STUDY OF PROTEINS SECRETED BY GROWING *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

The growing unaerated cells of *Saccharomyces cerevisiae* secreted a number of proteins in the extracellular broth. The amount and the gel electrophoretic profiles of these secretory proteins differed with the stage of the growth and the sampling position of the fermentation vessel.

INTRODUCTION

STUDY of protein secretion has remained an active area of investigation after the publication of Blobel hypothesis[1,2]. According to current thinking most of the secretory proteins are synthesized with a 15–20 amino acid (mainly hydrophobic) extension at the N-terminal end. This sequence of amino acids called a signal sequence plays a pivotal role in facilitating the protein secretion and later it is cleaved off by a specific protease[3]. In bacteria, many secretory proteins e.g. alkaline phosphatase, β-lactamase, etc have been thoroughly studied. The information gained from these studies has been used to advantage in fusing the structural sequences of the genes of interest with the appropriate regulatory sequences of the genes of the secretory proteins such that the hybrid gene products are secreted in the medium and become amenable to easy recovery[4,5]. In yeast, an ideal host for gene cloning in view of its ability for glycosylation, the studies of the secretory proteins have been limited.

Preliminary studies have indicated that the secretion of proteins during yeast growth is affected by the concentration of carbon dioxide and sodium chloride in the medium and by the rate of aeration[6]. It was also observed in these studies that significant differences in the extracellular protein concentration can occur between the top and the bottom of the fermentor. The observation is of interest since some spacially fractionated secretory protein products could lend themselves to recovery by the bubble fractionation obviating the need of processing total fermentor volumes. This paper deals with the identification of polypeptides secreted by non-aerobically growing *S. cerevisiae*. The bubbles were provided by *in situ* carbon dioxide generation. The regulatory gene sequences of distinct and prominent polypeptides identified in this study can be further assessed for their value in fusions with the gene sequences of interest.

MATERIALS AND METHODS

S. cerevisiae (Fleischmann's Baker's yeast, Jan. 18, 85 B) was inoculated (0.25% w/v) and grown at 32°C in a (1 L tall beaker) laboratory fermentor containing 800 ml synthetic medium[7]. The pH was held constant at 5. The fermentor was kept on a magnetic stirrer (3.8 cm stirring bar, 400 rpm). Approximately 5 ml broth samples were withdrawn from the top, middle (400 ml mark) and bottom points of the fermentor at the indicated times. In the samples, 0.1 ml of 10% (w/v) sodium dodecyl-sulphate (SDS) was mixed and the contents were immediately spun at 3100 rpm for 15 min at room temperature. The supernatant was removed with a Pasteur pipette without disturbing the pellet and stored at 4°C for various assays. The pellet was used for the determination of cell dry weight. The Coomassie Blue dye binding microassay was used for the determination of proteins[8,9]. The results of determinations with this method were identical with the results of determinations by the method of Lowry *et al*[10]. Polypeptides were resolved on 12% (w/v) acrylamide–SDS gels in a slab apparatus[11]. The buffer (62.5 mM Tris base containing, w/v, 20% sucrose, 0.001% bromophenol blue and 10% SDS, pH 6.8) and the sample (or a mixture of standard molecular weight markers) were mixed in equal proportions and the solution was heated at 100°C for 5 min before loading (50 μl) on the gels. Electrophoresis was performed at 25 mA in 25 mM Tris base – 190 mM glycine – 3.47mM SDS – 9 mM β-mercaptoethanol at pH 8.5 until the dye marker had moved three-quarters of the way down.

The polypeptide bands were visualized by silver staining[12]. All five sample reported in figures 1 and 2 were run on the same gel slab.

RESULTS AND DISCUSSION

The results of extracellular protein concentrations in a 10 hr non-aerated batch are given in table 1. The growth reached a plateau by the end of the fermentation and there was no lysis of cells. Larger amounts of proteins are secreted during early growth followed by a stabilization at a lower level. The lowering in extracellular concentration of proteins with advance in time may result from their increased degradation. The differences in protein levels at various positions in the fermentor are apparent (more than two fold differences) in spite of stirring, although there are no apparent gradients in pH and glucose concentrations. It is possible that a slight fractionating effect is introduced as the secretory proteins rise with the bubbles[13]. No discernible difference in cell concentration by position was observed (at a given time).

