EXTRACELLULAR PROTEASE PRODUCTION BY B. AMYLOLIQUEFACIENS

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INTRODUCTION

B. amylo1iquefaciens produces large amounts of two major secretory proteases: alkaline protease and neutral protease. Protease production by this organism is very unusual in its response to inhibitors of transcription. Using the antibiotics rifampicin and actinomycin D, which block transcription, we have demonstrated the presence of an apparent mRNA pool, capable of supporting protease production for about sixty minutes after addition of the antibiotics [1].

To examine this phenomenon at the molecular level required hybridization studies of protease mRNA production using a cloned gene probe.

ALKALINE PROTEASE mRNA PRODUCTION BY B. AMYLOLIQUEFACIENS

The gene for the alkaline extracellular protease of B. amylo1iquefaciens was isolated using a cDNA probe synthesized using a synthetic oligonucleotide primer mixture specific for a short (14 nucleotide) segment of the gene (as derived from the protein sequence) and total mRNA isolated from the organism. The entire gene is contained within a 2.9 kb HindIII restriction fragment of DNA cloned in pBR322 (pBAP1).

This fragment has been completely sequences, and the gene sequence corresponds exactly with that of Wells et al., 1983 [2]. The protease is synthesized with a propeptide of as yet unclear function preceding the sequence of the mature protein. The cleavage site of the signal peptide has recently been determined by Vasantha and Thompson, 1986 [3].

The cloned alkaline protease gene was used as a probe to examine mRNA production by B. amylo1iquefaciens, using the technique of Northern analysis.

Protease production is subject to repression by high levels of amino acids in the incubation medium. A build-up of alkaline protease mRNA occurs during incubation of cells in low amino acids medium (LAA) for various times, after incubation of these same cells in high amino acids medium (HAA) (repressive) for 75 minutes (Figure 1). Two transcripts are evident, the most prominent (1.4 kb) is of a size consistent with that
Fig. 1. Effect of rifampicin addition at zero and 75 minutes on protease production by washed cells of B. amyloliquefaciens in the presence of low and high amino acid levels. Cells were harvested at OD₆₀₀ of 3.6, washed twice and resuspended to the same cell density in either low (0.025%) or high (0.5%) casamino acids medium and incubated with shaking at 30°C, rifampicin (0.5 μg/ml) was added at zero and 75 minutes to 20 ml samples of these cells. 1 ml samples were taken, centrifuged and the supernatants assayed for protease activity. (-——-) Low casamino acids, no addition of drug. (———) High casamino acids, no addition of drug. (—Δ—Δ—) High casamino acids, rifampicin added at zero minutes, an identical curve was obtained in low casamino acids medium. (—○—○—) High casamino acids, rifampicin added at 75 minutes. (—□—□—) Low casamino acids, rifampicin added at 75 minutes. Arrows indicate time of rifampicin addition.

predicted from the DNA sequence of the cloned gene (Figure 2). The identity of the minor species (1.5 kb) is unclear, and remains to be investigated.

The decay of alkaline protease mRNA which occurs during prolonged incubation in low amino acids medium (LAA) was examined. Messenger RNA build-up was allowed to proceed for 30 minutes, at which time rifampicin was added to block transcription, and further time samples were taken. Using a scanning laser densitometer to measure band densities, a half-life of approximately 20 minutes was determined for the 1.4 kb transcript (Figure 3).

The findings presented here are consistent with the concept of a pool of unusually stable mRNA being responsible for the prolonged synthesis of alkaline protease in the presence of transcription inhibitors. The neutral protease needs to be examined in a similar manner.