Chemosphere 135 (2015) 418–426

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Saccharomyces cerevisiae SHSY detoxifies petroleum *n*-alkanes by an induced CYP52A58 and an enhanced order in cell surface hydrophobicity ☆

Abdulsamie Hanano [a,*], Mouhnad Shaban [a], Ibrahem Almousally [a], Mahmoud Al-Ktaifani [b]

[a] Department of Molecular Biology and Biotechnology, Atomic Energy Commission of Syria (AECS), B.P. Box 6091, Damascus, Syria
[b] Department of Chemistry, Atomic Energy Commission of Syria (AECS), B.P. Box 6091, Damascus, Syria

HIGHLIGHTS

- A PCO-grown *S. cerevisiae* expresses an induced microsomal protein of 59 kDa.
- A new gene of *CYP52A* family is cloned and therefore referred as *CYP52A58.*
- CYP52A58 preferentially catalyzes the terminal hydroxylation of *n*-hexadecane.
- PCO-grown yeast has a modified composition in cell wall mannoproteins.
- The content of unsaturated fatty acids is increased in PCO-grown SHSY.

ARTICLE INFO

Article history:
Received 4 September 2014
Received in revised form 3 November 2014
Accepted 5 November 2014
Available online 27 November 2014

Handling Editor: Frederic Leusch

Keywords:
Saccharomyces cerevisiae SHSY
n-Alkanes
CYP52A58
Detoxification

ABSTRACT

Environmental hydrocarbon contamination has a serious hazard to human health. Alkanes, the major component of hydrocarbons, can be consumed by various species of yeast. We previously identified a new strain SHSY of *Saccharomyces cerevisiae* with a remarkable ability to utilize the petroleum crude-oil (PCO) in aqueous solution. The current study demonstrated that the *n*-alkanes-assimilation activity of *S. cerevisiae* SHSY was related to an induced microsomal protein of 59 kDa approximately. The identified ORF encoded a protein of 517 amino acids and shared 93% sequence identity with an alkane-inducible hydroxylase CYP52A53 isolated from *Scheffersomyces stipitis* CBS. It was therefore referred as CYP52A58. The catalytic activity of the recombinant CYP52A58 was confirmed by the hydroxylation of *n*-alkanes, it showed an optimal mono-terminal hydroxylation activity toward *n*-hexadecane. Moreover, the ability of the yeast to use *n*-alkanes was accompanied with an increasing level in cell wall mannoproteins. Two differential protein bands were detected in the mannoproteins extracted from PCO-grown yeast. In parallel, a significant increase in the fatty acids content with a high degree of unsaturation was subsequently detected in the PCO-grown yeast. This study characterizes a safe and potential microorganism to remove *n*-alkanes from the aquatic environment.

1. Introduction

Petroleum crude-oil (PCO) hydrocarbons are known to have a worrying risk on organisms. Their contaminations in soil and aquifer are therefore of great environmental concern. The toxicity of alkanes, a major component of petroleum hydrocarbons, to microorganisms, plants, animals and humans is well established. In humans for example, high concentrations of inhaled alkanes can result in anesthetic effects or narcosis (Zhu et al., 2005). Biological detoxification of hydrocarbons in the environment can take place by microorganisms including bacteria, filamentous fungi, and yeasts (Hamamura et al., 2006; Kanaly and Harayama, 2010). Especially, *n*-alkanes-detoxifying microorganisms were given an increasing attention last two decades. Herein, few yeast genera, namely, *Candida, Yarrowia,* and *Pichia* were isolated from petroleum contaminated environments and their potential abilities to degrade *n*-alkanes were studied. Conventionally, the capability of several yeast species to use *n*-alkanes is mediated by specific enzymatic systems, the initial attack being catalyzed by oxygenases

☆ Database: nucleotide sequence data are available in the GenBank databases under the accession number KF595150.

* Corresponding author. Tel.: +963 11 2132580; fax: +963 11 6112289.
E-mail address: ashanano@aec.org.sy (A. Hanano).

http://dx.doi.org/10.1016/j.chemosphere.2014.11.011
0045-6535/

(Fritsche and Hofrichte, 2000). Yeast cytochrome P450 alkane hydroxylases (CYP450ALKs), classified in the family CYP52s, were presented to act as key enzymes in *n*-alkanes degradation pathway (Van Beilen and Funhoff, 2007). These cytochrome P450s, identified as microsomal enzymes, have been isolated from various yeast species such as *Candida* sp. and *Yarrowia* sp. and their catalytic activities have been described to be involved in the terminal mono-oxygenation of *n*-alkanes chain giving corresponding alcohols, as the initial step of the *n*-alkane degradation pathway (Schunck et al., 1989; Iida et al., 1998). The regulation of the *CYP450ALK* genes expression in yeasts was extensively studied, their expression found to be highly induced by *n*-alkanes and repressed strongly by glycerol and weakly by glucose (Iida et al., 2000). Interestingly, Hirakawa et al. have identified an upstream activating sequence that provides the transcriptional induction in response to *n*-alkanes in the *ALK1* promoter, named therefore an Alkane-Responsive Element 1 (ARE1) (Hirakawa et al., 2009). ARE1 contains an E-box-like sequence, and ARE1-like sequences are found in the promoters of several genes involved in *n*-alkane assimilation, including a subset of *ALK* genes. Moreover, the functions of *YAS1* and *YAS2*, encoding basic helix-loop-helix (bHLH) transcription factors, as essential activators for the transcriptional induction of *ALK1* by *n*-alkanes have been reported (Hirakawa et al., 2009).

