

Production of Humanlike Recombinant Proteins in *Pichia pastoris*

From Expression Vector to Fermentation Strategy

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Ever since Invitrogen of Carlsbad, CA (www.invitrogen.com) acquired the distribution rights in 1993 for the *Pichia pastoris* yeast protein expression system, *P. pastoris* has gained widespread popularity, as witnessed by an increasing number of publications (Figure 1). The expression system was originally developed as a single-cell protein production platform by Philips Petroleum of Bartlesville, OK, and later adapted for heterologous protein expression (1, 2). It is freely distributed among academic research laboratories, although its use for commercial production requires a license from the current patent holder, Research Corporation Technologies of Tucson, AZ (www.rcotech.com).

Renewed interest in yeast and fungal expression systems in general and the *P. pastoris* expression system in particular has been spurred by a growing demand for scalable and cost-

effective humanlike therapeutic protein manufacturing systems. The goal is to achieve high fermentation yields in processes that extend for days instead of the weeks required for mammalian expression systems. Today the *P. pastoris* system is licensed to more than 160 companies in the biotechnology, pharmaceutical, vaccine, animal health, and food industries. More than 500 heterologous proteins have been expressed in this host (3, 4).

The first of those proteins entered human clinical trials in 1996, followed by an increasing number of candidate therapeutic proteins and antigens (5). All yeast-based biopharmaceutical products currently on the market in the United States and Europe are manufactured in *Saccharomyces cerevisiae*; however, a recombinant DNA hepatitis B vaccine and interferon alpha derived from *P. pastoris* have been marketed in India since 1999 and 2002 respectively by Shanta Biotech (www.shantabiotech.com). Similarly, a recombinant human insulin was approved in India in 2003 and marketed by the joint venture of Shanta Biotech and Biocon (www.biocon.com).

Bipha, a subsidiary of Yoshitomi Pharmaceutical (now Mitsubishi Pharma Corporation) has been manufacturing recombinant Human Serum Albumin (rHSA) since 2000 using *P. pastoris* in its Chitose, Japan, factory. The capacity of this plant is an impressive one million vials of rHSA a year, the eq actually increase capacity to over 40 tons a year (6; subsequent

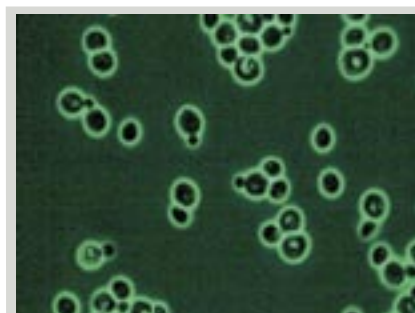


Photo 1 JAMES CREGG, KECK GRADUATE INSTITUTE, CLAREMONT CA (WWW.KGIE.EDU)

references 7–17 are called out in Table 2). The dosage/vial of 12.5 g is extremely high compared with other biopharmaceuticals and truly represents a testimony to the high expression levels of *P. pastoris* and establishment of large-scale fermentation technology: up to 80,000-L reactors.

AN EMERGING EXPRESSION SYSTEM

P. pastoris is a robust unicellular methylotropic yeast (Photo 1). It combines the unique advantages of prokaryotic growth characteristics and expression levels with the ability to perform posttranslational protein modifications available only in eukaryotic systems (Table 1). Several heterologous proteins expressed in *P. pastoris* have reached expression levels as high as grams per liter. Table 2 lists several therapeutically relevant proteins — including vaccines, antibody fragments, hormones, cytokines, and matrix proteins — along with their achieved expression levels.

In the absence of glycerol, *P. pastoris* uses methanol as its carbon

PRODUCT FOCUS: THERAPEUTIC PROTEINS

PROCESS FOCUS: PROTEIN EXPRESSION (PRODUCTION), FERMENTATION, SCALE-UP

WHO SHOULD READ: PROCESS DEVELOPMENT, MANUFACTURING, AUTOMATION, FACILITY PLANNERS

KEYWORDS: *P. PASTORIS*, METHYLOTROPIC YEAST, METHANOL UTILIZATION PHENOTYPES, AOX1 AND AOX2 PROMOTERS, GLYCOSYLATION ENGINEERING, BATCH AND FED-BATCH, DO CASCADE, METHANOL SENSOR

LEVEL: INTERMEDIATE