
بسم الله الرحمن الرحیم

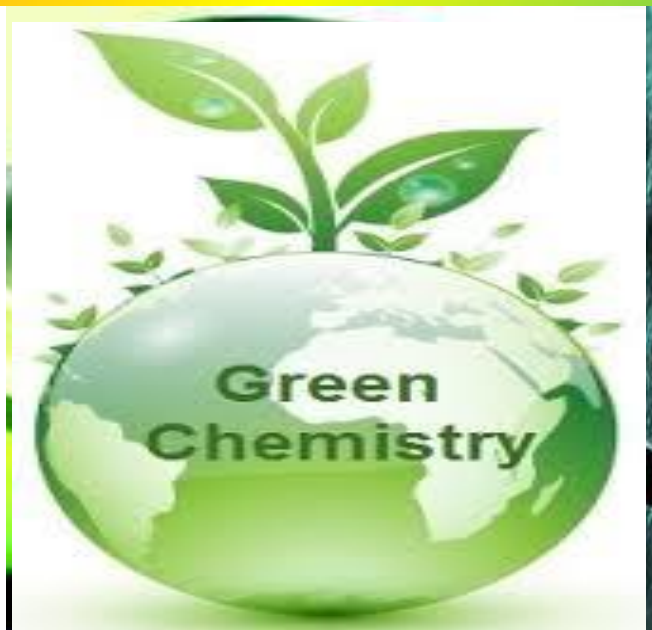




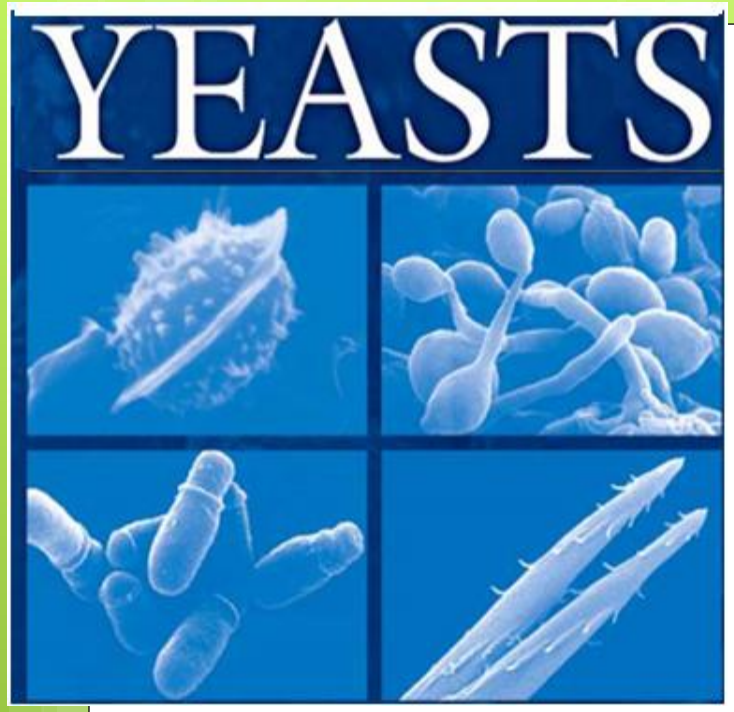
شیمی
سبز

Yeasts: Green Catalysts in Biotransformation Processes









The English word yeast meaning “foam”, direct references to the fermentation processes

Yeast are single-celled microorganisms that are classified, along with molds and mushrooms, as members of the Kingdom Fungi.

Yeasts can be defined as those fungi whose asexual growth predominantly results from budding or fission

basidiomycetous ascomycetes

Ascomycetes (e.g. *Saccharomyces*, *Candida*) or Basidiomycetes (e.g. *Filobasidiella*, *Rhodotorula*).

Yeast Habitats

- Atmospheric Yeasts
- Aquatic/Marine Yeasts
- Terrestrial Yeasts

Isolation and maintenance of yeasts

Enrichment: Temperatures (20-25), Acidified medium (pH 3.5-5), Osmotic media (high concentration of glucose: 2-50% glucose), Use of antibiotics and other selective compounds (Antibiotics: tetracycline at 50 ppm, combination of penicillin G and streptomycin at 150-500 ppm, chloramphenicol at 100-300 ppm) or Antifungals include cycloheximide (100-500 ppm), cyclosporine A (4-10 ppm), pimaricin (5-100 ppm), Rose Bengal (25 ppm), Dichloran (2 ppm).

Dichoran (2, 6 dichloro-4-nitroanilline) Rose Bengal Chloramphenicol Agar (glucose, peptone, nutrients, …)

Isolation: YPD/YTD (glucose-yeast extract/peptone or tryptone Agar)

Maintenance of yeast cultures: Distilled water, Glycerol stock, Lyophilization, Liquid Nitrogen Preservation)

In 1972, cells of *Rhodotorula rubra* were transported to outer space on the Apollo 17 mission. The cells were exposed to the vacuum of space and subsequently stored in sterile water for return to earth. After 27 years in aqueous suspension the cells were shown to be viable (Volz and Parent 1998)

Total number of known-fungal recognized: 150000 (2004)
Approximately 200 fungal pathogens (*candida*, *Cryptococcus* spp.) were recognized

approximately , 149 genera and 1,500 recognized yeast species listed in the latest edition of The Yeasts: a Taxanomic Study (2011).

Of all these yeast species, only about a dozen is used at industrial scale, and some 70 – 80 species have been shown at laboratory/pilot scale to possess potential value in biotechnology (Kurtzman et.al., 2011; Deak, 2009).

Fungi species has been estimated to be as high as 1,500,000 compared to 270,000 and 1,000,000 estimated numbers of species of plants and bacteria, respectively (Boekhout and Samson 2005). Thus, fungi are among the richest kingdoms on earth with respect to biodi- versity, but much work is needed to understand their potential to provide valuable industrial resources.

5000000

Composition of the Cell Wall

Macromolecule	% of Wall Mass[a]
Mannoproteins	30–50
β-1,6-Glucan	5–10
β-1,3-Glucan	30–45
Chitin	1.5–6

Molecular chracterization: ITS1-5.8S-ITS2

Morphology, Pigmentation

	Fermentation	Growth reactions and other characteristics
	Glucose	Glucose
	Galactose	Inulin
	Sucrose	Sucrose
	Maltose	Raffinose
	Lactose	Melibiose
	Raffinose	Galactose
	Trehalose	Lactose
		Trehalose
		Maltose
		Melezitose
		Methyl-α-D-glucoside
		Soluble Starch
		Cellobiose
		Salicin
		L-Sorbose
		L-Rhamnose
		D-Xylose
		L-Arabinose
		D-Arabinose
		D-Ribose
		Methanol
		Ethanol
		Glycerol
		Erythritol
		Ribitol
		Galactitol
		D-Mannitol
		D-Glucitol

Growth reactions and other characteristics
myo-Inositol
DL-Lactate
Succinate
Citrate
D-Gluconate
D-Glucosamine
N-Acetyl-D-glucosamine
Hexadecane
Nitrate
Nitrite
Vitamin-free
2-Keto-D-gluconate
5-Keto-D-gluconate
Saccharate
Xylitol
L-Arabinitol
Arbutin
Propane 1,2 diol
Butane 2,3 diol
Cadaverine
Creatinine
L-Lysine
Ethylamine
50% Glucose
10% NaCl/5% glucose
Starch formation
Urease
Gelatin liquefaction
Cycloheximide 0.01%
Cycloheximide 0.1%
Growth at 19°C
Growth at 25°C
Growth at 30°C
Growth at 35°C
Growth at 37°C
Growth at 40°C
Growth at 45°C
CoQ (Main component)
Mol% G + C (Ave.)

Yeasts: GRAS and Probiotic/Prebiotics
Since 1950 *S. boulardii*: treatment of intestinal diseases

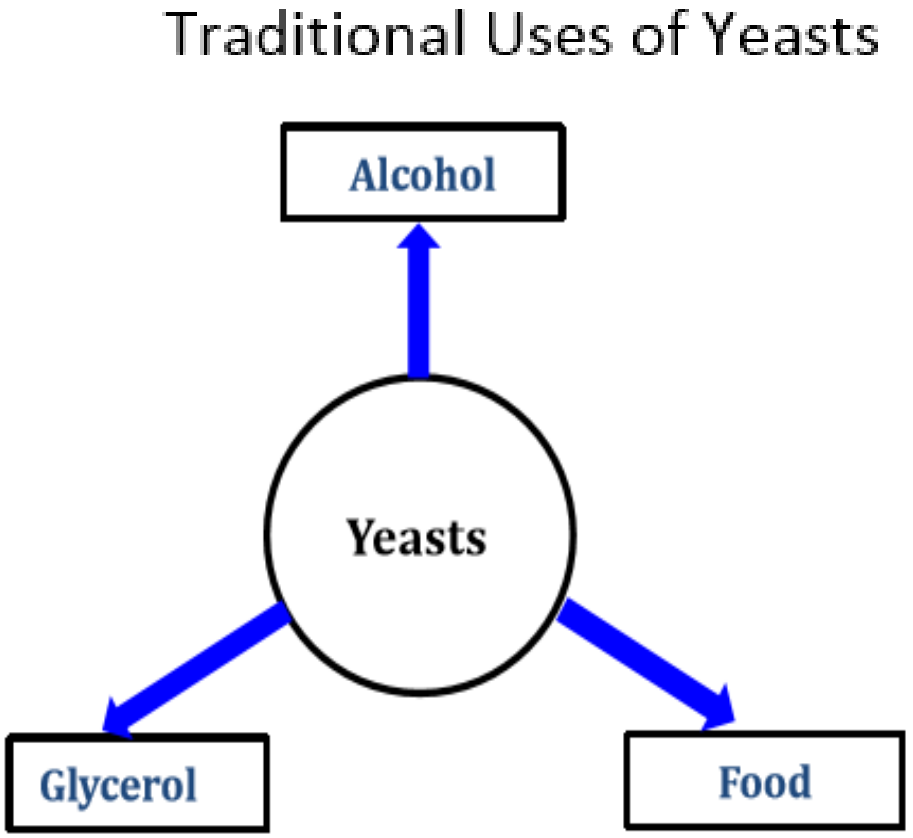
Saccharomyces spp.

Yarowia spp.

Kluveromyces spp.

