AROMATIC AMINO TRANSFERASE I GENE (ARO8) IN THE *Saccharomyces cerevisiae* STRAINS

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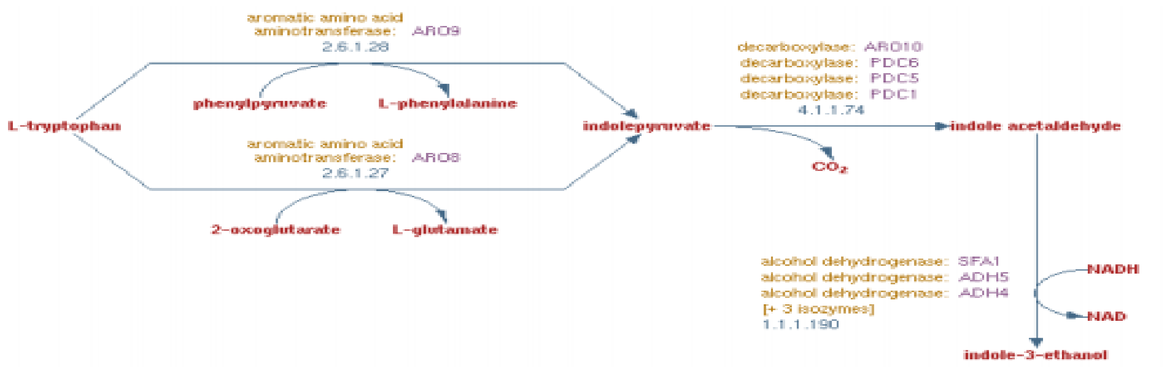
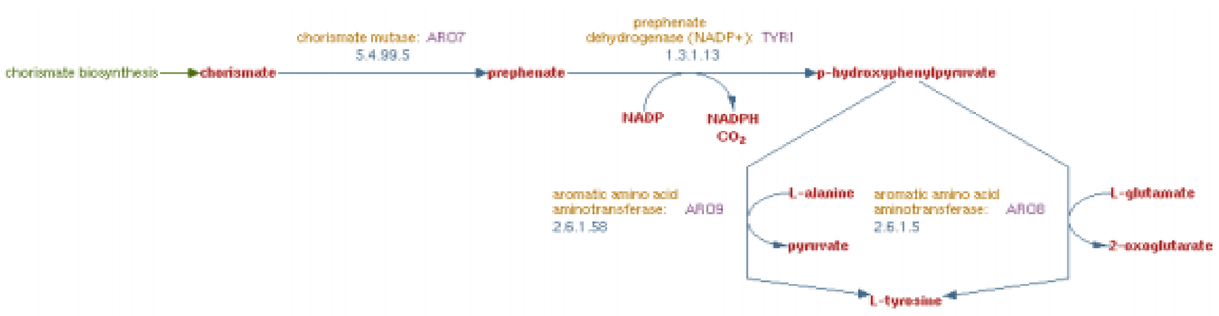
Abstract

Saccharomyces cerevisiae is a commonly used microorganism in the food industry as it produces the desired taste and aroma properties in brewing, winemaking and bread production. High alcohols formed by the metabolism of *Saccharomyces cerevisiae*, esters, carbonyl compounds, sulfur-containing compounds, etc. Aroma ingredients give the desired sensory properties to the products. Aroma agents are formed by various metabolic pathways such as Ehrhlic pathway. The activity of aromatic aminotransferase I catalyzing the transamination step, which is the initial step of the Ehrhlic pathway, was investigated by real time polymerase chain reaction method for *Saccharomyces cerevisiae* HUF16M1C0004. *Saccharomyces cerevisiae* HUF16M1C0004 was grown at 25°C in YPD Broth medium (1% yeast extract, 2% peptone and 2% glucose, pH 5.3) for 48 hours. Samples were taken at the end of the 4th, 8th, 24th and 48th hours of growth. Cell number of dilutions (10^{-1} 10^{-2} 10^{-3} 10^{-4} 10^{-5}) which were prepared from sample were calculated by using Thoma chamber and spectrophotometer. Absorbance of dilutions measured at 640 nm with Spectrophotometer by using cell-free YPD broth as reference. Culture whose initial concentration is 1 x 10^{7} cfu / ml were incubated at 25 °C for 24 hours. DNA extraction was performed with the Eurx GeneMATRIX Bacterial and Yeast Genomic DNA Purification Kit (EURxLtd,Poland). At the end of the DNA extraction, nucleic acid amount of DNA templates and A260 / 280 values were obtained with NanoDrop Spectrophotometer. Primers were designed at Primer 3 software. LightCycler FastStart DNA Master SYBR Green I was used for the RT-PCR analysis. Standard curve graph was formed by logarithm of nucleic acid concentrations and Cq values obtained by RT-PCR method. This method can be used to identify the activity of the ARO8 gene in other *Saccharomyces cerevisiae* strains for further studies.

