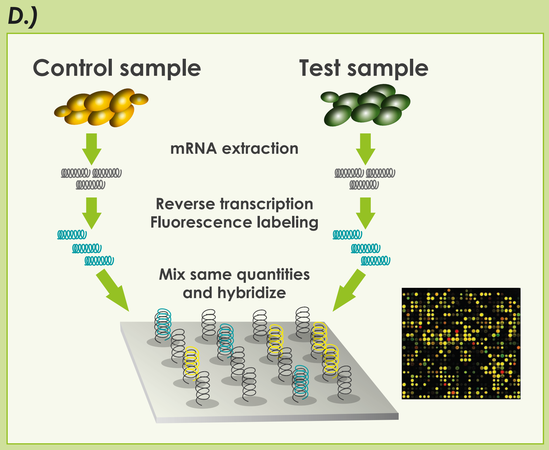


Microarray shed light on the Modification of Yeast HOG Pathway

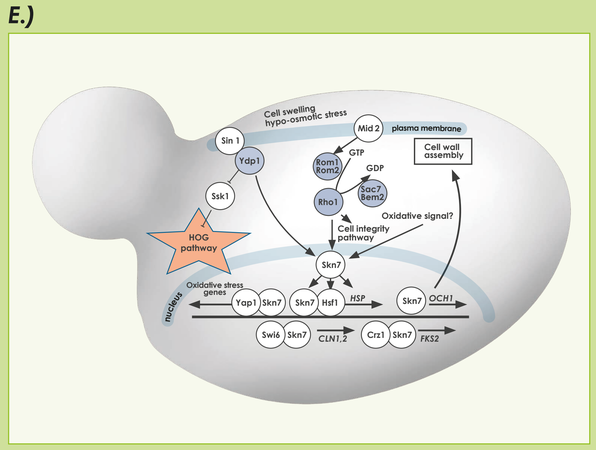
Results and Discussion

The staggered mutagenesis and selection procedures play the key role in the modification of the yeast genetics. The comparison of wild-type and modified yeast by microarray technologies showed clearly how genes were altered (D). Among other mutations, several genes of the HOG (High Osmolarity Glycerol) pathway were involved (E).

D.)



E.)



Beyond that molecular biological perspective, organoleptic impacts on wine are of important interest when this new wine yeast will be applied on grape juice. Hereby, the production of glycerol instead of ethanol (F) provides further advantages by balancing the alcoholic strength and astringency. Glycerol confers smoothness on the palate and give the impression of full-bodied wines. The overall flavor intensity is positively influenced by glycerol. For the bitter tastes, glycerol was reported to suppress astringency and roughness perception of a wine [3]. Nevertheless, random mutagenesis adversely affected the vitality of the new clone. In order to counterbalance this drawback, a support by complex nutrition is required and consists of organic and inorganic nitrogen sources, as well as vitamins and minerals.

F.)

Yeast	Gluc. g/L	Fruct. g/L	Sugar tot. g/L	Alc. g/L	Glyc. g/L	Acet. acid g/L	Malic acid g/L	Acetald. mg/L	Succ. acid g/L
LA-HOG	0,67	0,64	1,31	94,6	12,9	0,21	2,53	14	1,47
Comp.	0,84	0,86	1,70	99,7	8,7	0,25	2,48	16	1,35
Ref.	0,59	0,68	1,27	102,4	6,4	0,26	2,58	15	0,98

Glossary and abbreviations: *LA HOG: Marketed wine yeast Oenoferm®/ErboFerm™ LA HOG (Low alcohol - High Osmolarity Glycerol [2]) Comp.: Comparator (marketed yeast claimed to reduce alcohol); Ref.: Reference (conventional wine yeast); Gluc.: Glucose; Fruct.: Fructose; tot.: total; Alc.: Alcohol; Glyc.: Glycerol; Acet.: Acetic; Acetald.: Acetaldehyde; Succ.: Succinic.*

References
[1] Fröhlich J, Pfannebecker J (2007) Species-independent DNA fingerprint analysis with primers derived from the NotI identification sequence. Patent application WO/2007/131776
[2] Fröhlich J, Eck J, Meurer G, Naumer C, Büscher J, Mampel J (2016) Method of producing yeast mutants and the use thereof. Patent WO/2016/128296
[3] Jones PR, Gawel R, Francis IL, Waters EJ (2008) The influence of interactions between major white wine components on the aroma, flavour and texture of model white wine. Food Qual Prefer 19: 596–607

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ZIM Zentrales Innovationsprogramm Mittelstand

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