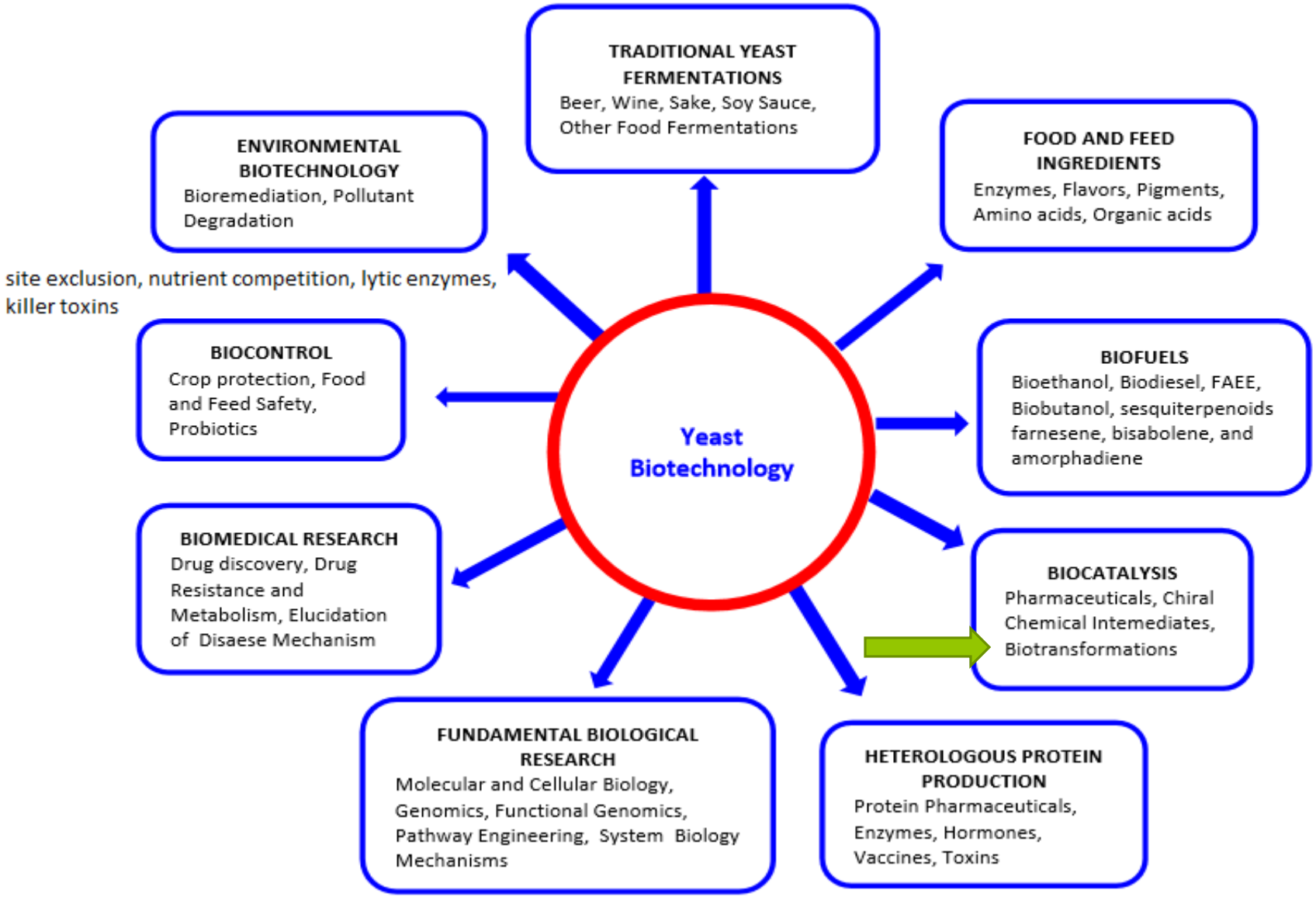
Candida spp.

Marine yeasts



Biotechnologically Important Yeast sp. **(Johnson, 2013a,b)**

Ascomycetous	**Basidiomycetous**
Saccharomyces cerevisiae	*Rhodotorula* spp.
Schizosaccharomyces pombe	*Rhodosporidium* spp.
Kluyveromyces lactis	*Trichosporon* spp.
Kluyveromyces marxianus	*Xanthophyllomyces dendrorhous*
Schwanniomyces occidentalis	*Cryptococcus* spp.
Lipomyces spp.	*Phaffia rhodozyma*
Saccharomycopsis spp.	
Debaryomyces hansenii	
Ogataea polymorpha	
Komagataella pastoris	
Scheffersomyces stipitis	
Pichia spp.	
Yarrowia lipolytica	
Candida spp.	
Blastobotrys adeninivorans	


TRADITIONAL YEAST FERMENTATIONS
Beer, Wine, Sake, Soy Sauce, Other Food Fermentations
FOOD AND FEED INGREDIENTS
Enzymes, Flavors, Pigments, Amino acids, Organic acids
ENVIRONMENTAL BIOTECHNOLOGY
Bioremediation, Pollutant Degradation
site exclusion, nutrient competition, lytic enzymes, killer toxins
BIOCONTROL
Crop protection, Food and Feed Safety, Probiotics
Yeast Biotechnology
BIOFUELS
Bioethanol, Biodiesel, FAEE, Biobutanol, sesquiterpenoids farnesene, bisabolene, and amorphadiene
BIOMEDICAL RESEARCH
Drug discovery, Drug Resistance and Metabolism, Elucidation of Disaese Mechanism
BIOCATALYSIS
Pharmaceuticals, Chiral Chemical Intemediates, Biotransformations
FUNDAMENTAL BIOLOGICAL RESEARCH
Molecular and Cellular Biology, Genomics, Functional Genomics, Pathway Engineering, System Biology Mechanisms
HETEROLOGOUS PROTEIN PRODUCTION
Protein Pharmaceuticals, Enzymes, Hormones, Vaccines, Toxins

Industrial Enzymes from Yeasts **(Johnson & Echavarri-Erasun, 2011)**

Enzyme	Yeast	Industry
Chymosin	*Kluyveromyces spp.* *Saccharomyces cerevisiae*	Food processing
α-Galactosidase	*Saccharomyces spp*	Feed applications
L-Glutaminase	*Zygosaccharomyces rouxii*	Therapeutic Analytical
Inulinases	*Candida spp.* *Kluyveromyces marxianus*	Food applications
Invertase	*Saccharomyces cerevisiae*	Food applications
Lactase	*Candida pseudotropicalis* *Kluyveromyces spp.*	Food processing
Lipase	*Candida rugosa* *Pseudozyma antarctica A, B* *Geotrichum candidum* *Trichosporon fermentum* *Yarrowia lipolytica*	Food processing Flavors, Wastewater Degreasing, Bioremediation Therapeutic, Detergent
L-Phenylalanine ammonialyase	*Rhodotorula spp.* *Rhodosporidium spp.*	Pharmaceutical
Phenylalanine dehydrogenase	*Candida boidinii*	Pharmaceutical
Phytase	*Ogataea polymorpha*	Feed Nutrition

Examples of Industrial Recombinant Enzymes Produced in Yeasts

Enzyme	Recombinant Yeast Host
Chymosin	*Kluyveromyces lactis*
Glycolate oxidase	*Komagataella pastoris*
Phytase	*Komagataella pastoris*

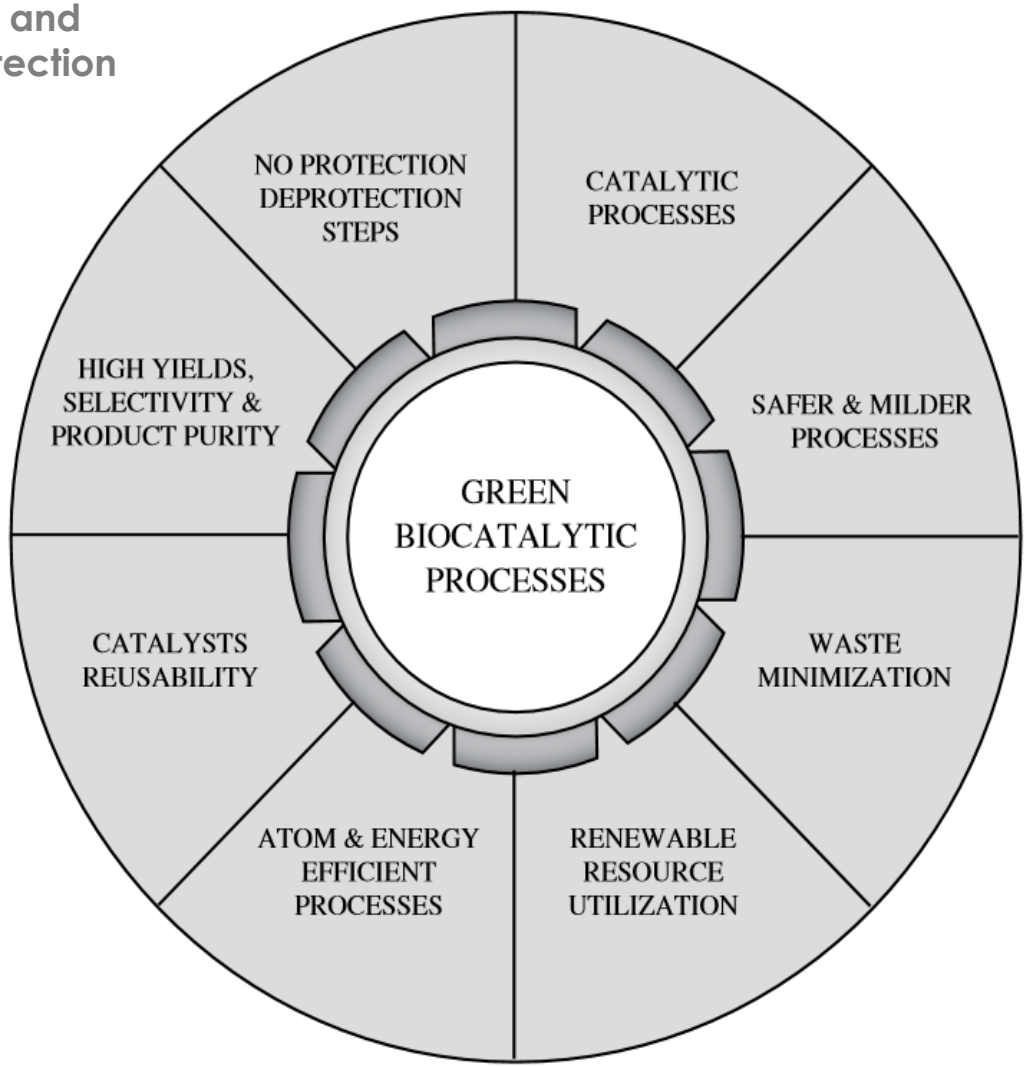
Comodity Chemicals Produced by Yeasts **(Amaral, 2008; Branduardi and Porro, 2012; Sauer et.al., 2008)**

Products	**Yeast**
Amino acids including lysine, methionine, phenylalanine, and proline	*Saccharomyces cerevisiae* and *Rh. glutinis*
Erythritol	*C. magnoliae, Moniella spp.* *C. peltata, and a Candida* sp.
Mannitol	Various yeast sp.
Glycerol	*C. glycerinogenes* *S. cerevisiae*
Astaxanthin	*Xanthophyllomyces dendrorhous, Phaffia rhodozyma*
α-ketoglutaric, pyruvic, citric and isocitric acids,	Yarrowia lipolytica
Gluconic acid	*Aureobasidum pullulans*
2-phenylethanol	*Pichia fermentans*
Pyruvic acid	*Candida glabrata*
Riboflavin	*Pichia guilliermondii*
Glycolipids and surfactants, Sophorolipids	Basidiomycetous yeasts, *Pseudozyma, Candida, Kurtzmanomyces*

Green chemistry: Healthy chemical reactions with safe products and maximum efficiency,

minimum consumption of matter and energy,

Green chemistry "clean chemistry".
introduced in the mid-1990s by Anastas and colleagues of the US Environmental Protection Agency (EPA).

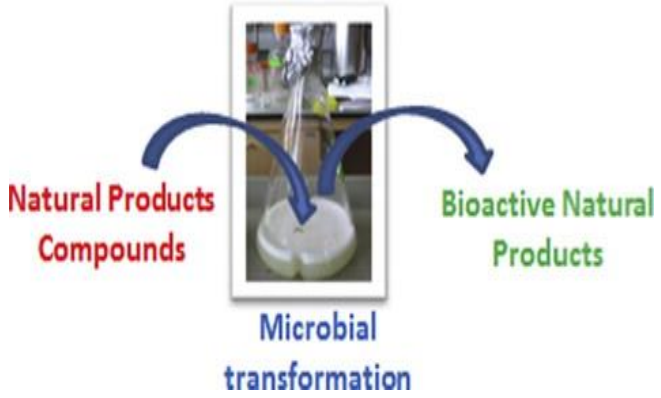


Microbial Biotransformation/Bioconversions ?

Fermentation?

Applications?

Advantages/disadvantages?

