BRAZILIAN JOURNAL OF MICROBIOLOGY

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Industrial Microbiology

An original method for producing acetaldehyde and diacetyl by yeast fermentation

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ARTICLE INFO

Article history:
Received 2 June 2015
Accepted 29 February 2016
Available online 25 July 2016
Associate Editor: Jorge Gonzalo Farias Avendano

Keywords:
Acetaldehyde
Diacetyl
YAD culture medium
Yeast fermentation

ABSTRACT

In this study a natural culture medium that mimics the synthetic yeast peptone glucose medium used for yeast fermentations was designed to screen and select yeasts capable of producing high levels of diacetyl and acetaldehyde. The presence of whey powder and sodium citrate in the medium along with manganese and magnesium sulfate enhanced both biomass and aroma development. A total of 52 yeasts strains were cultivated in two different culture media, namely, yeast peptone glucose medium and yeast acetaldehyde-diacetyl medium. The initial screening of the strains was based on the qualitative reaction of the acetaldehyde with Schiff's reagent (violet color) and diacetyl with Brady's reagent (yellow precipitate). The fermented culture media of 10 yeast strains were subsequently analyzed by gas chromatography to quantify the concentration of acetaldehyde and diacetyl synthesized. Total titratable acidity values indicated that a total titratable acidity of 5.5 °SH, implying culture medium at basic pH, was more favorable for the acetaldehyde biosynthesis using strain D15 (*Candida lipolytica*; 96.05 mg L^{-1} acetaldehyde) while a total titratable acidity value of 7 °SH facilitated diacetyl flavor synthesis by strain D38 (*Candida globosa*; 3.58 mg L^{-1} diacetyl). Importantly, the results presented here suggest that this can be potentially used in the baking industry.

Introduction

Yeasts are commonly used as starter cultures for increasing product-specific aroma production during various fermentation processes (cheese, kefir, sourdough, wine, beer, etc.), as they are capable of synthesizing natural flavors like acetaldehyde (ethanal) or diacetyl (2,3-butanedione) which in turn serve to enhance the quality of the food.[1] Acetaldehyde is the most important carbonyl compound produced during alcoholic fermentation with final concentrations typically varying between 10 and 200 mg L^{-1} depending on technological factors

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http://dx.doi.org/10.1016/j.bjm.2016.07.005

such as culture medium composition, pH, fermentation temperature, aeration and SO_2 concentration, and on the yeast strain used.[2,3] Acetaldehyde is found in a variety of foods and beverages such as cheese, yogurt, beer, and wine.[4,5] Generally, acetaldehyde levels reach their peak values in the early fermentation phase; it is then partly reutilized by the yeasts during the rest of the fermentation process.[6] Acetaldehyde is responsible for the green apple aroma in alimentary products and, usually, the added acetaldehyde is produced by chemical synthesis. Contrarily, by adding using acetaldehyde produced by biosynthesis renders the product safe for human consumption, and the inadvertent addition of the byproducts of chemical acetaldehyde synthesis can be avoided. Diacetyl is almost exclusively synthesized by lactic acid bacteria and is the key flavor compound naturally produced by the *Leuconostoc* sp.[7,8] Diacetyl is an important flavoring compound that determines specific characteristics of products such as fermented milks and, at very low concentrations (up to $5\,mg\,L^{-1}$), is also responsible for the characteristic "buttery" aroma of milk products.[9] Furthermore, it is known that while *Saccharomyces cerevisiae* produces $132.4\,mg\,L^{-1}$ acetaldehyde, less than $1\,mg\,L^{-1}$ diacetyl is produced during red wine fermentation.[10–12] Thus, by using a potential fermentation mix that can produce both acetaldehyde and diacetyl during the fermentation process, in place of a chemically synthesized product, it is possible to confirm to the current global trend of replacing synthetic products with naturally obtained products, while concurrently satisfying consumer demand for natural compounds in alimentary products.

