



# 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

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Yeast Habitats Atmospheric Yeasts Aquatic/Marine Yeasts Ascomycetes (e.g. Saccharomyces, Candida ) or Basidiomycetes (e.g. Filobasidiella, Rhodotorula).

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 at 1,500,000
 5000000

 compared to 270,000 and 1,000,000 estimated numbers of species of
 5000000

 plants and bacteria, respectively (Boekhout and Samson 2005). Thus,
 5000000

 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 <sup>a</sup>		
Mannoproteins	30-50		
β-1,6-Glucan	5-10		
β-1,3-Glucan	30-45		
Chitin	1.5-6		

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#### Molecular chracterization: ITS1-5.8S-ITS2

#### Morphology, Pigmentation

	Fermentation	Growth reactions and other characteristics			
_	Glucose Galactose Sucrose Maltose Lactose Raffinose Trehalose	Glucose Inulin Sucrose Rafinose Melibiose Calactose Lactose Lactose Melezitose Melezitose Methyl-o-p-glucoside Soluble Starch Cellobiose Soluble Starch Cellobiose Salicin L-Sorbose D-Xrlose D-Arabin			
Growth reactions and other characteristics					
	<i>myo</i> -Inositol DI-Lactate Succinate Citrate D-Gluconate D-Glucosamine N-Acetyl-D-glucosamine Hexadecane Nitrate	vitamin-rree 2-Keto-D-gluconate 5-Keto-D-gluconate 5-Keto-D-gluconate 5-Keto-D-gluconate 5-Keto-D-gluconate Arbutin Arbutin Arbutin Arbutin Arbutin Cadaverine Cadaverine Cadaverine Cadaverine Ethylamine 50% Glucose 50% Glu			

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

### **Traditional Uses of 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	



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

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

#### 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,	Saccharomyces cerevisiae and Rh. glutinis			
phenylalanine, and proline				
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).





# **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*.

# advantages drawbacks

biotransformation

1) Tag natural

- 2) Operation under mild conditions
- 3) High substrate selectivity
- 4) Green chemistry

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



#### Advantages of Whole-cell biocatalysts

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

► The 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- D-amino acid oxid cephalosporanic acid		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 0 00 000	high-fructose corn syrup	glucose isomerase
> 100 000	lactose-free milk	lactase
> 10 000	acrylamide	nitrilase
	cocoa butter	lipase
> 1 0 0 0	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-camitine	de hyd rase/
		hydroxylase
	L-DOPA	β-tyrosinase
	L-malic acid	fumarase
	(S)-methoxyisopropyl- amine	lipase
	( <i>R</i> )-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	E2: FDH (1.2.1.2)	(S)-2-Amino-5-(1,3-dioxolan-	Bristol-Myers Squibb,
or Pichia pastoris	[E1: PheDH From Thermoactinomyces intermedius]	2-yl)-pentanoic acid	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 obae (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



# Vanillin

# llin 🦳



### Importance

# **Commercial types exist**

Structure

History

Worldwide annual consumption /demand



#### 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)





Spreading the beans in the sun after sweating



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 <sup>-1</sup> )	
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	





#### 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) <u>Tag "nature-identical"=artificial products</u>
- 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**







### **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





# Eugenol(C<sub>10</sub>H<sub>12</sub>O<sub>2</sub>)

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







### 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







#### Vanillin production from eugenol and isoeugenol

Microorganism	Final molar yield (%)	Initial substrate	Reaction time (h)	Conversion system	References
Compliant and a complete DOM 2010/	00.5	00 <del></del> //	010	·	Dala a ala a mit av
Serratia marcescence DSM 30126	20.5	20 g/i	212	growing culture	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





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



Va	nillin 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%



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



Amycolatopsis Streptomyces Serratia More than 10%

Pilot plant (20 liter fermenter)







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.

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

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







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.



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