The samples of the middle position of the fermentor were analyzed for polypeptides on polyacrylamide-SDS gels (figure 1) at various stages of growth. The individual polypeptide peaks tend to be larger for the 7 hr sample than for the 4.5 and 10 hr samples, in keeping with the observation for total

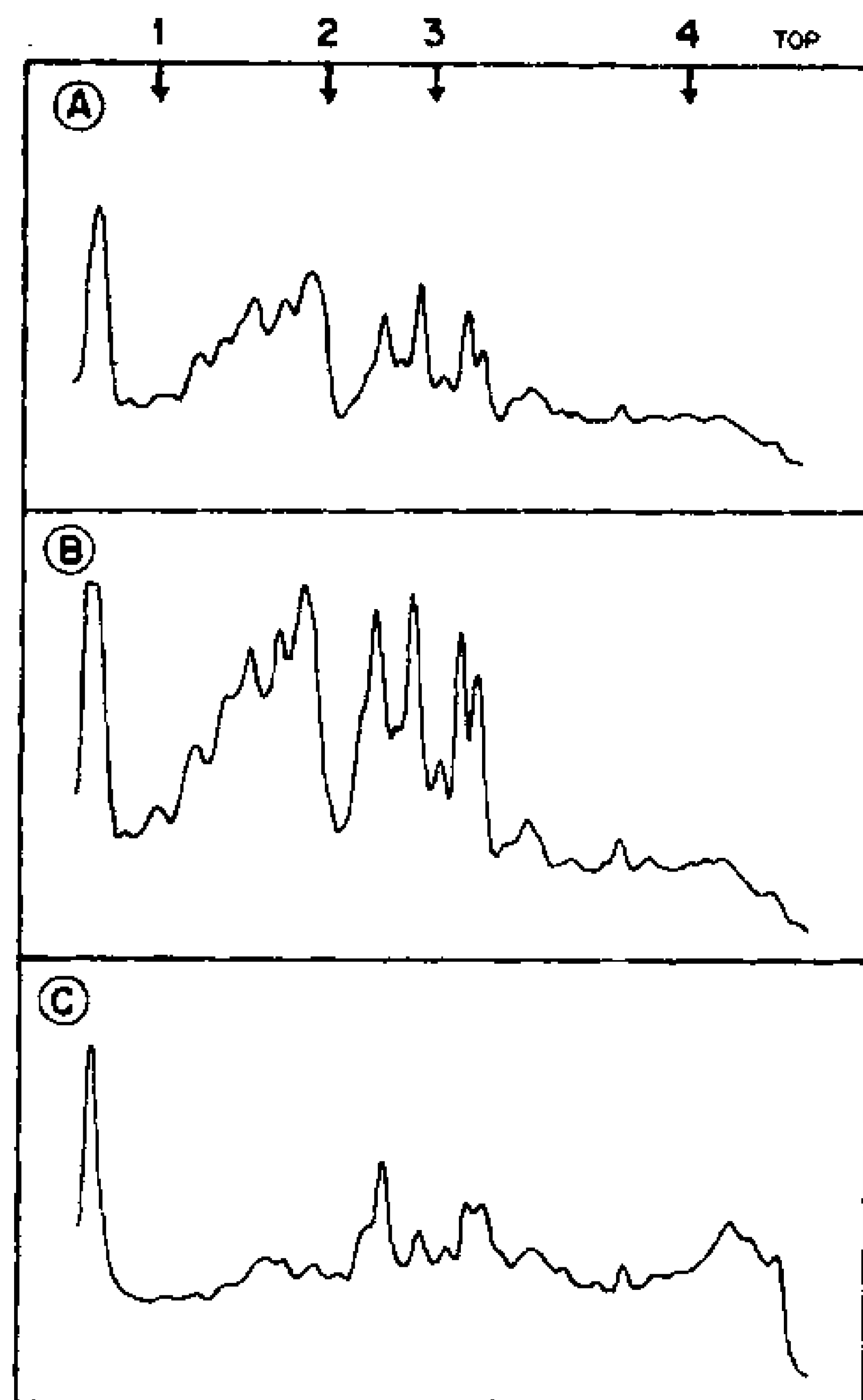

Figure 1. Densitometer tracing of gels showing the profile of polypeptides in samples taken from the middle of the fermentor after 4.5 hr (panel A), 7 hr (panel B) and 10 hr (panel C) of non-aerated growth. Arrows indicate the positions of 1. lysozyme (14.4 kD), 2. ovalbumin (45 kD), 3. albumin from bovine serum (66 kD), and 4. β-galactosidase (116 kD) run on a separate lane of the electrophoresis gel slab.

Table 1 *Amount of yeast secretory proteins in samples taken from top, middle and bottom of the fermentor during various stages of the growth*

Sampling time (hr)	Dry weight* mg ml^{-1}	Protein μg ml^{-1}		
		Top	Middle	Bottom
4.5	5.0	313	304	163
7.0	10.1	169	361	181
10.0	7.9	141	130	114

* Same cell concentration at all three positions.