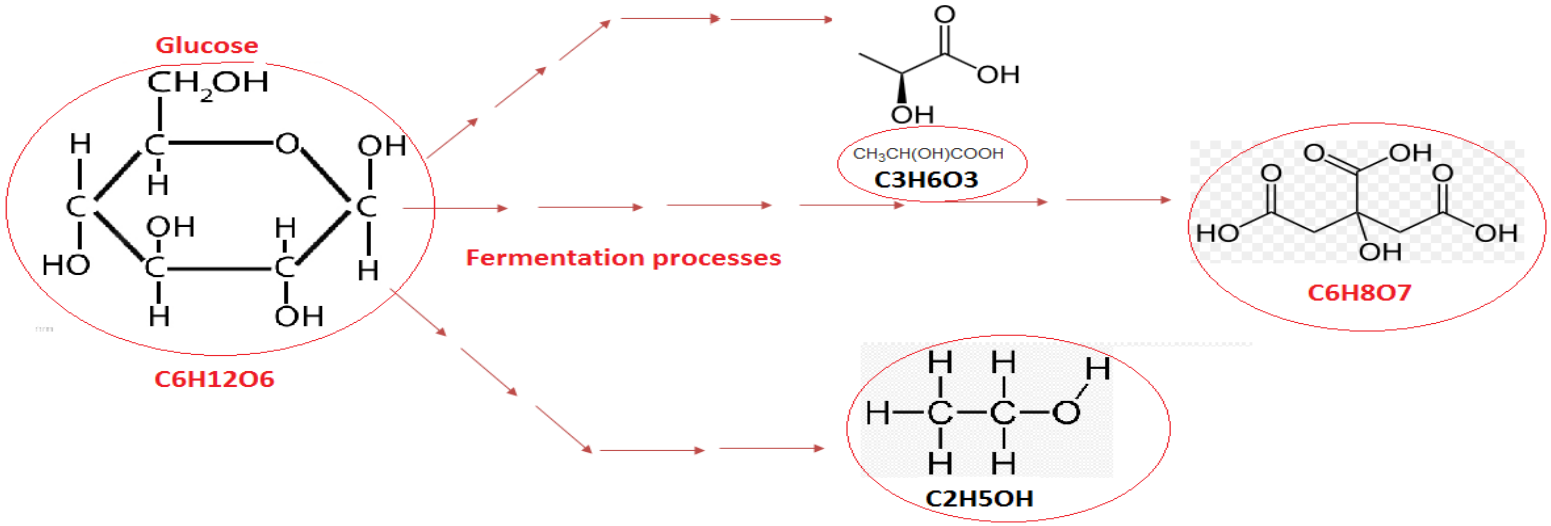


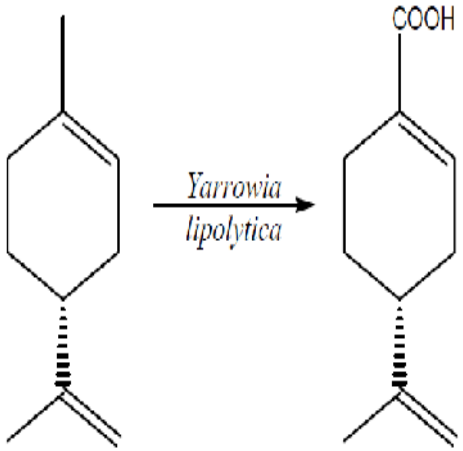
Biotransformation?

- **biotransformation** :Use of biocatalysts for synthesis of an organic chemical or destruction of an unwanted chemical

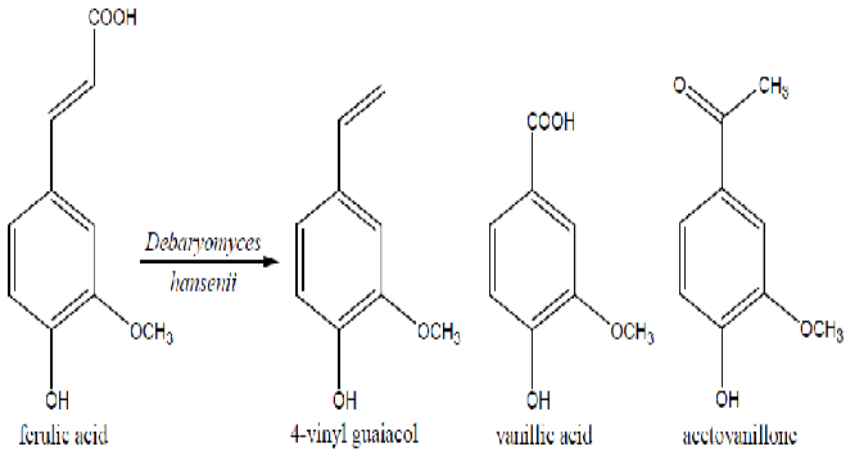
- **Types of biocatalysts:**
  1) Prokaryotic/Eukaryotic cells (WC)
  2) Enzymes (Oxidoreductases, Transferases, Hydrolases, Lyases, Isomerases, Ligases)

- Difference between fermentation and biotransformation?
- Application of biotransformation?
- Industrial biotransformation catalyzed by WC/Enzymes?





Biotransformation of (+)-limonene to perillic acid promoted by *Y. lipolytica*.



Biotransformation of ferulic acid into higher value added products by *Debaryomyces hansenii*.

biotransformation

advantages

1) Tag natural
2) Operation under mild conditions
3) High substrate selectivity
4) Green chemistry

drawbacks

1)Low water solubility of the precursors

2)Toxicity of precursors and products

3)Metabolic diversity



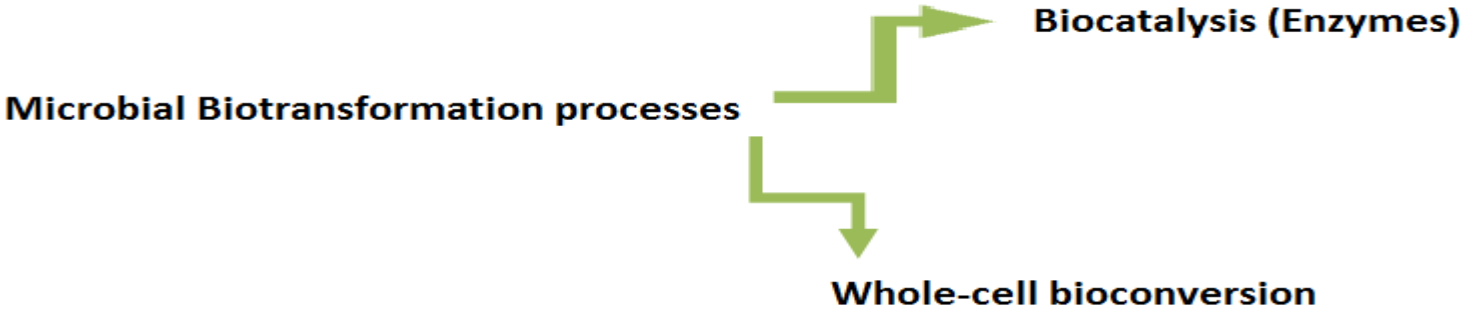
Why microbial Biotransformation?

Advantages of microbial biotransformation:

1) Simplicity of screening procedures
2) High ratio of surface area to volume
3) Metabolic diversity
4) The facility to adapt
5) Ease of genetic manipulation
6) Relatively high yield

Disadvantages of plant biotransformation:

1) Very low yield
2) Complicate of genetic manipulation
3) Metabolic pathway?
4) Relatively slow growth



Disadvantages: Chemical synthesis: using non-biological catalysts

1. relatively low catalytic efficiency for many reactions
2. lack of enantiomeric specificity for chiral synthesis
3. need for reaction conditions of high temperature, low pH and high presure
4. extensive use of organic solvents with consequent formation of organic waste and pollutants

Advantages of **microbial biotransformation** processes for chemical process:

1. mild reaction conditions: operation at temperatures from 20 to 80, neutal or slightly acidic pHs, and atomospheric conditions

2. green chemistry e.g. solvent often water

3. very high enantioselectivity/ regioselectivity



Advantages of Whole-cell biocatalysts

►More convenient and stable sources than purified enzymes, , with no **need for costly purification and coenzyme addition**.

►Th**e number of enzymes commercially available on the market (free or immobilized) is still quite limited**, particularly for some types of uncommon substrates, namely some compounds of pharmaceutical interest.

►When the enzymes are kept within their natural environment **(whole cell cytoplasm) lesser inactivation usually occurs**

Some representative industrial biotransformation catalyzed by whole cells

Product	Biocatalyst	Operating since	Company
vinegar	bacteria	1823	various
L-2-methylamino-1-phenylpropan-1-ol	yeast	1930	Knoll AG, Germany
L-sorbose	*Acetobacter suboxydans*	1934	various
prednisolone	*Arthrobacter simplex*	1955	Schering AG, Germany
L-aspartic acid	*Escherichia coli*	1958	Tanabe Seiyaku Co., Japan
7-ADCA	*Bacillus megaterium*	1970	Asahi Chemical Industry, Japan
L-malic acid	*Brevibacterium ammoniagenes*	1974	Tanabe Seiyaku Co., Japan
D-*p*-hydroxyphenylglycine	*Pseudomonas striata*	1983	Kanegafuchi, Chemical Co., Japan
acrylamide	*Rhodococcus sp.*	1985	Nitto Chemical Ltd, Japan
D-aspartic acid and L-alanine	*Pseudomonas dacunhae*	1988	Tanabe Seiyaku Co., Japan
L-carnitine	*Agrobacterium sp.*	1993	Lonza, Czech.Rep.
2-keto-L-gulonic acid	*Acetobacter sp.*	1999	BASF, Merck, Cerestar, Germany

Some representative industrial biotransformation catalyzed by Enzymes

Product	Biocatalyst	Operating since	Company
L-amino acid	aminoacylase	1954, 1969	Tanabe Seiyaku Co. Ltd., Japan
6-aminopenicillanic acid	penicillin acylase	1973	SNAMProgetti and others*
low lactose milk	lactase	1977	Central del Latte, Milan, Italy (SNAMProgetti technology)
7-amino-cephalosporanic acid	D-amino acid oxidase	1979	Toyo Jozo and Asahi Chemical Industry, Japan

Process	Biocatalyst	Operating since	Company
fat interesterification	lipase	1979, 1983	Fuji Oil, Unilever
ester hydrolysis	lipase	1988	Sumitomo
transesterification	lipase	1990	Unilever
aspartame synthesis	thermolysin	1992	DSM
acylation	lipase	1996	BASF

Biocatalysis

Biotransformations on an Industrial Scale

t/a	product	enzyme
> 1 000 000	high-fructose corn syrup	glucose isomerase
> 100 000	lactose-free milk	lactase
> 10 000	acrylamide	nitrilase
	cocoa butter	lipase
> 1 000	nicotinamide	nitrilase
	D-pantothenic acid	aldonolactonase
	(*S*)-chloropropionic acid	lipase
	6-aminopenillanic acid	penicillin amidase
	7-aminocephalosporanic acid	glutaryl amidase
	aspartame	thermolysin
	L-aspartate	aspartase
	D-phenylglycine	hydantoinase
	D-*p*-OH-phenylglycine	hydantoinase
> 100	ampicillin	penicillin amidase
	L-methionine, L-valine	aminoacylase
	L-carnitine	dehydrase/ hydroxylase
	L-DOPA	β-tyrosinase
	L-malic acid	fumarase
	(*S*)-methoxyisopropyl-amine	lipase
	(*R*)-mandelic acid	nitrilase
	L-alanine	L-aspartate-β-de-carboxylase

Industrial biotransformations employing wild type yeast whole-cell biocatalysts (adapted from **Pscheidt & Glieder, 2008)**

Yeast strain	Enzyme name	Product	Company
Zygosaccharomyces rouxii	Alcohol NAD+ oxidoreductase	Benzodiazepine	Eli Lilly and Company, USA
Geotrichum candidum	Dehydrogenase, NADPH-dependent	chiral β-hydroxy ester	Bristol-Myers Squibb, USA
Candida sorbophila	Dehydrogenase	(R)-amino alcohol	Merck & Co, Inc., USA
Pichia methanolica	Reductase	Ethyl-5-(S)-hydroxyhexanoate and 5- (S) hydroxyhexanenitrile	
Candida boidinii or *Pichia pastoris*	E2: FDH (1.2.1.2) [E1: PheDH From *Thermooctinomyces intermedius*]	(S)-2-Amino-5-(1,3-dioxolan-2-yl)-pentanoic acid	Bristol-Myers Squibb, USA
Trigonopsis variabilis ATCC 10679	D-Amino acid oxidase	L-6-Hydroxynorleucine	Bristol-Myers Squibb, USA
Baker's yeast (= Saccharomyces cerevisiae)	Reductase	(*R*)-2,2,6-trimethylcyclohexane-1,4-dione	Hoffmann La-Roche, CH
Cryptococcus laurentii (E1) [and Achromobacter oboe (E2)]a	E1: Lactamase (3.5.2.11) and [E2: Racemase	L-Lysine	Toray Industries Inc., Japan
Saccharomyces cerevisiae	Pyruvate decarboxylase	PAC ➔ ephedrine and pseudoephedrine	Krebs Biochemicals & Industries Ltd., India
Candida rugosa	Enoyl-CoA hydratase	R)-β-Hydroxy-n-butyric acid and (R)-β-Hydroxy-isobutyric acid	Kanegafuchi Chemical Industries Co., Ltd., Japan
Rhodotorula rubra	L-Phenylalanine ammonia-lyase	L-phenylalanine	Genex Corporation, USA

