# TEKNOLOGI FERMENTASI DENGAN KHAMIR

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## Khamir

- Termasuk kapang, namun berbentuk sel tunggal/uniseluler. Dari kelompok *Ascomycetes* dan *Basidiomycetes*
- Tersebar luas di alam. Ada yang bermanfaat adapula yg merugikan bagi manusia.
- Manfaat: untuk pembuatan roti, bir, wine, cider, sake, brem, tape, bioethanol, kecap, fermentasi teh, pakan, dsb.
- Produksi enzim? Produksi minyak? Produksi flavor?

# Wine Fermentation

- Banyak penelitian menunjukkan bahwa dalam fermentasi wine alami terjadi suksesi *Hanseniaspora* ke *saccharomyces*.
- Beberapa genus khamir yang juga ditemukan antar lain *Metschnikowia, Candida, Torulaspora, Lachancea / KluyVaromyces dan Zygosaccharomyces.*
- *Oleh karena itu, fermentasi anggur adalah proses mikroba yang kompleks, di mana kondisi fisikokimia dan interaksi mikroba memengaruhi pertumbuhan dan metabolisme mikroorganisme yang terlibat.*

# Wine fermentation

- Inokulasi dengan kultur terseleksi mis *Saccharomces cerevisiae ditujukan untuk memperbaiki proses fermentasi* dan diharapkan dapat menjamin kualitas produk
- Pemberian inoculum dalam jumlah yang cukup dianggap dapat menghambat khamir yang tidak diharapkan. Namun demikian, seringkali khamir non-*Saccharmyces* berkontribusi terhdap pembentukan flavor.
- Dikembangkan fermentasi multi starter *Sacc – non Sacc*

# Beberapa aspek yg mendukung multi starter fermentation

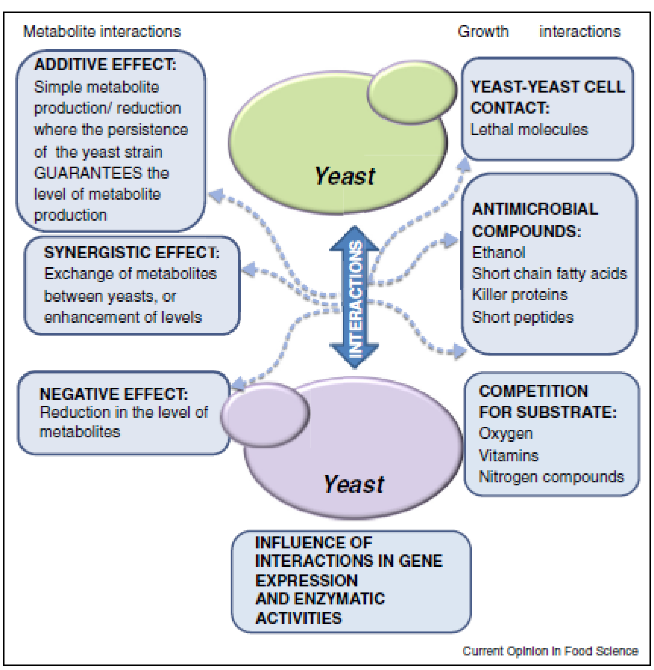
(i) Modifikasi beberapa senyawa analitik tertentu, seperti peningkatan kandungan gliserol, peningkatan keasaman total, atau mengurangi kandungan asam asetat wine;

(ii) peningkatan profil analitik wine (ester, thyol volatile);

(iii) pengurangan kandungan etanol wine;

(iv) Kontrol mikroflora pembusuk dalam wine; dan

(v) peningkatan kualitas dan kompleksitas keseluruhan wine.