Introduction

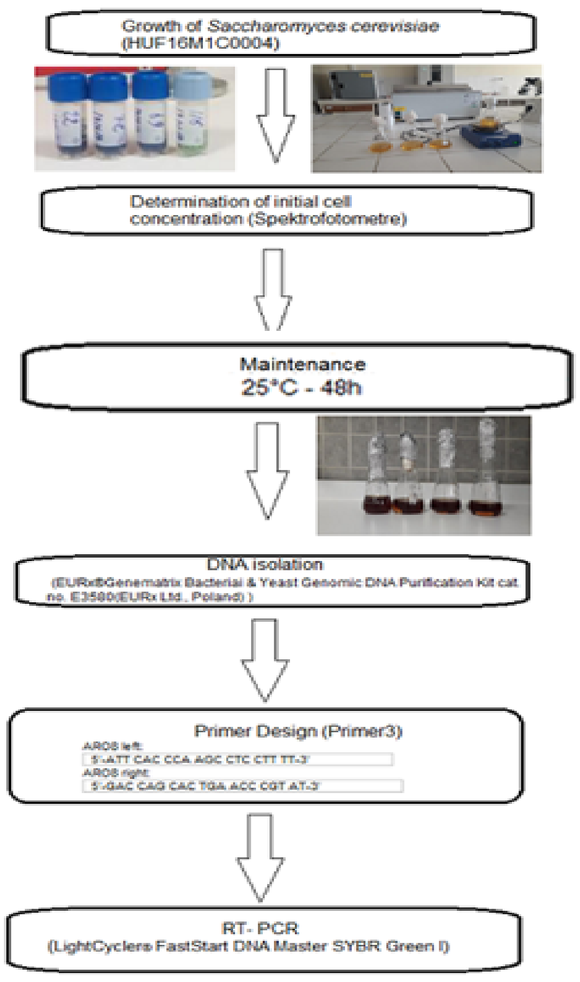


Aromatic amino acid aminotransferase I (ARO8) and Aromatic amino acid aminotransferase II (ARO9) in the degradation of tryptophan.



Aromatic amino acid aminotransferase I (ARO8) and Aromatic amino acid aminotransferase II (ARO9) in the tyrosine biosynthesis.

Methodology



Results

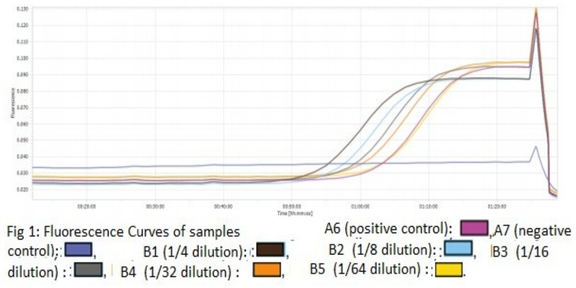


Fig 1: Fluorescence Curves of samples

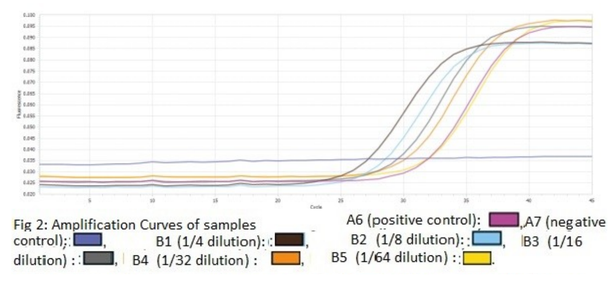
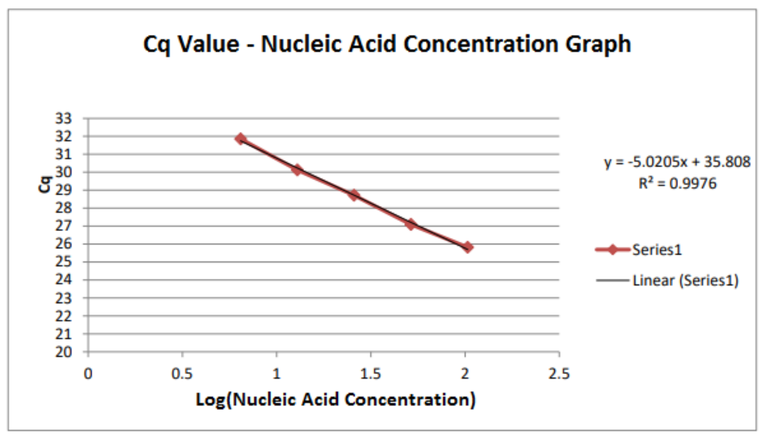


Fig 2: Amplification Curves of samples

Color	Position	Sample	Cq	Call	Sample Type	Standart	Cq Average	Cq Error
	A6	6	31.33	Positive	Positive control	-	31.33	0.00
	A7	7	-	Negative	Negative control	-	-	0.00
	B1	13	25.82	Positive	Standard	-	25.82	0.00
	B2	14	27.09	Positive	Standard	-	27.09	0.00
	B3	15	28.72	Positive	Standard	-	28.72	0.00
	B4	16	30.13	Positive	Standard	-	30.13	0.00
	B5	17	31.86	Positive	Standard	-	31.86	0.00



Position	Nucleic acid concentration	Cq Value	Logarithm of nucleic acid concentration	Nucleic acid concentration
A4	Unknown	32,26	0,705179283	5,072 ng/µl
A5	Unknown	31,27	0,902390438	7,987 ng/µl

Conclusion

The Cq value which are a fractional number of cycles, is threshold amount of fluorescence at the PCR kinetic curve. In this study, Cq values of dilutions (1/4, 1/8, 1/16, 1/32, 1/64) were determined by using RT- PCR. Standard curve graph was formed by logarithm of nucleic acid concentrations and Cq values obtained by RT-PCR method. Equation of standard curve graph was calculated as y= -5.0205x +35.808 and R^2 value was found as 0.99.

Nucleic acid concentration of unknown samples was calculated by using standard curve graph equation and Cq values.This method can be used to identify the activity of the ARO8 gene in other *Saccharomyces cerevisiae* strains for further studies.

Acknowledgement

The study was carried out with dear technical assistants Merve Ezeroğlu, Fatma Kelkitli.

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