Advantages of Yeasts as Green Biocatalysts in Biotransformation Reactions

1. Abundance in soils and aquatic habitants

2. Fast growth rate and high biomass production

3. Presence of thick cell wall that protects from physical damaging during fermentation/bioconversion processes

4. High level of compatibility with the surroundings

5. High Enymatic contents

6. Metabolic diversity

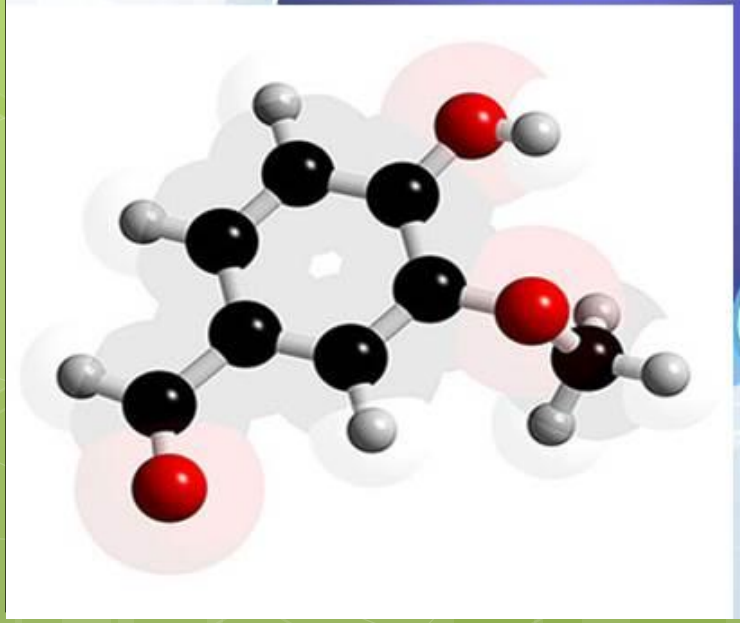
7. Simple downstream (Flocculation,..)

8. Certain species have GRAS= safe organisms for food and pharmaceutical industries

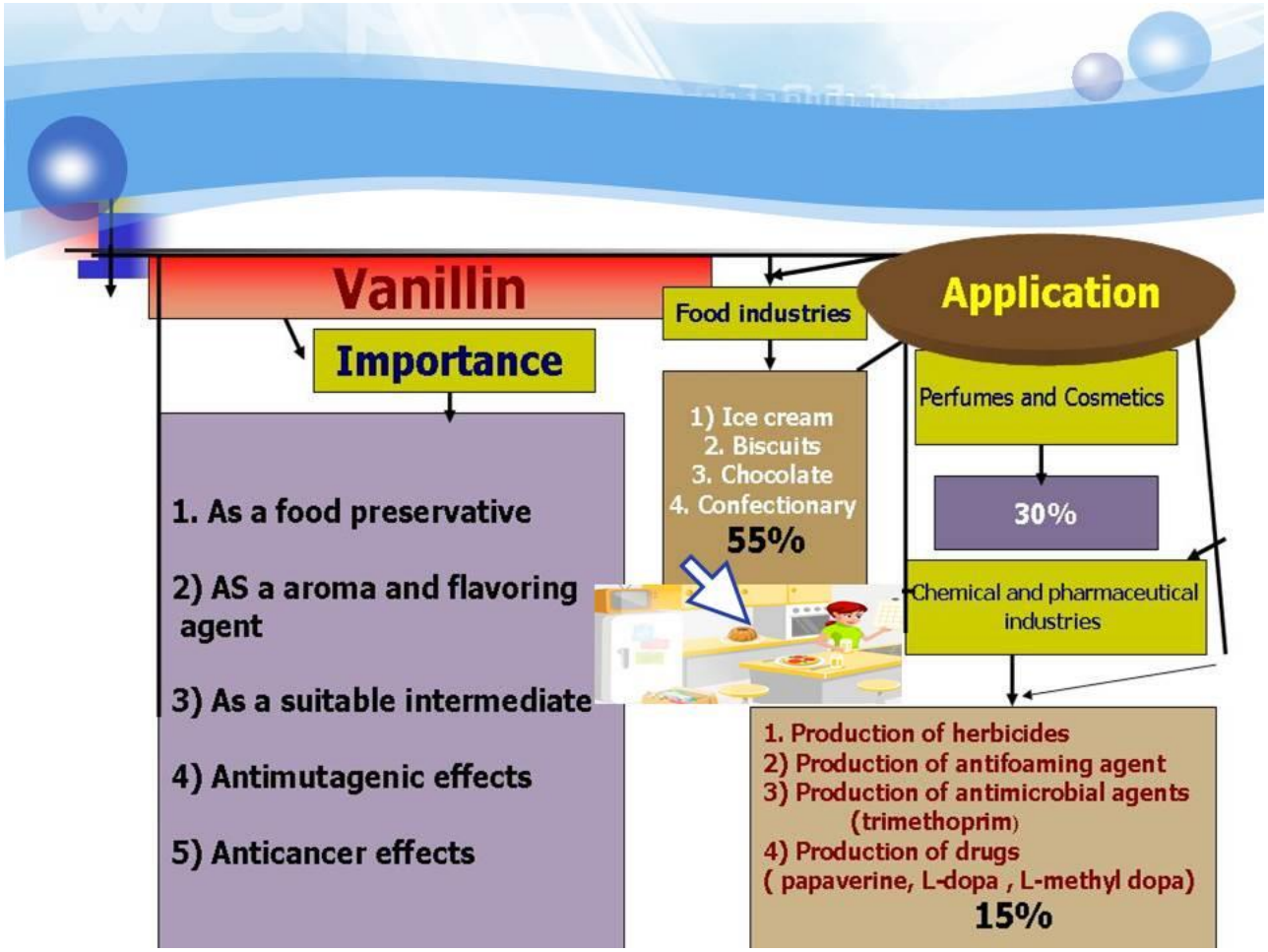
9. Sterospecificity

10. aqueous-organic system

Vanillin



- History
- Structure
- Importance
- Commercial types exist
- Worldwide annual consumption /demand


Vanillin
Importance
1. As a food preservative
2) AS a aroma and flavoring agent
3) As a suitable intermediate
4) Antimutagenic effects
5) Anticancer effects
Food industries
1) Ice cream
2. Biscuits
3. Chocolate
4. Confectionary
55%
Application
Perfumes and Cosmetics
30%
Chemical and pharmaceutical industries
1. Production of herbicides
2) Production of antifoaming agent
3) Production of antimicrobial agents (trimethoprim)
4) Production of drugs
(papaverine, L-dopa , L-methyl dopa)
15%

Synthesis of vanillin from vanilla

Vanillin is present in trace amounts (ppm) in potato parings and tobacco. But the main source for natural vanillin is the vanilla plant.
Vanilla plant: orchidaceaea (110 species) only three are important commercially (vanilla planifolia, vanilla pompona and vanilla techitensis)

Production of vanilla from vanilla beans:
1) curing(heat-treating , drying and maturation)
2) Alcoholic extraction





Disadvantages

1) Limitation growing areas
2) Laborious (tedious curing process)
3) Time consuming (culture complexity)
4) Low concentration (2% w/w)
5) Expensive (1200-4000 dollar/kg)
6) Contain more than 200 other flavor compounds
7) Artificial pollination
8) Climate fluctuations of harvest yields



Annual production of cured pods : 2200-2400 tons

alternatives sources

The curing process involves:
Killing
Sweating
Drying
Conditioning
(8-10 month)

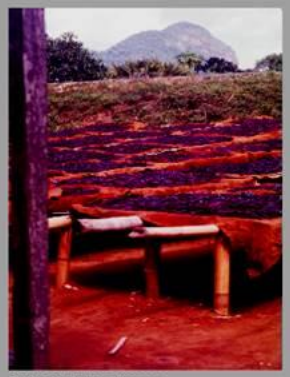


Killing the beans





Spreading the beans in the sun after sweating



Drying on trays in the sun



Drying on trays in the sun

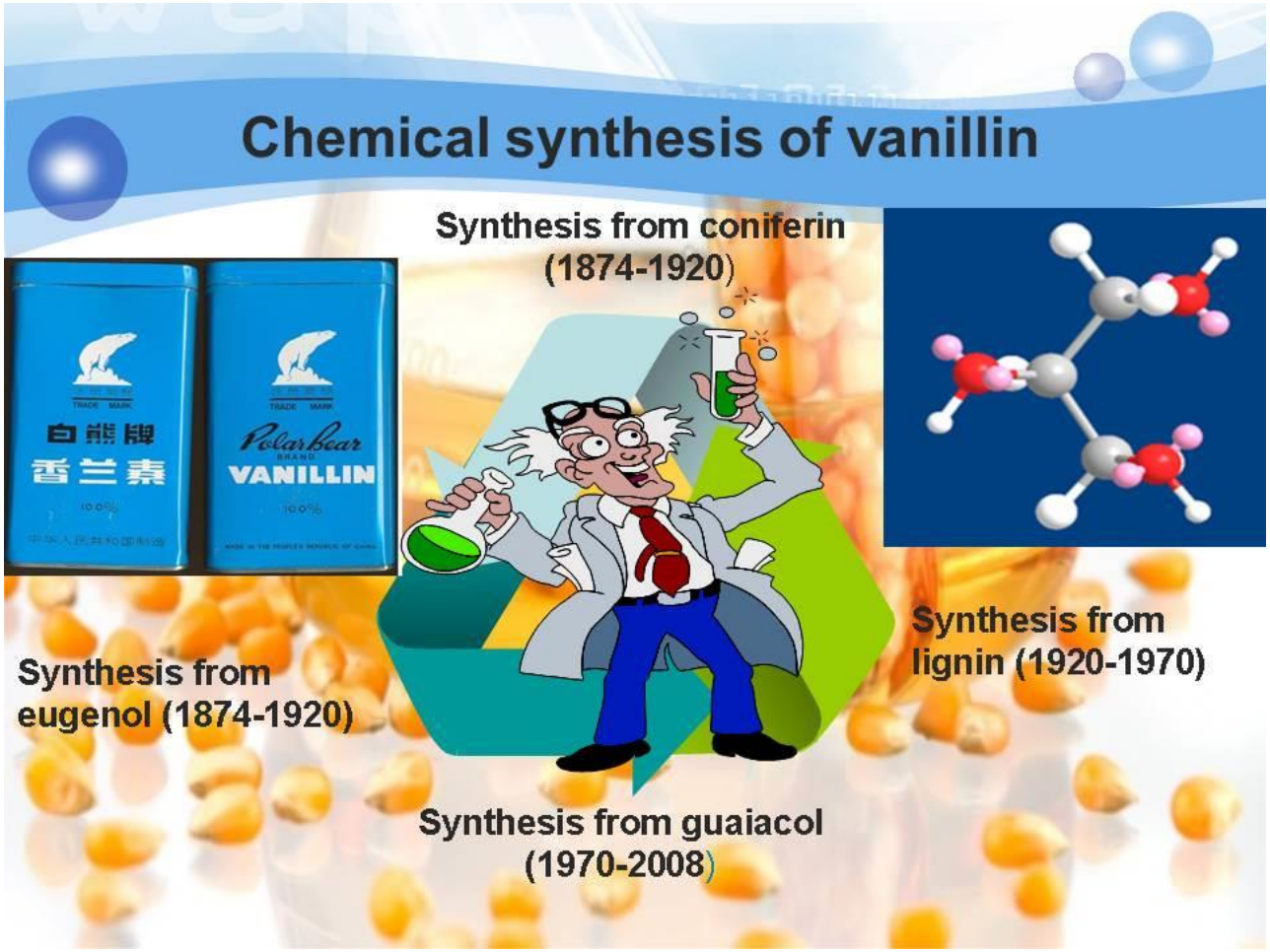


Maturing the cured vanilla in crates and packing in small bunches

Market estimation of vanilla pod production from *Vanilla fragrans* and *V. planifolia* for flavor use in 1999

Country	Production (t a^{-1})
Madagascar	950–1400
Indonesia	150–300
Comoros	150–200
Tonga	30–40
Mexico	20–40
Uganda	20–40
Fr. Polynesia	20
Réunion	20
India	10–30
China	10–20
Costa Rica/Guatemala	3–5
Others	300–800
Worldwide	2200–2400




Chemical synthesis of vanillin
Synthesis from coniferin
(1874-1920)
白熊牌
香兰素
Polarbear
VANILLIN
Synthesis from
eugenol (1874-1920)
Synthesis from
lignin (1920-1970)
Synthesis from guaiacol
(1970-2008)

Companies for vanillin(31)

1)China (20) 2) Turkey 3)Netheland 4) Romania 5) Egypt 6) Mexico .