Therefore, the main objective of this study was to identify yeasts capable of biosynthesizing high amounts of acetaldehyde and diacetyl and to assess their proposed use as natural products in fermentation products during industrial production. This is necessary because any product on the market that already contains this aroma cannot be further used in patisserie products.

Materials and methods

Yeast strains

The yeasts strains utilized in this study were isolated from various sources in the laboratories of BIOALIMENT (biotechnology applied in food industry – integrated center for research and education), "Dunarea de Jos", University of Galati, Faculty of Food Science and Engineering. Every strain was assigned a unique code in the MIUG collection of the "Dunarea de Jos" University of Galati, and simultaneously, a unique code for the microorganism collection of the "Petru Poni" Institute of Macromolecular Chemistry (ICMPP). Pure strains were stored at −80 °C in YPG culture medium supplemented with 20% glycerol.[13] Further, the taxonomic classification of the isolated yeasts was determined and flavor production was followed by gas chromatography (GS).

Culture conditions

In this study, we designed and used a natural culture medium, yeast acetaldehyde-diacetyl medium (YAD), that could mimic the synthetic YPG culture medium, to select yeast strains capable of producing high levels of diacetyl and acetaldehyde. Importantly, the sodium citrate, manganese and magnesium sulfate, which are part of the YAD culture medium, are consumed by the microorganisms during growth and biosynthesis, thereby rendering the YAD culture medium 'natural'. For selecting yeast strains capable of producing diacetyl and acetaldehyde, we designed a culture medium with the following composition: $10\,g\,L^{-1}$ dextrose; $50\,g\,L^{-1}$ yeast extract; $5\,g\,L^{-1}$ sodium citrate; $10\,g\,L^{-1}$ whey powder; $0.05\,g\,L^{-1}$ manganese sulfate; $0.2\,g\,L^{-1}$ magnesium sulfate. The pH was adjusted between 6 and 7 using 0.1 N HCl. The culture medium was sterilized at 120 °C for 20 min, and this culture medium was designated YAD (yeast diacetyl acetaldehyde). The dormant yeasts were activated by inoculating them in standard YPG medium and transferred to YAD culture medium during the log growth phase by inoculating 5 mL of YAD culture medium with a 20% yeast suspension that was activated in YPG medium for 24 h and had an absorbance of 0.5 at 600 nm. The cultures were incubated at 30 °C for 48 h under static conditions in order to reduce the evaporation of volatile substances. After fermentation, the medium was filtered to remove cells and subjected to Gas chromatography for identification and quantification of acetaldehyde and diacetyl production.

Identification of the yeast strains using ID 32 C™

ID 32 C™ is a standardized system of yeast identification that uses 32 miniaturized assimilation tests and a database. The ID 32 C™ strip consists of 32 cupules, each containing a dehydrated carbohydrate substrate. A semi-solid, minimal medium is added along with a suspension of the yeast to be tested. After 24–48 h of incubation, growth in each cupule was read using the ATB Expression, the mini API instrument, or visually. Identification was performed using the Apiweb™ identification software.

Detection of diacetyl and acetaldehyde in culture medium

Detection of aldehydes with Schiff's reagent

Schiff's test is a qualitative test for the presence of aldehyde functional groups wherein, a colorless Schiff reagent turns into a characteristic violet color when aldehyde groups are present in the sample added.[14] The reaction was performed by adding 500 μL of Schiff's reagent to the 5 mL of medium fermented for 48 h at 30 °C. The media samples were assigned scores between 0 and 5 based on reaction intensity at 5 s after reagent addition, with 0 denoting no acetaldehyde production; 1–3 denoting low production of acetaldehyde, 4 denoting good production of acetaldehyde, and 5 denoting very good production of acetaldehyde. Only samples with high aldehyde content (score 5) were further tested to determine if acetaldehyde was indeed present in high amounts.

Detection of diacetyl with Brady's reagent

2,4-Dinitrophenylhydrazine (DNPH, Brady's reagent) is a chemical compound which can be used to qualitatively detect the presence of ketone or aldehyde functional groups. The test is considered positive if a yellow (aliphatic), orange or red (aromatic) precipitate is present. DNPH does not react