Yeast at Lonza AG (I)

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Lonza is the leading custom manufacturer for small and large molecules. Products include organic fine chemicals, nucleotides, secondary metabolites, glucans, peptides, therapeutic proteins and monoclonal antibodies.

The broad product range requires a very broad range of hosts and production organisms. Besides mammalian cells (CHO, NSO), bacterial cells, and deuteromycetes also **yeast strains** are required in manufacturing. The performance of the production strain has a direct influence on both the fermentation and down stream processing. Most process problems (e.g. low product titers, by-product formation, poor productivity, complex media requirements, too many unit operations, low yields etc.) can be traced back to the low quality of the production strain.

Organism	Lonza	Established	Therapeutic Proteins	Fine Chemicals*
Prokaryotes	✓	✓	Lonza	Lonza
Yeasts	✓	✓	Lonza	Lonza
Actinomycetes	✓	✓	Lonza	Lonza
Plant cell culture	no	partly	no	no
Transgenic plants	no	partly	no	no
Insect cell culture	no	yes	no	no
Mammalian cell culture	✓	✓	Lonza	no
Transgenic animals	no	partly	no	no

Effect of strain characteristics, the process and the plant on the overall process outcome

The strain is the single most important ingredient for a successful bioprocess.

	Process flexibility	QP	Sterility	Cost
STRAIN	High	High	Medium	High
PROCESS				
Culture Medium	Low	Medium	Medium	Medium
Parameters	Low	Medium	Medium	Low
PLANT				
Fermentation	Low	Low	High	I & D
DSP	High	Low	-	I & D and Y

Qp= Volumentric Productivity; I & D= Interset & Depreciation; Y= Yield

Why should one use yeast as production strains in biocatalyis?

Higher eukaryotes (plants and animals) are a vast source of potential biocatalysts. The use of yeast may be advantageous for the expression and production of these eukaryotic enzymes.

As of today, most reports on yeast biocatalysis are on oxido-reduction steps, such as the asymmetric reduction of carbonyl groups like prochiral ?-ketoesters. Other less frequently used yeast enzymes are lipases, glycosyltransferases or cyclisation enzymes.

However, also the total biosynthesis of hydrocortisone by a yeast from simple carbon sources was reported [NatureBiotechnology **21**:143-149 (2003)]

Why should one use yeast as production strain for proteins?

Depending on the peptides or proteins structural complexity, Lonza considers different strategies for cGMP manufacture, as there are:

– Solid phase chemial synthesis
– Liquid phase chemical synthesis
– Recombinant technology (mammalian or microbial)
– A combination of above mentioned technologies

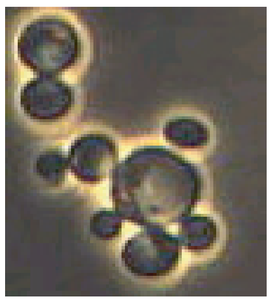
Lonza is using different microbial hosts and expression systems such as:

– Escherichia coli
– Bacillus sp.
– Pseudomonas putida
– Pichia pastoris
– Pichia angusta (Hansenula polymorpha)

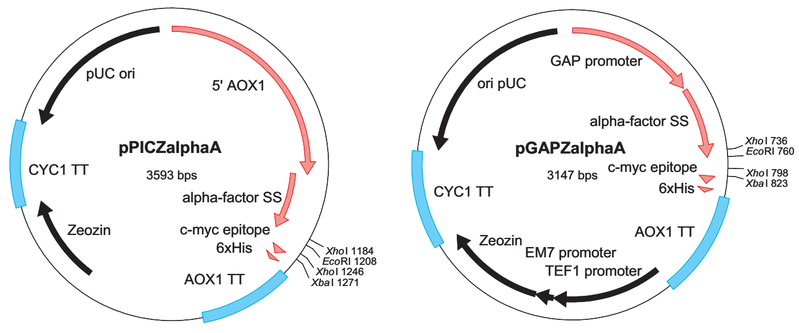
The reasons for using yeast are:

– Unicellular eukaryote
– GRAS organism
– Easy cultivation and simple nutritional requirements facilitate high-cell-density fermentation
– Secretion of functional proteins into the culture medium
– Easy purification from the culture medium
– Postranslational modifications possible if necessary

Yeast genetics is well established based on the available genomic sequences, and based on the variety of different expression vectors and suitable recombinant methods for specific strain backgrounds which facilitate efficient genetic engineering. Consequently, yeast strains have become increasingly important for large-scale production of therapeutic proteins and peptides.



Pichia pastoris

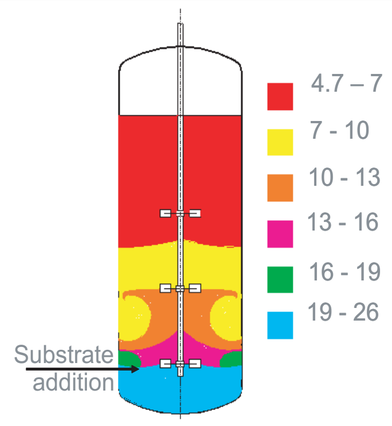


Cloning vectors for Pichia pastoris with different promoters as examples

However, very high productive yeast processes require also particular care of the fermentation process. Oxygen limitations, heat transfer limitations, by-product formation and other aspects are important issues when it comes to bioprocess scale-up and industrial manufacturing. Biomass concentrations during fermentation can reach several hundred grams of dry cell weight per L. Oxygen up-take rates of 5-10 mmol/g/h, high nutrient demand and heat evolution become a concern. For example, as much as half of the available enthalpy in the oxidised substrate is given off as heat, leading to enormous heat evolution.

$$Q_W = V_0 \cdot \mu \cdot X / Y_H$$

Q_W= rate of microbial heat formation [kcal h^{-1}]; V_0 = working volume [l]; μ= growth rate of microorganism [h^{-1}]; X= Biomass [g/l]; YH = microbial heat formation coefficient [$g_{biomass}$. kcal].



Concentration distribution in a 15m^3 fermentor of an educt fed into the reactor.

Lonza has established a cooperation programme with the VSCHT in Prague and HS Wädenswil to address process engineering questions for highly productive yeast processes. Similar work was carried out earlier with bacterial processes to determine e.g. optimal reactor design for feeding. This will be extended to the specific request of yeast, particularly