Disadvantages





1) Environmentally unfriendly processes
2) Lack substrate selectivity/specificity
3) Tag "nature-identical"=artificial products
4) Operation under extreme conditions (T/pH/pressure)

Natural products: (according to EEC and Us legislations) natural products those produced from natural sources by physical extraction or by living cells or their components, including enzymes having GRAS status.

- ►In Germany in 2000 about 80% vanillin and other flavors used were natural reflecting the increasing health of the customers.
- ►The US annual consumption of vanillin beans, all of which are imported from foreign countries, is 1200-1400 tons with a market value of about 100 million dollars.

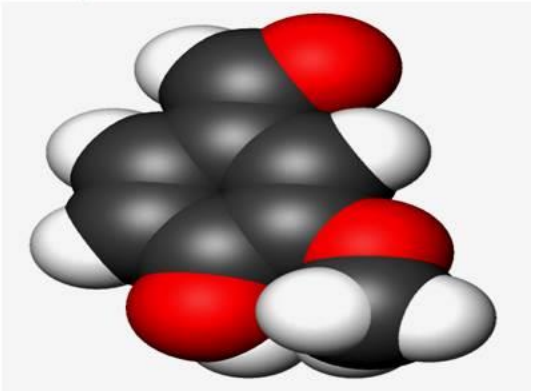
- Biotechnological production (1985) : 1) limited supply and high price of annual vanilla , 2) high demand for natural vanillin

- **1) production of vanillin by de novo synthesis (unsuccessful)**
- **2) production of vanillin by biotransformation.**

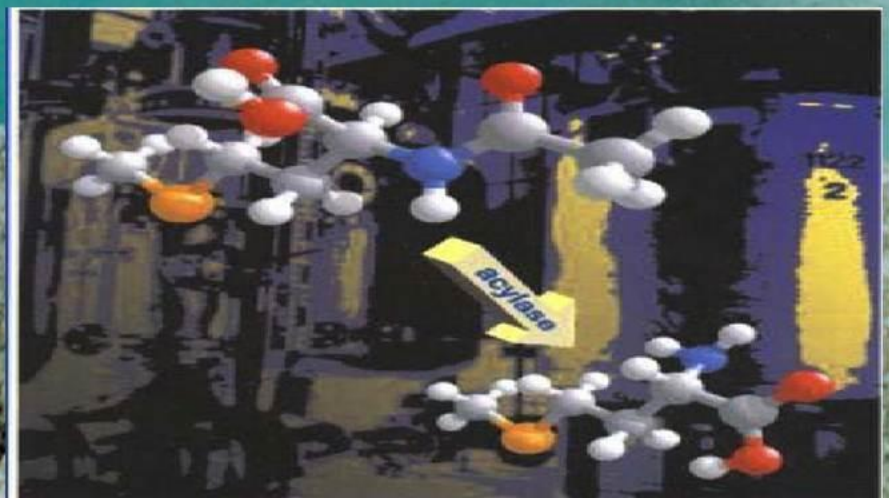
Determination of the origin of vanillin

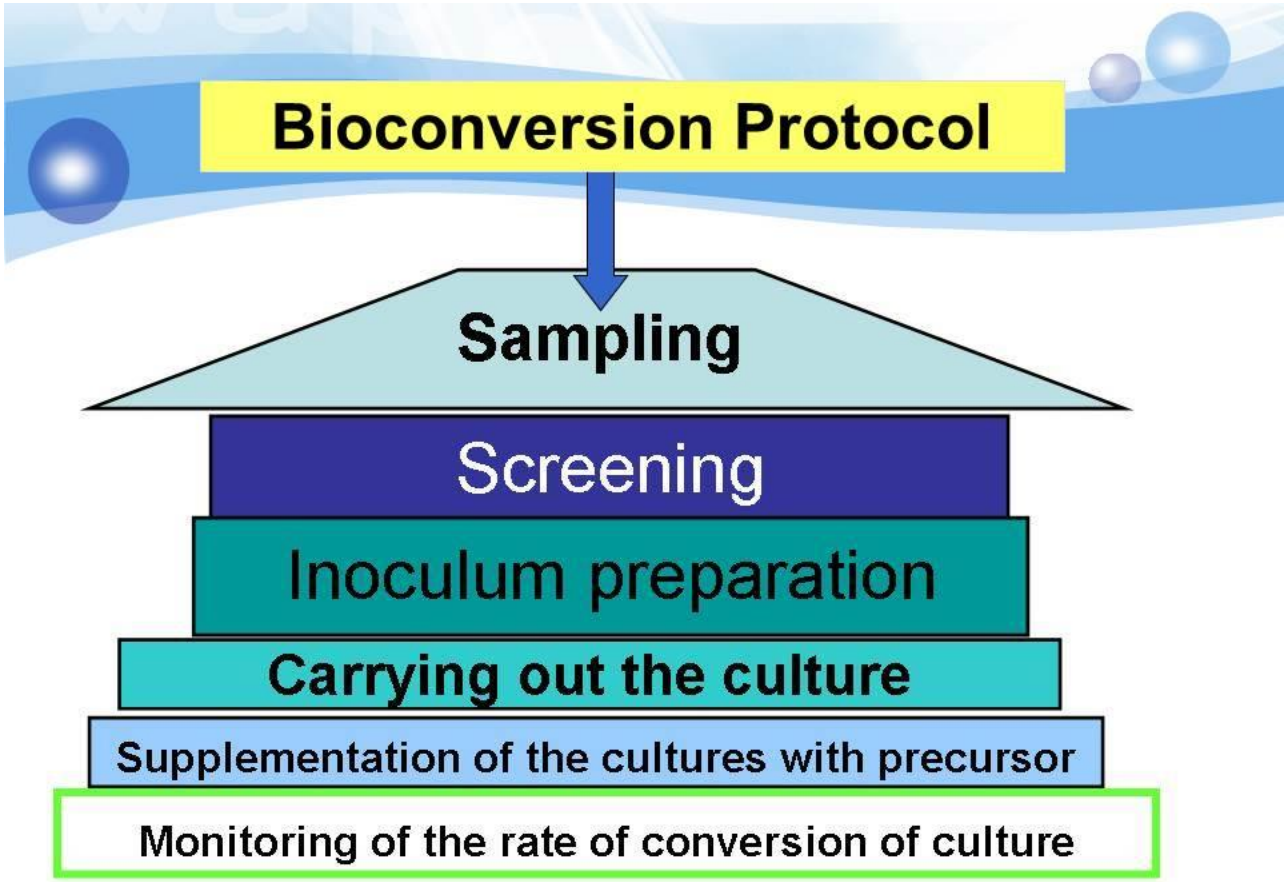
1) NMR(isotope-ratio)

2) XRD (peaks intensities)



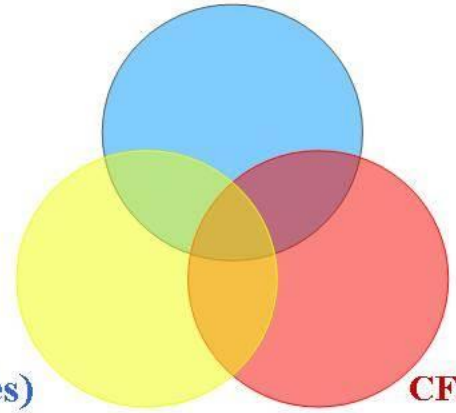
Microbial Bioconversion




Bioconversion Protocol
Sampling
Screening
Inoculum preparation
Carrying out the culture
Supplementation of the cultures with precursor
Monitoring of the rate of conversion of culture

Types of inoculum and culture

Whole cells (growing/resting or spore)



CD (cell derbies)

CFE (cell-free extract)

Culture in: semi synthetic/synthetic or complex media
These media contains carbon and nitrogen sources , nutrients , trace elements and if appropriate vitamins

Precursors

Starting materials (obtained from natural sources)