## Wine fermentation

Tipe interaksi khamir dalam fermentasi campur

# Interaksi pertumbuhan Khamir - khamir

**Table 1**

**Killer toxins and other antimicrobial compounds involved in multi-starter fermentation.**

| Killer yeast | Killer toxin | Sensitive strain | Application in mixed fermentation | Reference |
|---|---|---|---|---|
| *Saccharomyces cerevisiae* strain "Prise de mousse" | K2 type | *Saccharomyces cerevisiae* | Control of fermentation (*S. cerevisiae* wild yeast) | [25] |
| *Saccharomyces cerevisiae* CCMI 885 | AMPs[a] | *Hanseniaspora guilliermondii*<br>*Torulaspora delbrueckii*<br>*Kluyveromyces marxianus*<br>*Dekkera bruxellensis* | Biocontrol | [24*] |
| *Tetrapisispora phaffii* | Kpkt | *Hanseniaspora uvarum* | Control during pre-fermentation stage | [26] |
| *Wickerhamomyces anomalus* | Pikt | *Brettanomyces/Dekkera* | Biocontrol of *Brettanomyces/Dekkera* | [41] |
| *Kluyveromyces wickerhamii* | Kwkt | *Brettanomyces/Dekkera* | Biocontrol of *Brettanomyces/Dekkera* | [41] |
| *Pichia membranifaciens* | PMKT2 | *Brettanomyces bruxellensis* | Biocontrol of *Brettanomyces/Dekkera* | [27] |
| *Ustilago maydis* | KP6 | *Brettanomyces bruxellensis* | Biocontrol of *Brettanomyces/Dekkera* | [42] |

[a] Antimicrobial peptides.

# Interaksi pertumbuhan Khamir - khamir

**Table 2**

**Recent yeast interactions described in mixed wine fermentation.**

| Yeast species | Interactions | References |
|---|---|---|
| *S.cerevisiae/S. bombicola* | Modification of ADH1 and PDC1 gene expression in *S. cerevisiae* | [36*] |
| *S.cerevisiae C. zemplinina* | Decrease in terpene and lactone content | [15*] |
| *S.cerevisiae/C. zemplinina* | Reduction in acetic acid in must with high sugar content | [37] |
| *S.cerevisiae/C. zemplinina* | Increase in glycerol content with yeast hulls | [38] |
| *S.cerevisiae/T. delbrueckii* | Reduction in acetic acid<br>Increase in 2 phenyl ethanol<br>Increase in isoamyl acetate | [7,23] |
| *S. cerevisiae/L. thermotolerans* | Increase in total acidity<br>Increase in glycerol content<br>Increase in 2-phenyl ethanol and esters | [12] |
| *S. cerevisiae/M. pulcherrima* | Synergistic effects on aromatic profiles of mixed fermentation | [7,15*] |
| *S. cerevisiae/H. vinae* | Increase in 2-phenylethyl acetate | [31] |
| *S. cerevisiae/Z. florentinus* | Increase in 2-phenylethyl acetate, 2-phenyl ethanol and polysaccharides | [11] |

## Fermentasi Bioetanol

- Fermentasi alcohol menjadi hal yang menarik untuk mengubah limbah menjadi bioenergy.
- Banyak limbah yang masih mengandung gula yang dapat dimanfaatkan menjadi bioethanol
- Konversi laktosa dari whey susu memunkinkan dikembangkan menjadi bioethanol seperti halnya perkembangan bioethanol generasi kedua yang menggunakan lignoselulosa sebagai bahan baku.

## Fermentasi Bioetanol

- Fermentasi langsung dari whey ke etanol tidak layak secara ekonomi karena kadar laktosa rendah sehingga menghasilkan titer etanol rendah (2-3% v / v), membuat proses distilasi terlalu mahal.
- Bagaimana cara meningkatkan kadar gulanya?
- Selama 30 tahun terakhir, banyak penulis telah membahas produksi etanol dari laktosa, sebagian besar merujuk pada *Kluyveromyces fragilis, K. marxianus dan C. mseudotropicalis*

# Beberapa Pustaka fermentasi bioethanol dari laktosa

- Mathematical modelling of ethanol production as a function of temperature during lactic-alcoholic fermentation of goat's milk after hydrolysis and transgalactosylation of lactose https://doi.org/10.1016/j.measurement.2018.11.070
- Production of ethanol from lactose in a bioreactor integrated with membrane distillation (https://doi.org/10.1016/j.desal.2013.01.026)
- Ethanol production from lactose in a fermentation/pervaporation system https://doi.org/10.1016/j.jfoodeng.2006.01.071

