Also, the genetic instability of plasmids is more of a problem in *B. subtilis* than in *E. coli.* Finally, the high levels of excretion that have been observed with native *B. subtilis* proteins have not yet been obtainable with heterologous protein (i.e., foreign proteins produced from recombinant DNA).

Other gram-positive bacteria that have been considered as hosts include *Streptococcus cremoris* and *Streptomyces* sp. These other systems are less well characterized than *B. subtilis.*

Both gram-negative and gram-positive bacteria have limitations on protein processing that can be circumvented with eucaryotic cells.

14.3.4. Lower Eucaryotic Cells

The yeast, *Saccharomyces cerevisiae,* has been used extensively in food and industrial fermentations and is among the first organisms harnessed by humans. It can grow to high cell densities and at a reasonable rate (about 25% of the maximum growth rate of *E. coli* in similar medium). Yeast are larger than most bacteria and can be recovered more easily from a fermentation broth.

Further advantages include the capacity to do simple glycosylation of proteins and to secrete proteins. However, *S. cerevisiae* tends to hyperglycosylate proteins, adding large numbers of mannose units. In some cases the hyperglycosylated protein may be inactive. These organisms are also on the GRAS list, which simplifies regulatory approval and makes yeast particularly well suited to production of food-related proteins.

Generally, the limitations on *S. cerevisiae* are the difficulties of achieving high protein expression levels, hyperglycosylation, and good excretion. Although the genetics of *S. cerevisiae* are better known than for any other eucaryotic cell, the range of genetic systems is limited, and stable high protein expression levels are more difficult to achieve than in *E. coli.* Also, the normal capacity of the secretion pathways in *S. cerevisiae* is limited and can provide a bottleneck on excreted protein production, even when high expression levels are achieved.

The methylotrophic yeasts, *Pichia pastoris* and *Hansenula polymorpha,* are very attractive hosts for some proteins. These yeasts can grow on methanol as a carbon-energy source; methanol is also an inducer for the AOX 1 promoter, which is typically used to control expression of the target protein. Very high cell densities (e.g., up to 100 g/l) can be obtained. Due to high densities and, for some proteins, high expression levels, the volumetric productivities of these cultures can be higher than with *E. coli.* Protein folding and secretion are, also, often better than in *E. coli.* These yeasts do simple glycosylation and are less likely to hyperglycosylate than *S. cerevisiae.* Like many host systems, their effectiveness is often a function of the target protein. The disadvantages of the methylotrophic yeast are due to the high cell density and rate of metabolism, which creates high levels of metabolic heat that must be removed and high oxygen demand. Effective induction of expression, while maintaining cell activity, requires very good process control due to methanol's dual rate as growth substrate and inducer. Further, high levels of methanol are inhibitory (i.e., substrate inhibition), which also demands good process control. Scale-up to large reactors often is very challenging, since heat removal, oxygen supply, and process control are typically more difficult in large reactors with longer mixing times (see Chap-