propenylbenzenes

Eugenol

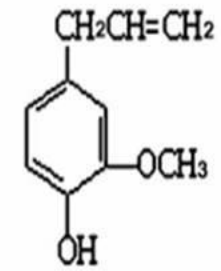
Isoeugenol

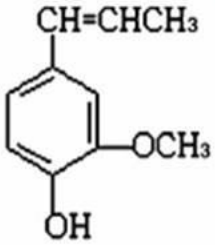
Ferulic acid

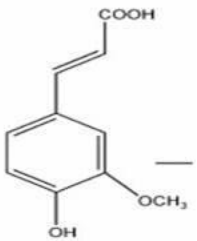
Vanillic acid

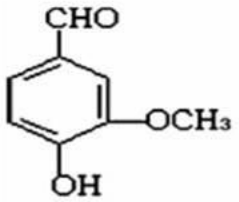
Safrole

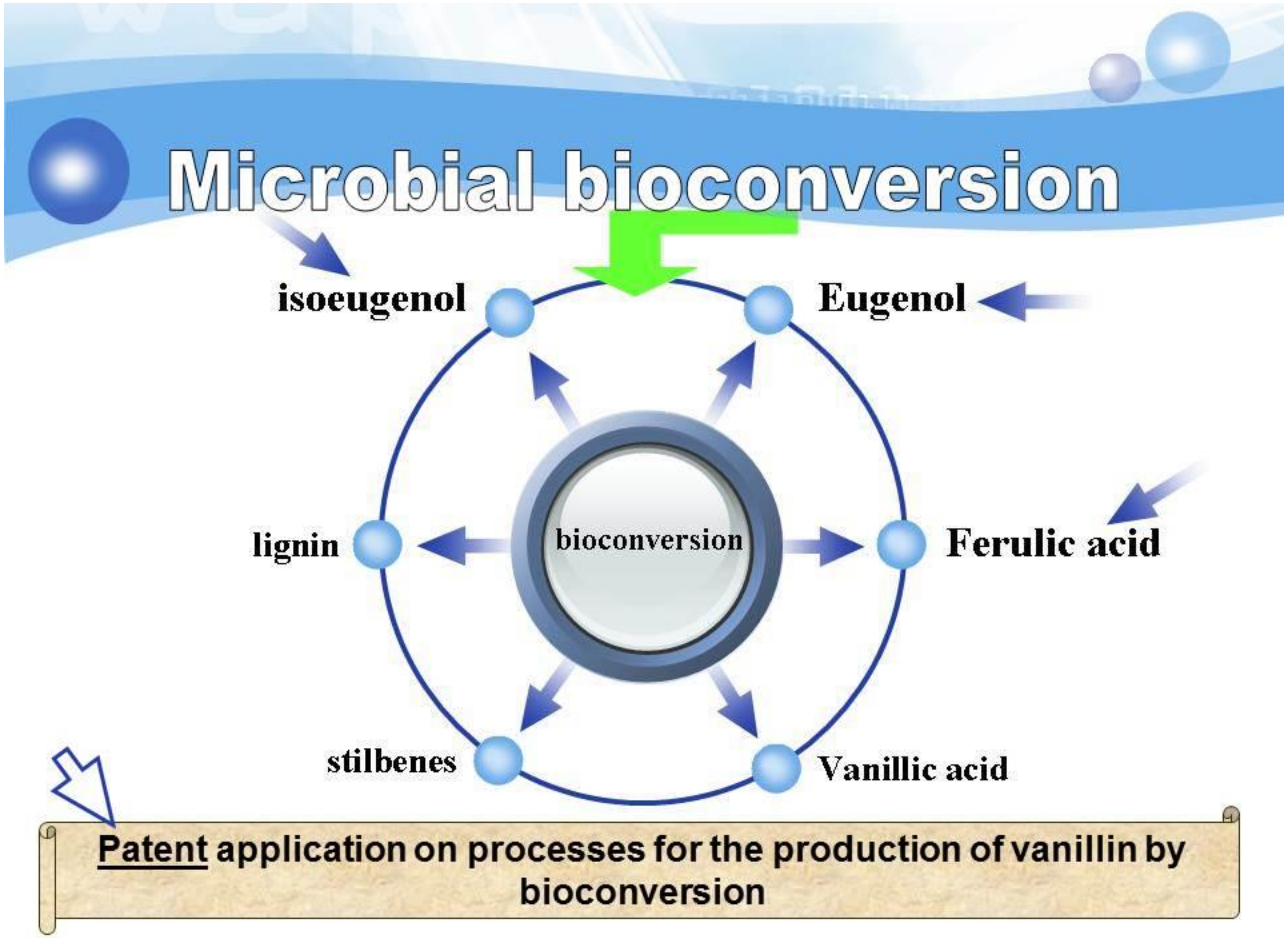
isosafrole










Microbial bioconversion
isoeugenol
Eugenol
lignin
bioconversion
Ferulic acid
stilbenes
Vanillic acid
Patent application on processes for the production of vanillin by bioconversion

Eugenol($C_{10}H_{12}O_2$)

Eugenol: the name comes from a scientific name for the clove ***(Eugenia aromaticum=Syzygium aromaticum)***

Occurrence: in the essential oils of plants.
Extract from (leaves, stem and bud) clove, cinnamon, calamus, pimento and camphor) (70-90%)

Current market price: a cheap substrate, commercially available (5 dollar/kg)

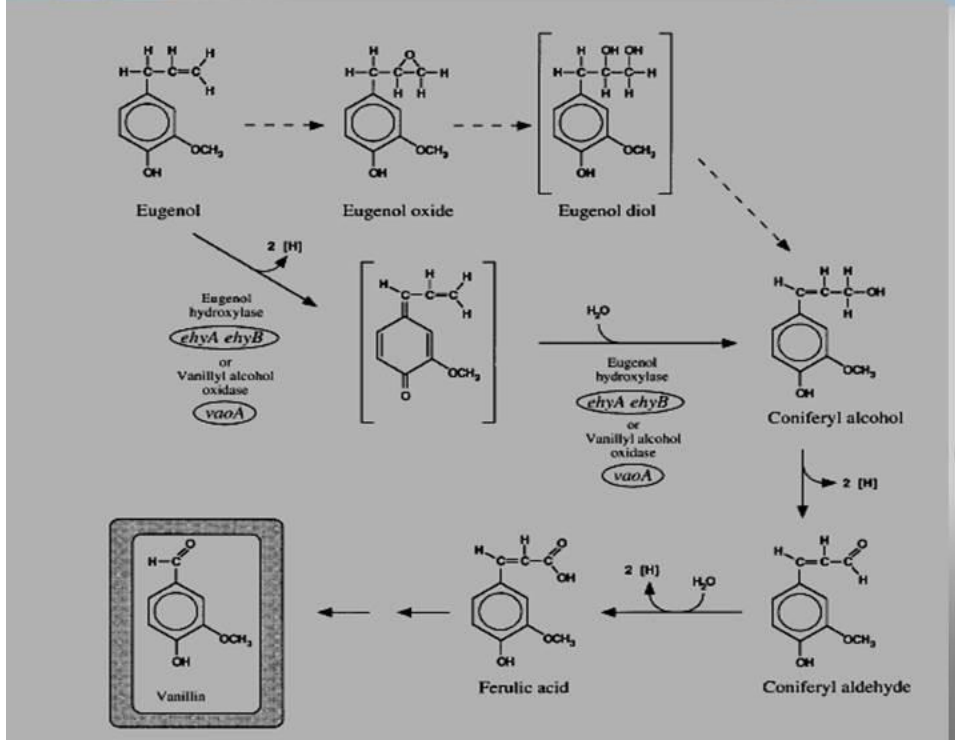
Bioconversion to vanillin (bacteria/fungi)
First report: *Corynebacterium .SP* (Tadasa,1977)

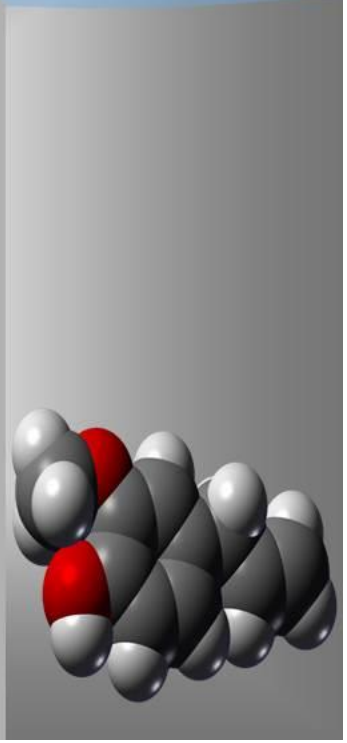
General pathways of eugenol transformation to vanillin





General pathways of eugenol transformation to vanillin





Isoeugenol

Occurrence: oil essential of clove tree (15-20%)

1)Extract from clove directly
2)Obtained from eugenol by isomerization
3) Obtain from eugenol under basic condition

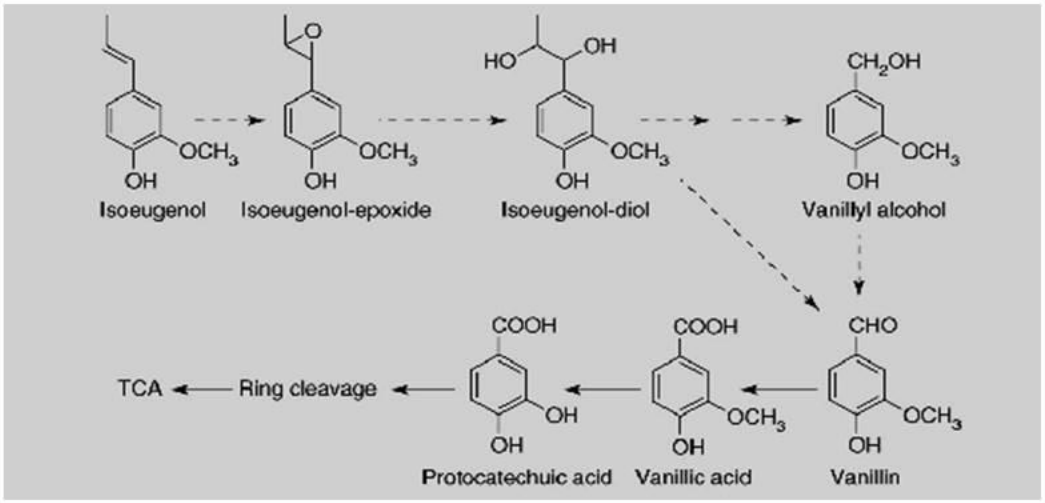
Current market price: 9 dollars/kg)

Bioconversions to vanillin:
First report: *A.niger* ATCC1942(Abraham et al.,1988)

General proposed pathways of Isoeugenol transformation to vanillin



General proposed pathways of Isoeugenol transformation to vanillin



Vanillin production from eugenol and isoeugenol

Microorganism	Final molar yield (%)	Initial substrate	Reaction time (h)	Conversion system	References
Serratia marcescence DSM 30126	20.5	20 g/l	212	growing culture	Rabenhorst and Hopp 1991
Rhodococcus rhodochrous MTCC 289	58	1 g/l	72	whole cell	Chatterjee et al. 1999
Bacillus subtilis B2	14.0	1 % (v/v)	48	cell free extract	Shimoni et al. 2000
Bacillus subtilis HS8	14.7	10 g/l	96	whole cell	Zhang et al. 2006
Lysinibacillus fusiformis SW-B9	5.83	60 % (v/v)	72	aqueous-organic system	Zhao et al. 2015
Pseudomonas putida IE27	71.0	150 mM (10% DMSO)	24	resting cell	Yamada et al. 2007
Pseudomonas chlororaphis CDAE5	12.6	10 g/l	24	growing culture	Kasana et al. 2007
Bacillus pumilus S1	40.5	10 g/l	150	growing culture	Hua et al. 2007
Bacillus fusiformis CGMCC 1347	17.4	50 g/l	72	addition of 12.5 g HD-8 resin	Zhao et al. 2006
***Candida galli* PGO6**	**48.7**	**1 g/l**	**30**	**resting cell**	**Ashengroph et al. 2011**
Psychrobacter sp. CSW4	13.8	10 g/l	48	resting cell	Ashengroph et al. 2012
Recombinant E. coli BL21 (DE3)	81	230 mM (10% DMSO)	6	-	Yamada et al. 2008

Ferulic acid

► Ferula foetida

Occurrence: ferulic acid is an abundant hydroxycinnamic acid
.woods and lignocelluosic residues such as corn cob, wheat bran, rice bran, bagasse/pulp

Current market price: a relatively expensive, commercially avaiable (30-50 dollars/kg)

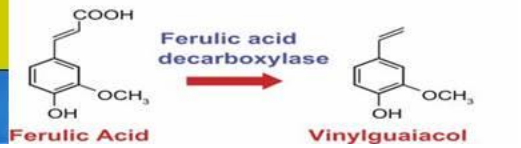
Bioconversions to vanillin:
First report: white-rot fungi (Gupta et al., 1981)

Importance: a suitable precursor for :1) 4-vinylguaiacol (the commercial cost is nearly 40 times more than ferulic acid)
2) Vanillin

General pathways (with respect to the initial reaction)

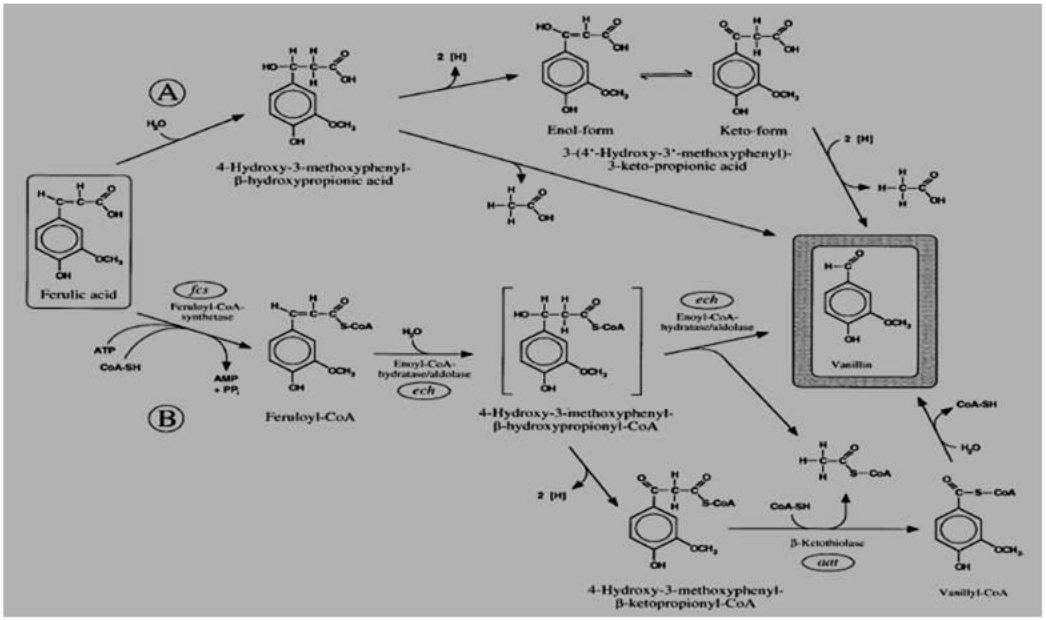
1) non-oxidative decarboxylation
2) Side reduction chain
3) Coenzyme-A-independent deacetylation
4) Coenzyme-A- dependent deacetylation