# Fermentasi Teh

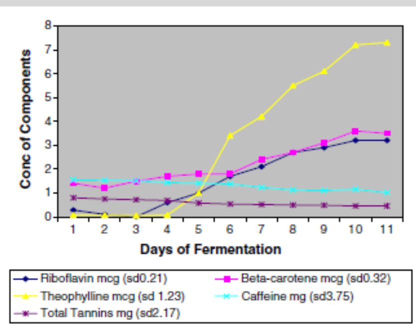



Fig. 1. Yeast fermentation of black tea concentrations are per ml. Series: (1) riboflavin; (2) β-carotene; (3) theophylline; (4) caffeine; (5) total tannins.

- Teh 2 g + sukrosa 2 g diberi air mendidih dan dibiarkan selama 15 menit dalam keadaan tertutup.
- Air disaring dan disterilkan kemudian ditambah khamir *Debaryomyces hansenii* MTCC 1073 dan difermentasi selama 10 hari pada suhu 25 C dan 150 rpm

## Theophylline

- Theophylline adalah bronkodilator Xanthine.
- Dosis pengobatannya, sebagai bronkodilator, adalah 0,18-1,0 g setiap hari.
- Secangkir teh hitam mengandung 0,014 mg theophylline dan asupan hariannya adalah 0,042 mg,
- Teh fermentasi mengandung 1,44 mg theophilin per cangkir dan asupan hariannya akan menjadi 4,32 mg, yang sangat membantu, karena meningkatkan neurostimulasi dan dapat menjadi bagian dari dosis terapeutik untuk pasien pada perawatan teofilin
- Bronkodilator adalah kelompok obat yang digunakan untuk meredakan gejala akibat penyempitan saluran pernapasan, seperti batuk, atau sesak napas. Asma dan penyakit paru obstruksi kronis (PPOK) adalah dua kondisi yang sering diobati dengan bronkodilator

## Khamir probiotik / Probiotic Yeast (PY)

- PYs mampu mentolerir pH rendah dan garam empedu, dan mereka menunjukkan aktivitas antimikroba terhadap patogen dan agen pembusuk
- Khamir resisten terhadap agen antibakteri, dan kurangnya elemen genetik yang dapat ditransfer antara bakteri dan khamir menyebabkan PY dipilih untuk digunakan selama perawatan antibiotik.

