Chapter 5
Candida famata (Debaryomyces hansenii)

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Abstract  Debaryomyces hansenii (teleomorph of asporogenous strains known as Candida famata) belongs to the group of so named ‘flavinogenic yeasts’ capable of riboflavin oversynthesis during starvation for iron. Some strains of C. famata belong to the most flavinogenic organisms known (accumulate 20 mg of riboflavin in 1 ml of the medium) and were used for industrial production of riboflavin in USA for long time. Many strains of D. hansenii are characterized by high salt tolerance and are used for ageing of cheeses whereas some others are able to convert xylose to xylitol, anti-caries sweetener. Transformation system has been developed for D. hansenii. It includes collection of host recipient strains, vectors with complementation and dominant markers and several transformation protocols based on protoplasting and electroporation. Besides, methods of multicopy gene insertion and insertional mutagenesis have been developed and several strong constitutive and regulatable promoters have been cloned. All structural genes of riboflavin synthesis and some regulatory genes involved in this process have been
identified. Genome of *D. hansenii* has been sequenced in the frame of French National program ‘Genolevure’ and is opened for public access.

**Keywords** Riboflavin, *D. hansenii*, *C. famata*, flavinogenic, transformation, insertional mutagenesis

### 5.1 Introduction

*Candida famata* (teleomorph: *Debaryomyces hansenii*) is osmotolerant yeast able to grow in the presence of high concentrations of NaCl. The yeast tolerates 4 M of the salt, whereas growth of *Saccharomyces cerevisiae* is completely inhibited by 1.7 M NaCl (Onishi, 1963; Prista et al., 1997). Both *C. famata* and *D. hansenii* strains are able to overproduce riboflavin (vitamin B<sub>2</sub>) in iron-deficient media (Gadd and Edwards, 1986; Shavlovsky and Logvinenko, 1988). Some *C. famata* mutants are the most flavinogenic organisms known (Heefner et al., 1988, 1992, 1993; Stahmann et al., 2000). Strains of *D. hansenii* and *C. famata* are found in habitats with high salinity levels, such as sea water, brines, salted food (cheeses, sausages) (Norkrans, 1966; Seiler and Busse, 1990; Lépingle et al., 2000). Ability of *D. hansenii* to grow in the presence of high NaCl concentrations resulted in a designation of the species as halotolerant (or, according to some authors: halophilic) yeast. Some *D. hansenii* strains are considered as potential producers of xylitol (Parajo et al., 1996; Roseiro et al., 1991). Osmotolerance of *D. hansenii* is advantageous for some biotechnological applications; it allows quasi-non-sterile production and high product/educt concentrations, conditions which should reduce production costs (Breuer and Harms, 2006). *D. hansenii* (*C. famata*) belongs to the monophyletic clade containing organisms that translate CTG as serine instead of leucine (Fitzpatrick et al., 2006). This chapter mainly is focused on the flavinogenic strains of the anamorph *C. famata*.

Complete sequence of *D. hansenii* genome has been published (http://cbi.labri.fr/Genolevures/elt/DEHA) and is available for public use (Dujon et al., 2004). It opens new opportunities for study and elucidation of molecular mechanisms of halotolerance and riboflavin overproduction in *D. hansenii* (*C. famata*) and using this yeast in basic and applied research.

Recently, the review on *D. hansenii* has been appeared (Breuer and Harms, 2006). This useful review contains the comprehensive data on many aspects of physiology, biochemistry, genetics and potential biotechnological applications of *D. hansenii*. However, in spite of the fact that the only industrial biotechnological application of *D. hansenii* to date is the use of the mutant strain of anamorph *C. famata* for riboflavin production (at Archer Daniels Midland Co. in USA), authors did not pay attention on this important aspect. They also did not mention on development of transformation system for *C. famata* and cloning structural and regulatory genes involved in riboflavin synthesis. Our review aims to fill these gaps.