GENETIC ASPECTS OF USING L-CARNITINE FOR THE PRODUCTION OF PROTEIN BIOMASS BY THE UNCONVENTIONAL YARROWIA LIPOLYTICA YEAST

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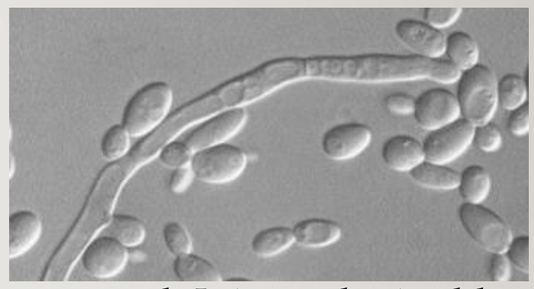

Introduction

L-carnitine or γ-trimethylamino-β-hydroxybutyric acid is a ubiquitous water-soluble quaternary amine compound. It is synthesized by most eukaryotic organisms, including some yeast, from amino acids: lysine as a precursor and methionine or S-adenosyl methionine as a methyl donor. In humans, endogenous synthesis of L-carnitine occurs chiefly in the liver. However, it must be complemented through dietary uptake. Since it is regarded to be a quasi-nutrient or conditionally essential nutrient, L-carnitine deficiencies sometimes cause life-threatening disorders. As an important factor in cellular metabolism, L-carnitine binds fatty acids and transfers them to the mitochondria for β-oxidation required for generation of energy. Without L-carnitine, the mitochondrial inner membrane is impermeable to fatty acids. L-carnitine transports long- to short-chain fatty acids out of the peroxisome, where β-oxidation is started, into the mitochondria, where the process is completed, by reversible esterification of the β-carbon hydroxylgroup with a fatty acid to form *O*-acyl-carnitine.

Aim of research

Two *Y. lipolytica* strains, namely the industrial A-101 strain and the standard reference strain from the American Type Culture Collection (ATCC), were examined for L-carnitine content in their biomasses depending on the medium, fat-rich biofuel waste, and fatty acid-poor YPD medium. Subsequently, the genes responsible for the production of L-carnitine were identified.

Methods

Y. lipolytica was cultured in two culture media: an industrial fat-rich SK medium and the chemically defined fatty acid-poor YPD medium (Difco). The SK medium is a waste from biofuel production, which is normally used by Skotan S.A for production of *Y. lipolytica* A-101 biomass rich in nutritional elements for commercial use. Biofuel is made through chemical reaction of vegetable oil with ethanol producing fatty acid esters (long-chain alkyl (methyl, ethyl, or propyl) esters). Crude biofuel waste consists of a mixture of vegetable oils with *degumming* and glycerol fractions (from 2% to 7% wt/wt). The sterile media were also supplemented with $FeSO_4$, trimethyllysine hydrochloride, and L-ascorbic acid.

Results

Free L-carnitine was detected in the biomass of both *Y. lipolytica* strains cultured in the YPD medium in the different culture conditions. The obtained biomasses of *Y. lipolytica* strains enriched in L-carnitine exhibit different sensitivities to temperature and pH.