## Potential applications of probiotic yeasts in the food industries and critical aspects for these applications

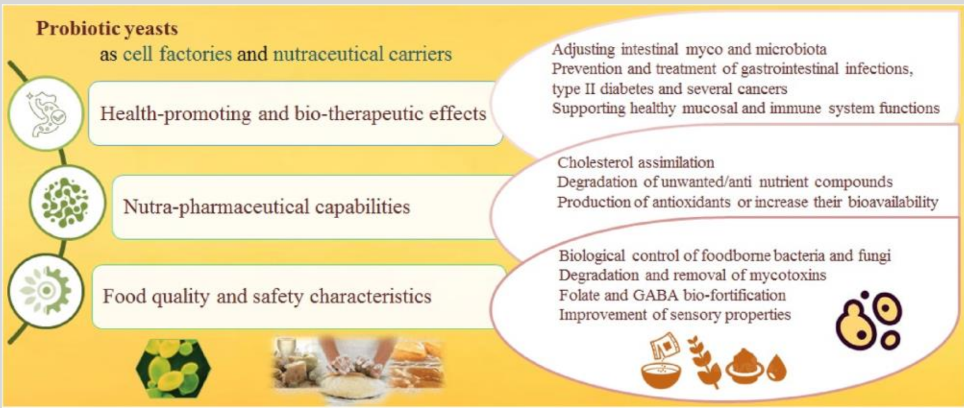



**Table 1**
Techno-functional capabilities of probiotic yeasts in foods.

| Probiotic yeast | Isolation source | Function or the produced food |
|---|---|---|
| Functional foods with improved sensory attributes | | |
| *S. cerevisiae* CCMA 0731, *S. cerevisiae* CCMA 0732 | Cocoa fermentation | Production of maize-based beverages as co-culture with *L. paracasei* LBC-81 with high volatile compounds |
| *Candida zeylanoides, Y. lipolytica, K. lactis* | Artisan dairies | Co-starters with LAB to develop functional cheeses with a distinctive aroma |
| *S. boulardii* | Commercial food supplements | Production of probiotic ice cream with improved rheological parameters and altered aroma profile |
| *S. boulardii* CNCM I-745 | Culture collection | Production of fermented green tea with elevated amounts of volatile compounds |

| | | |
|---|---|---|
| **Fortified foods with postbiotics and bioactive metabolites** | | |
| *D. hansenii, Torulaspora delbrueckii* | Greek-style black olives | Assimilation of d-galacturonic acid, lipolytic activity and hydrolyzing oleuropein |
| Probiotic *S. boulardii* combined with probiotic LAB | Dietary supplement sachet | Fermented soymilk with reduced glycosides and increased aglycones |
| *S. boulardii* | Commercial probiotic | Breakdown of dietary phytate and bio-fortification of folate |
| *Pichia membranaefaciens, Candida oleophila* | Portuguese brined olives | Lipolytic and high osmotolerance activities, ability to uptake oleuropein, absence of pectinolytic activity, produce B-complex vitamins* |
| *S. cerevisiae, C. tropicalis, Aureobasidium* sp., *Pichia manschuria* | Indian fermented foods (idli and jalebi batter) | Production of phytase, β-galactosidase, L-asparaginase, protease, lipase, vitamin $B_{12}$ and EPS, degradation of anti-nutrients |
| *P. kluyveri* | Peach and nectarine fruit | Production of prebiotic galactooligosaccharides |
| *P. kluyveri* LKC17, *Issatchenkia orientalis* OSL11, *P. kudriavzevii* OG32, *P. kudriavzevii* ROM11 | Cereal-based Nigerian traditional fermented foods | Protease, lipase and phytase activities, cholesterol removal and free radicals scavenging* |
| *S. cerevisiae* IFST 062013 | Fruit | Cholesterol assimilation, production of vitamin $B_{12}$ and glutathione in treated mice* |

| | | |
|---|---|---|
| **Other probiotic products containing probiotic yeasts** | | |
| *S. boulardii* MAY 796 | Culture collection | Controlled fermentation of sweet potato with enhanced amino acid, acid detergent fiber, crude fiber, neutral detergent fiber, protein and fatty acid levels |
| *Meyerozyma caribbica* 9D | Peel and spontaneously fermented pineapple pulp | Production of probiotic pineapple juice with greater sensorial acceptance, with different antioxidant and nutritional properties |
| *S. cerevisiae* ARDMC1 | Traditional rice/beer starter cake | Hypo cholesterolemic effects on Wistar rats fed a high-cholesterol diet |
| *S. boulardii* | Culture collection | Production of probiotic cake containing encapsulated PYs that survived during 90 storage days (with proper textural properties and sensorial attributes compared to the control) |
| *S. boulardii* | Commercial food supplement | Production of alcohol-free beers |
| *S. boulardii* | Commercial probiotic preparation | Antiradical activity and good survival in lentil and adzuki bean sprouts (as effective carriers) after *in vitro* digestion |
| *S. boulardii* | Culture collection | Production of a synbiotic yogurt by incorporation of inulin with improved textural and sensorial properties, as well as higher volatile compounds* |