Ferulic Acid — Vinylguaiacol

Coenzyme A- independent (A) and coenzyme A dependent deacetylation (B) of ferulic acid to yield vanillin. Involved enzymes and genes are indicated



Vanillin production from ferulic acid (2002-2008)

substrate	Microorganism	Yield(g/l)	Molar yield(%)
Ferulic acid	*Bacillus pumilus*	9.6g/l	49.7%
	Sporotrichum thermophile	0.7g/l	36%
	Streptomyces setonii	0.6 g/l	89%
	Pseudomonas flurescens	4.7 g/l	35%
	Pseudomonas putida	0.1 g/l	80%
	A.Niger and P.cinnabarinus (two stage process)	0.2 g/l	78%

Rhodia Organics

Beginning in 2000, Rhodia began marketing biosynthetic vanillin prepared by the action of microorganisms on ferulic acid extracted from rice bran. At $700/kg, this product, sold under the trademarked name Rhovanil Natural

Amycolatopsis
Streptomyces
Serratia
→ Problems scaling up
More than 10%

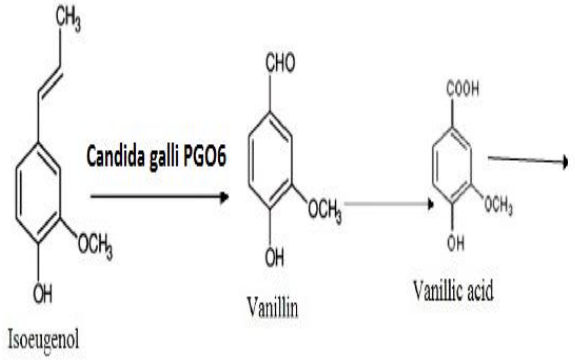
Pilot plant (20 liter fermenter)

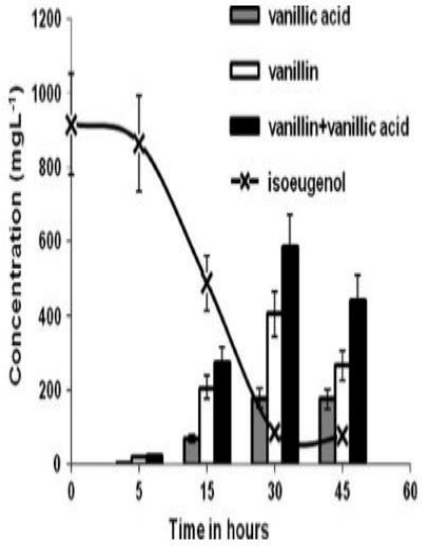


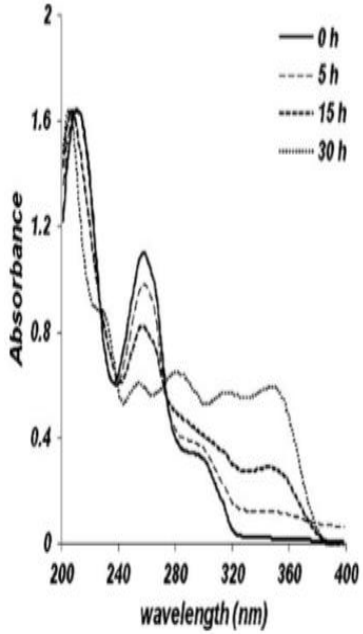
1000 tons

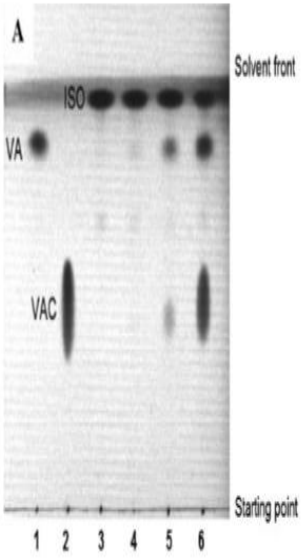
EXTRA RHOVANIL® PURE

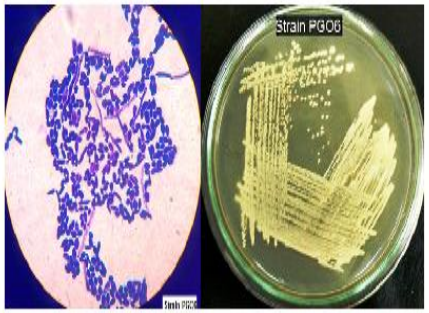

Rhodia
Usine de St Fons Chimie

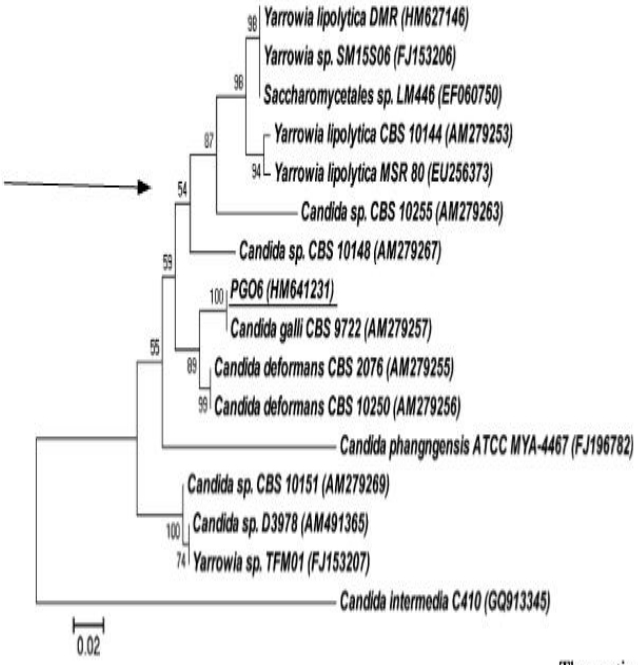


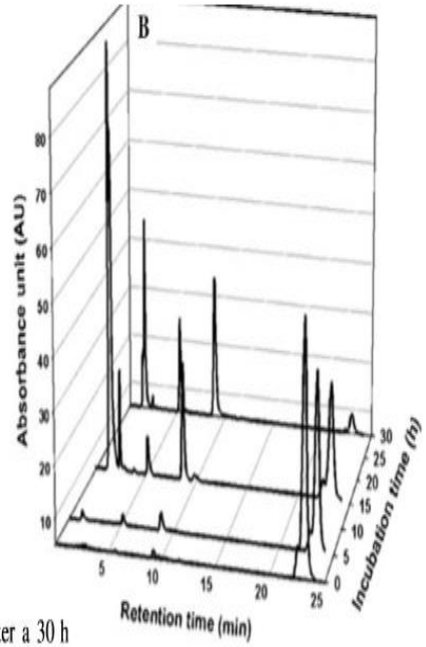












The optimal molar conversion of vanillin (48%) and vanillic acid (19%) was obtained after a 30 h incubation using 0.1% (v/v) of isoeugenol

Ashengroph, M.; Nahvi, I.; Zarkesh-Esfahani, H.; Momenbeik, F. *Candida galli* strain PGO6: A novel isolated yeast strain capable of transformation of isoeugenol into vanillin and vanillic acid. *Curr. Microbiol* **2011**, *62*, 990–998.

Theophylline (1,3-dimethylxanthine), a naturally occurring alkaloid, is found in small amounts in tea, coffee, and cocoa plants and is produced commercially for use in cosmetic products as a tonic and as an anticellulite product

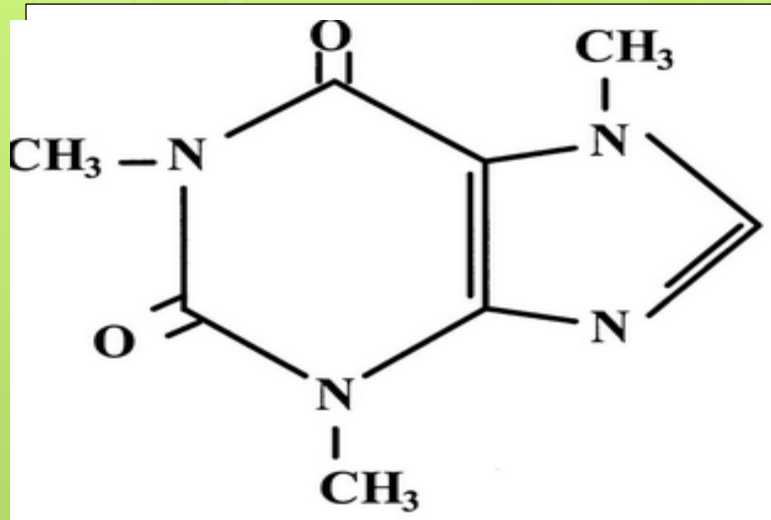
Theophylline is also used in the treatment of bronchial asthma, idiopathic apnea of prematurity, and dieresis, which is due to its desirable pharmacological properties such as diuretic, cardiac stimulation, and smooth muscle relaxation

Most of the theophylline sold commercially is prepared from dimethylurea and ethyl cyanoacetate by traditional chemical transformations. However, the chemical transformation of theophylline has several drawbacks such as high cost, low efficiency, and use of catalysts that are not environment friendly. Hence, the microbial transformation of caffeine offers a cleaner, more economical alternative for the natural production of theophylline

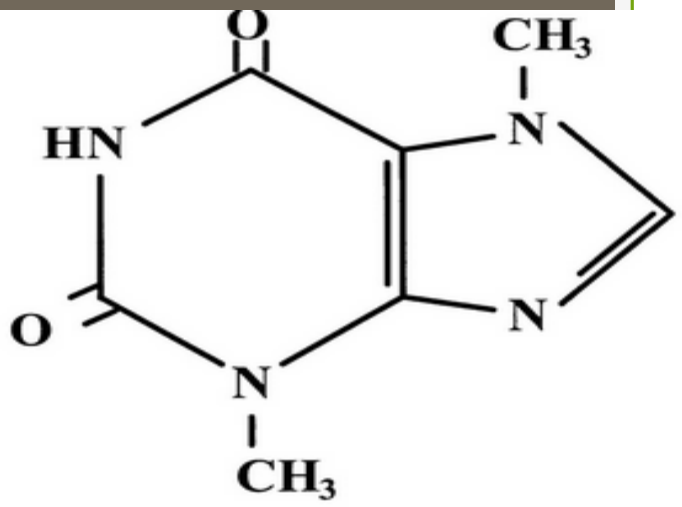
Caffeine (1,3,7-trimethylxanthine) is one of the most popular and commercially important plantderived purine alkaloids. It is found in over 100 plant species among which Coffea arabica (coffee), Camellia sinensis (tea), Cola nitida (Cola) and Theobroma cacao (cacao) genera are prominent .