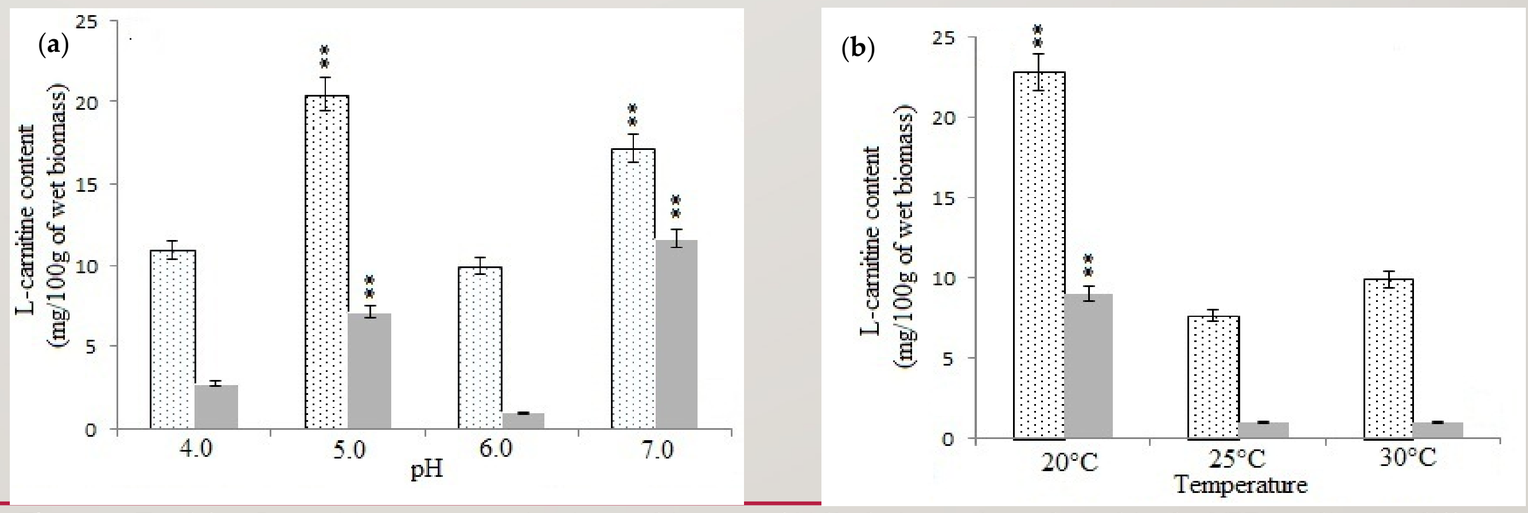

Figure 1. Total free L-carnitine concentration in wet biomass of *Y. lipolytica* strains cultured in the YPD medium at different conditions. (**a**) constant temperature (30°C) and variable pH values. (**b**) constant pH (6.0) values and variable temperature. *Y. lipolytica* ATCC 9793 (dotted squares); *Y. lipolytica* A-101 (filled squares). ***P*<0.01 indicate significant difference compared to reference cultivation.

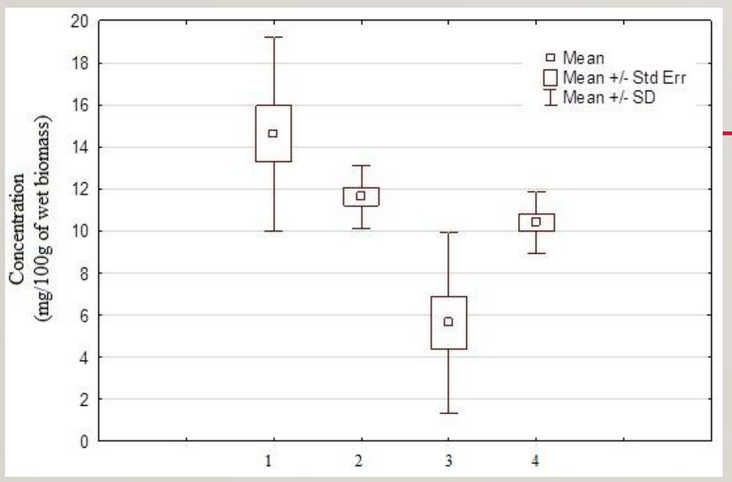

Figure 2. Average values of L-carnitine and protein production by *Y. lipolytica* A-101 and ATCC 9793 strains growing on YPD medium, shown as irrespective of the culture parameters. 1. L-carnitine concentration in *Y. lipolytica* ATCC 9793 biomass. 2. Protein content in *Y. lipolytica* ATCC 9793 biomass. 3. L-carnitine concentration in *Y. lipolytica* A-101 biomass. 4. Protein content in *Y. lipolytica* A-101 biomass.

Another oleaginous yeast *C. albicans* can synthesize carnitine *de novo*. Mutant strains with deletion of one of four genes determining the L-carnitine synthesis, were incapable of utilizing either acetate or ethanol as carbon therefore did not grow on fatty acids or their grow were strongly reduced. Identified enzymes involved in the carnitine biosynthesis pathway were, in sequence:

- trimethyllysine dioxygenase,
- hydroxytrimethyllysine aldolase,
- trimethylaminobutyraldehyde dehydrogenase,
- butyrobetaine dioxygenase.

Irrespective of the culture parameters, both of the strains produced comparable concentration of proteins but it existed significant difference in production of L-carnitine between these strains. *Y. lipolytica* ATCC 9793 produced significantly more L-carnitine in comparison with A-101 strain.

Medium	L-carnitine concentration (mg/100g wet biomass) Mean ± Standard deviation (SD)			
	Y. lipolytica A-101		*Y. lipolytica* ATCC 9793	
	Biofuel waste	YPD medium	Biofuel waste	YPD medium
unsupplemented	<1.00	2.80 ± 0.05	<1.00	9.93 ± 0.50
supplemented trimethyllysine (0.01 g/L), iron(II) (0.001 g/L), L-ascorbic acid (0.002 g/L)	<1.00	9.80 ± 0.49 **	<1.00	10.99 ± 0.55
supplemented trimethyllysine (0.1 g/L), iron(II) (0.01 g/L), L-ascorbic acid (0.002 g/L)	<1.00	10.74 ± 0.52 **	<1.00	12.41 ± 0.62 **

Table 1. L-carnitine concentration in the wet biomass of *Yarrowia lipolytica* strains cultured in biofuel waste and YPD medium. ***P* < 0.01 indicates significant difference compared with the reference unsupplemented cultivation. The yeast were cultivated 12 h, at 30° C, pH 6.0, 12 h.

Candida albicans gene	Gene product	*Yarrowia lipolytica* homologous sequences	Sequence identity of translated DNA sequences [%]
orf19.4316	trimethyllysine dioxygenase	YALI0C10604	49,2
orf19.6306	trimethylaminobutyraldhyde dehydrogenase	YALI0C03025	48.2
		YALI0E00264	48.6
orf19.6305	hydroxytrimethyllysine aldolase	YALI0A21417	50.6

Table 2. *Yarrowia lipolytica* and *Candida albicans* homologous sequences, essential for L-carnitine synthesis, sharing more than 40% identity.

Conclusion

- ✓ *Y. lipolytica* is genetically adapted for the biosynthesis of L-carnitine *de novo*
- ✓ The yeast is able to utilize almost the entire pool of free L-carnitine for its growth and protein production

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