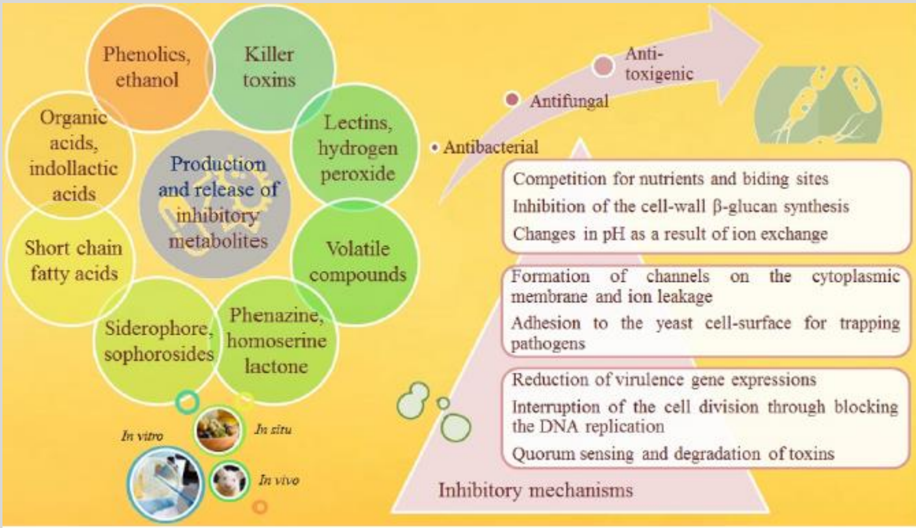



**Fig. 4.** Antimicrobial modes of action and inhibitory metabolites of probiotic yeasts.

**Table 3**
Inhibitory activity of probiotic yeasts against spoilage/pathogenic microorganisms or their toxins.

| Probiotic yeast | Isolation source | Studied pathogen | Inhibitory metabolite or mechanism |
|---|---|---|---|
| **Antibacterial effect** | | | |
| *S. cerevisiae* IFST062013 | Fruits | *B. subtilis, S. aureus, B. cereus, Bacillus polymyxa, Bacillus megaterium, Enterococcus faecalis, Salmonella typhi, Salmonella flexneri, K. pneumonia, Proteus vulgaris, E. coli, Vibrio cholera* and *P. aeruginosa* | Killer toxin, siderophore and strong biofilm |
| *S. boulardii* | Engineered yeast | *C. difficile* infection in mouse models | A single tetra-specific antibody that neutralized toxins and protection against infection agent |
| *G. candidum* LG-8 | Kefir | *P. aeruginosa* PAO1 | Adhesion of PY to the bacterium through its syrup-like EPS |
| *Rhodotorula mucilaginosa* UFMGCB 18377 | Different habitats of Antarctica | *Salmonella enterica Typhimurium* | Co-aggregation |
| *S. boulardii* CNCM I-745 | Culture collection | *C. difficile* | Reduction of biofilm thickness and its weakening in the bacterium |
| **Antifungal effect** | | | |
| *S. cerevisiae* | Dairy wastewater | *C. albicans* | Inhibitory effect of biosurfactant against biofilm producers |
| *S. cerevisiae, Saccharomyces bayanus* and *Wickerhamomyces subpelliculosa* | Foods and beverages | Antifungal activity against strains from the same species | Killer peptides |
| *Candida norvegica* 7A and *Galactomyces* reessii 34A | Natural fermentation of table olives | *Cryptococcus neoformans* | Not determined |
| Anti-toxigenic activity | | | |
| *S. cerevisiae* RC012, RC016, RC009 and RC012 strains | Culture collection | Ochratoxin A and zearalenone | Ability to reduce the levels of ochratoxin A (RC012 and RC016 strains) and zearalenone (RC009 and RC012 strains) |
| A mixture of PYs cocktail *S. cerevisiae* strains | Culture collection | Aflatoxin | Reducing aflatoxin level in rat sera and diminished the deleterious effect |
| *K. marxianus* VM003 | Culture collection | Aflatoxin $M_1$ | Adsorb and degrade aflatoxin $M_1$ to less toxic metabolites in milk |
| *S. cerevisiae* A8L2 | Culture collection | Aflatoxin $B_1$ | Adsorbent of aflatoxin $B_1$ in simulated fish intestinal tract conditions |