In spite of such distribution in beverages and food preparations, higher level of caffeine consumption through beverages and confectionary products led to higher risk of human health problems like headache, fatigue, abnormal muscle activity, heart irregularity, adrenal simulation, abnormal renal function and bone problems such as osteoporosis and alveolar bone loss in ligature-induced periodontitis

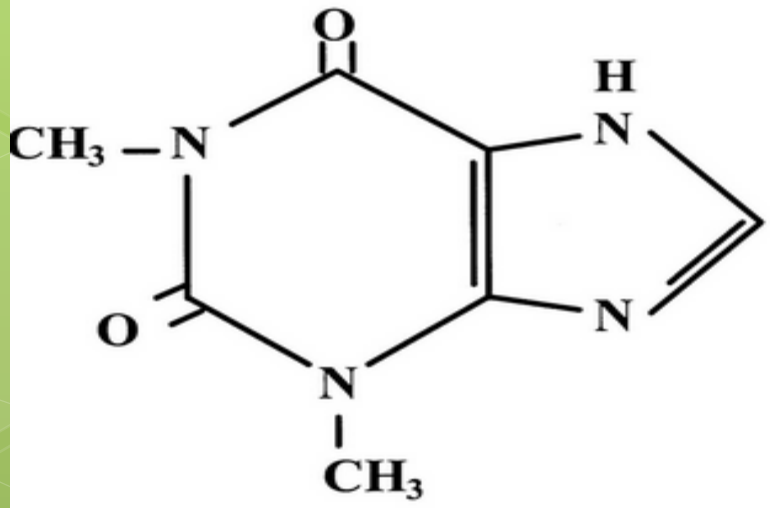
Additionally, caffeine as a by-product of decaffeination and from the coffee and tea processing plants is a **renewable and economic substrate** for the production of theophylline through biotransformation process



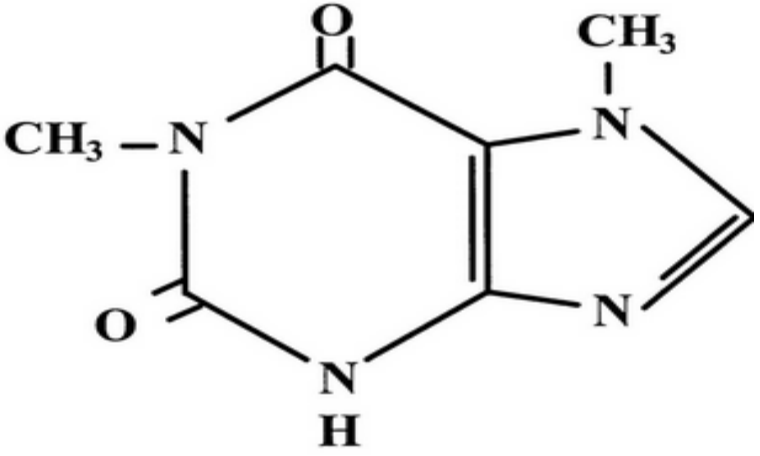
Caffeine
(1,3,7-trimethylxanthine)



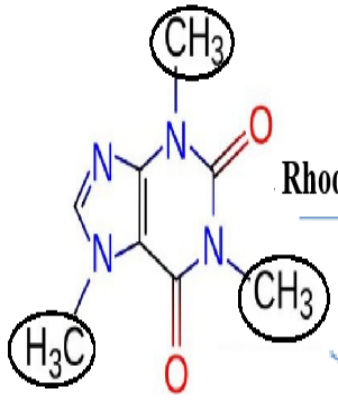
Theobromine
(3,7-dimethylxanthine)



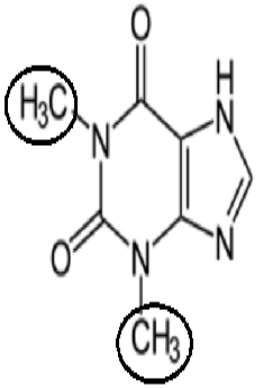
Theophylline
(1,3-dimethylxanthine)

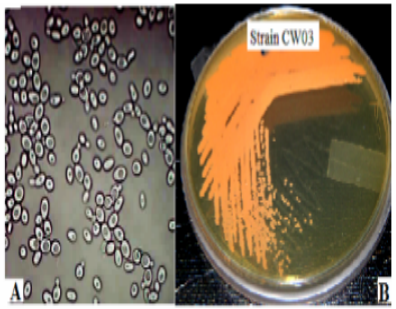


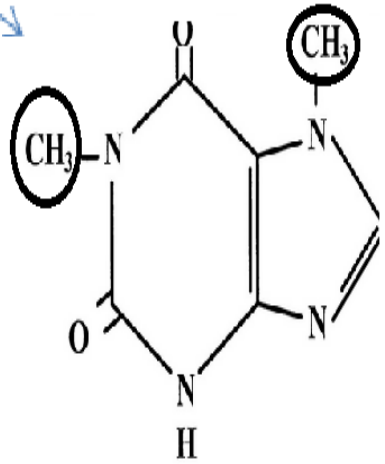
Paraxanthine
(1,7-dimethylxanthine)

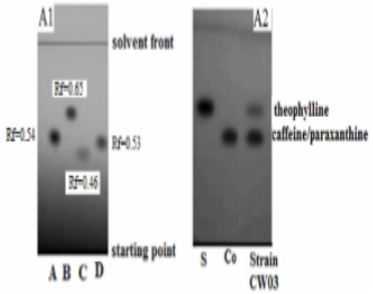


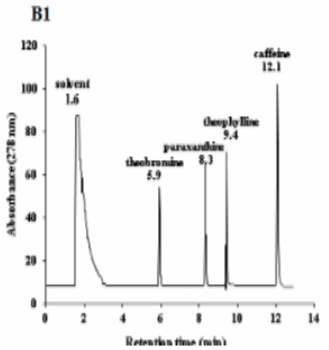
Rhodotorula sp. CW03

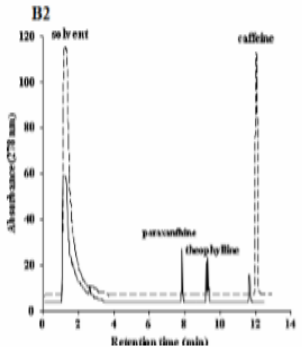


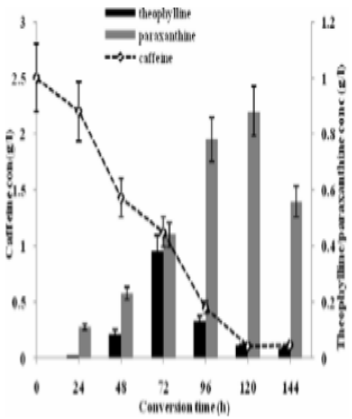










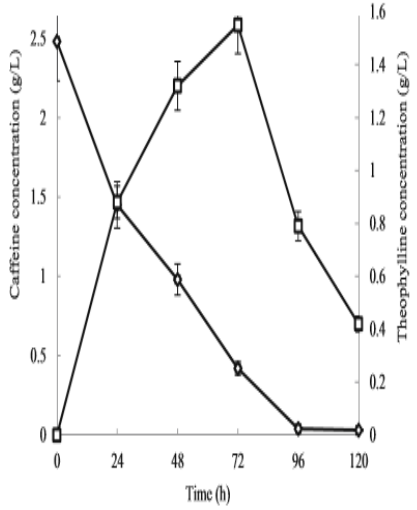


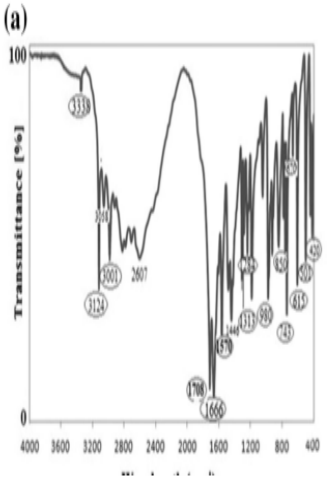
The results showed that under resting cell conditions a maximum concentration of theophylline 380 mg/l (molar yield of 16.4%) and paraxanthine 880 mg/l (molar yield of 37.9%) were obtained after 72 h and 120 h of conversion time, respectively.

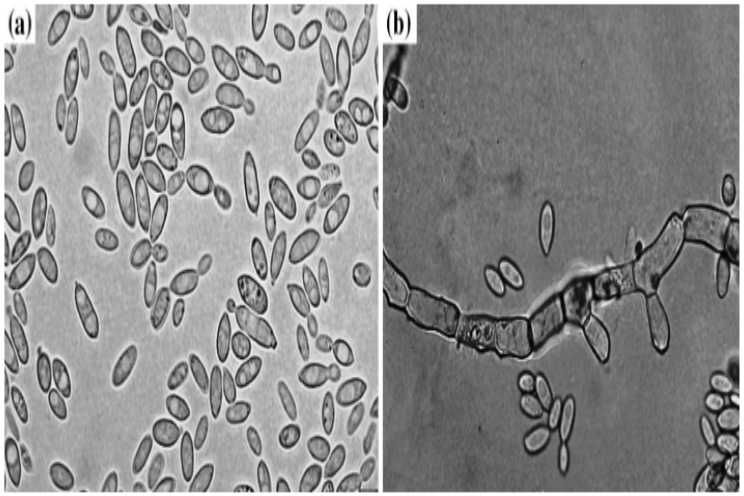
Ashengroph M, Haidarizadeh M, Borchaluei M (2015) Use of Resting Cells of Native Screened *Rhodotorula* sp. CW03 in Biotransformation of Caffeine to Theophylline and Paraxanthine. Scientific Journal of Hamadan University of Medical Science, 22(2): 83-92.

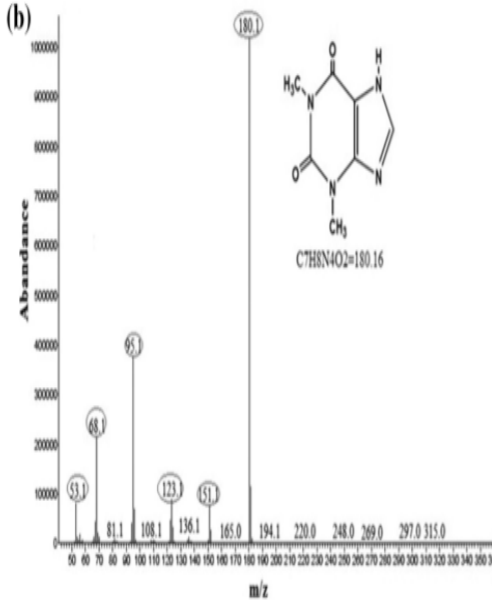
Caffeine → *Aureobasidium sp.* TeO12 → Theophylline

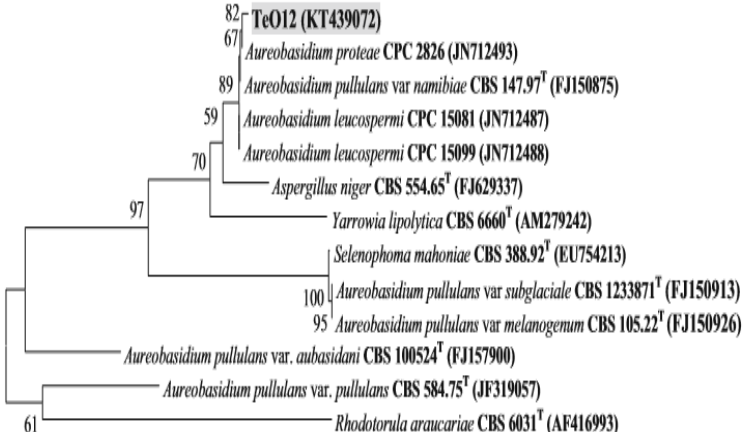
Under these optimal reaction conditions, the highest theophylline concentration of 1.55 g/L (molar yield of 67%) with an average degradation yield of the substrate of about 83% was obtained in the biotransformation process.

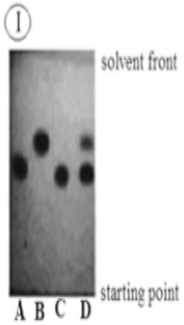


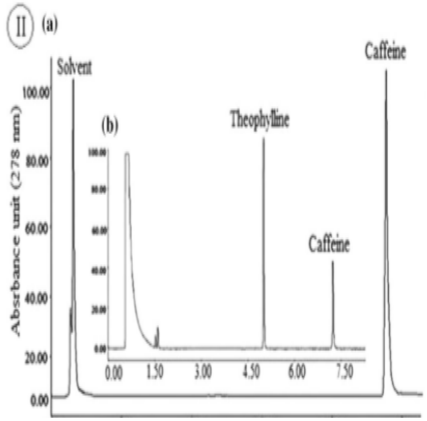












Ashengroph M (2017) A novel strain of *Aureobasidium* sp. TeO12 for theophylline production from caffeine. 3 Biotech 7(176) 1-8.