As alkanes are chemically very inert and hydrophobic molecules, their uptake by yeast poses challenge related to their low water solubility. This includes the production of surface-active compounds, known as biosurfactants, that enhance hydrocarbon solubility (Thanomsub et al., 2004). More, it was reported that when grew on hydrophobic compounds, including *n*-alkanes, few yeast species increased their surface hydrophobicity or produced surfactants and emulsifiers (Coimbra et al., 2009).

After a complete genome sequence was available to the public, the yeast *Saccharomyce cerevisiae* became an ultimate model organism for the study of biological processes in eukaryotes. Although the extensive history of *S. cerevisiae* as a natural factory for producing lipids, proteins, enzymes, and vitamins (Plessas et al., 2007), the capability of this microorganism to use naturally hydrocarbons has never been demonstrated. In a previous study, we isolated and identified a strain of *S. cerevisiae* SHSY with a remarkable ability to grow in the presence of PCO as a unique carbon sources (Hanano et al., 2013). Genetic approaches conducted to identify a *CYP52A* homolog gene harbored by SHSY. We hypothesized that the PCO-removal activity of the strain SHSY is mediated by an inducible *CYP52A* homolog gene. The current study is therefore constructed to achieve four main objectives: (i) subcellular localization and biochemical characterization of the PCO-degrading activity of *S. cerevisiae*, (ii) molecular identifying of the responsible *CYP52A* gene, (iii) expression and biochemical characterization of the recombinant protein, (iv) evaluation of cell wall composition and hydrophobicity when *S. cerevisiae* grows on hydrocarbons. The outcome of this work will significantly contribute to add a novel aspect to the biological functions of the yeast *S. cerevisiae*, as a potential microorganism to remove the petroleum hydrocarbons pollutants from the aquatic environment.

2. Materials and methods

2.1. Petroleum crude-oil, chemicals, yeast isolate and culture conditions

Light petroleum crude-oil (PCO) (light brown; pH, 5.2) was obtained from petroleum reservoir for oil field (TANAK), located in north western region of Syria. All chemicals, including *n*-alkanes and fatty acid standards, are purchased from Merck & Co., USA. The strain SHSY of *Saccharomyces cerevisiae* was previously isolated and identified by Hanano et al. (2013). For maintaining culture, the yeast was cultured in YPD (Yeast extract Peptone Dextrose) broth (HiMedia, India) at 28 °C. The PCO-dismantling yeast was cultured, for all experiments, using minimal salt medium (MSM) containing 5% (v/v) of PCO as a unique carbon source in a total volume of 100 mL. The culture was incubated at 28 °C on the rotary shaker at 200 rpm for 7, 14, 21 and 28 days. To study the induction of CYP52A expression by *n*-alkanes, the yeast was cultured as previously described using YPD medium containing 0.5% (v/v) of *n*-hexane (C_6H_{14}), *n*-hexadecane ($C_{16}H_{34}$) and *n*-tetracosane ($C_{24}H_{50}$) on the rotary shaker at 200 rpm for 7, 14, 21 and 28 days at 28 °C.

2.2. Estimation of yeast biomass and COP-degrading activity

Biomass was determined as described by Hanano et al. (2013) and expressed as g 100 mL^{-1} FW (fresh weight). Yeast PCO-degradation activity was estimated by determining the total hydrocarbons using a spectrophotometer (VIS 6315, JENWAY, UK) as described by Rahman et al. (2002). As control, parallel extractions were done from culture medium without yeast.

2.3. Preparation of yeast microsomes and determination their CYP450s content

Isolation of microsomal fraction from yeast cells was performed essentially as described by Record et al. (1998). The pellet corresponds to microsomes, was gently homogenized and suspended in 10 mM Tris–HCl, pH 8 containing 10% glycerol (v/v). Protein concentrations in each fraction was estimated by Bradford assay (Bio-Rad) using bovine serum albumin as a standard (Bradford, 1976). CYP450s content was determined in solubilized microsomes by the method of Omura and Sato (1964). CYP450s content was measured from the difference in the spectrum at 450 and 490 nm using an extinction molecular coefficient of $\varepsilon_{450-490} = 91\ mM^{-1}\ cm^{-1}$.

2.4. Quantitative real-time PCR

Real-time PCR was carried out in 48-well plates using a StepOne cycler from Applied Biosystems (USA) and according to DeCoste et al. (2011), 25-μL reaction mixtures contained 0.5 μM of each target (CYP52qF and CYP52qR) and reference (18SqF and 18SqR) genes primers (Table S1), 12.5 μL of SYBR Green PCR mix (Bio-Rad, USA) and 2.5 μL of 10-fold diluted cDNA. QPCR conditions were as described previously by Hanano et al. (2013). Each point was triplicated and the average of C_T was taken. Subsequently, the relative quantification $RQ = 2^{(-\Delta\Delta CT)}$ of target gene was calculated by the software of Applied Biosystems.

2.5. Genome walking and sequencing of S. cerevisiae CYP52A gene

The full-length *CYP52A* gene was identified by genome walking procedure using BD GenomeWalker universal kit (BD Biosciences). First, genomic DNA of *S. cerevisiae* SHSY was carefully isolated as previously described by Hanano et al. (2013). Two genome walking libraries were constructed by digesting 2.5 μg of genomic DNA using two different restriction enzymes *Dra*I and *Eco*RV according to manufacturer's protocol. After a step of DNA purification, a genome walking adaptor was ligated using 4 units of T4 DNA ligase to both ends of the DNA fragments. Ligation was carried out overnight at 16 °C, and two different DNA libraries were therefore constructed. Gene-specific primers were designed based on alignment of the conserved sequences corresponding to I-helix and heme-binding regions (Craft et al., 2003). The primers CYP52gwF and CYP52gwR (Table S1) were therefore designed for the genome walking PCR to amplify the DNA upstream and downstream of the