About 20% of all protein in *E. coli* is translocated across the inner membrane into the periplasmic space or incorporated into the outer membrane. As the reader will recall from Chapter 4, secreted proteins are made with a signal or leader sequence. The presence of a *signal sequence* is a necessary (but not sufficient) condition for secretion. The signal sequence is a sequence of amino acids attached to the mature protein, and the signal sequence is cleaved during secretion.

Many benefits are possible if a protein is secreted. Secretion eliminates an undesired methionine from the beginning of the protein. Secretion also often offers some protection from proteolysis. Periplasmic proteases exist in *E. coli,* but usually at a low level. They are most active at alkaline pH values, and pH control can be used to reduce target protein degradation. The environment in the periplasmic space promotes the correct protein folding in some cases (including the formation of disulfide bridges). Proteins in the periplasmic space can be released by gentle osmotic shock, so that fewer contaminating proteins are present than if the whole cell were lysed.

Even more attractive would be the extracellular release of target proteins. Normally, *E. coli* does not excrete protein (colicin and haemolysin are the two exceptions), but a variety of schemes to obtain excretion in *E. coli* are being developed. Strategies usually involve either trying to disrupt the structure of the outer membrane or attempting to use the colicin or haemolysin excretion systems by constructing a fusion of the target protein with components of these excretion systems. Excretion without cell lysis can simplify recovery and purification even more than secretion alone, while achieving the same advantages as secretion with respect to protein processing. Excretion also facilitates the potential use of continuous immobilized cell systems.

Excretion of normally cytoplasmic or human-designed proteins is problematic. Two preliminary reports for the excretion of normally cytoplasmic proteins have been made, but the general principles for the extension of excretion to cytoplasmic proteins are still being developed. At this time, the excretion of normally secreted proteins can be obtained in *E. coli* (and other cells) even when the protein is derived from animal cells. However, extension to nonsecreted proteins is difficult.

The lack of established excretion systems in *E. coli* has led to interest in alternative expression systems. Also, in some cases patent considerations may require the use of alternative hosts.

14.3.3. Gram-positive Bacteria

The gram-positive bacterium, *Bacillus subtilis,* is the best studied bacterial alternative to *E. coli.* Since it is gram positive, it has no outer membrane, and it is a very effective excreter of proteins. Many of these proteins, amylases and proteases, are produced commercially using *B. subtilis.* If heterologous proteins could be excreted as efficiently from *B. subtilis,* then *B. subtilis* would be a very attractive production system.

However, *B. subtilis* has a number of problems that have hindered its commercial adoption. A primary concern has been that *B. subtilis* produces a large amount and variety of proteases. These proteases can degrade the product very rapidly. Mutants with greatly reduced protease activity have become available, but even these mutants may have sufficient amounts of minor proteases to be troublesome. *B. subtilis* is also much more difficult to manipulate genetically than *E. coli* because of a limited range of vectors and promoters.