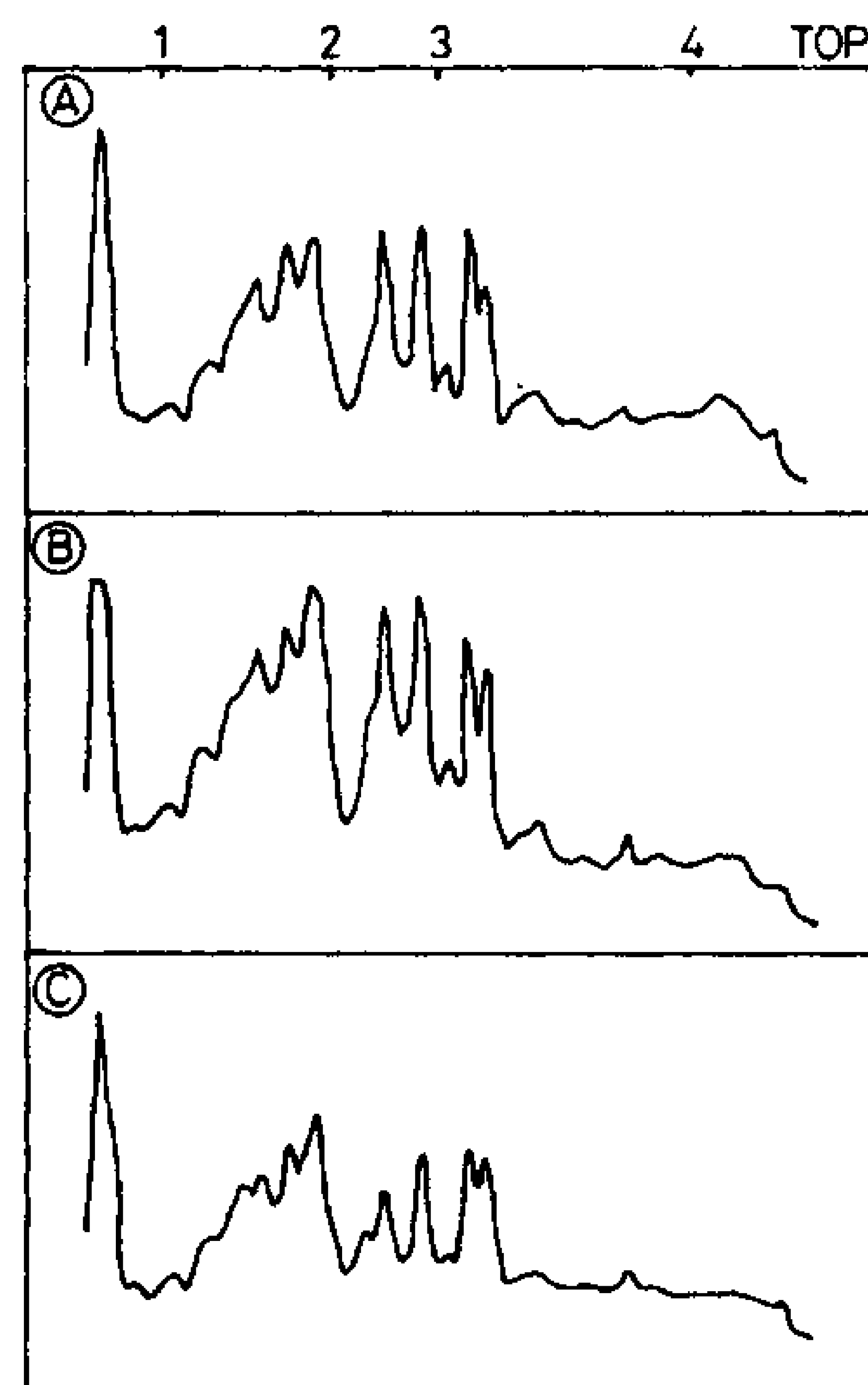

Figure 2. Densitometer tracing of gels showing the profile of polypeptides in samples taken at 7 hr at three different positions in the fermentor: Top (panel A), Middle (panel B) and Bottom (panel C) of non-aerated growth. Panel B (Middle position) is the same as panel B of figure 1 and links the two figures. Arrows indicate the same positions as for figure 1.

protein of the middle position, shown in table 1. Specific peaks vary greatly with time as expected.

The samples withdrawn from three positions of the fermentor during growth also were analyzed for polypeptides on polyacrylamide-SDS gels (table 2, figure 2). The summary of results at 7 hr indicates that approximately 10–13 major polypeptides with a wide spectrum of molecular weights (17.5 kD to 138 kD) are resolved on these gels. It is interesting to note that there are significant variations in the profile of polypeptides at three fermentor positions as well as the stages of the growth. The molecular weights of three most prominent polypeptides which comprised the bulk of total secretory proteins for each batch sample time are also given in table 2 in order of their abundance. The relative concentrations are qualitatively described by 'low medium and high' in table 3. As summarized in table 2, the most prominent polypeptides secreted during early growth (4.5 hr) are entirely different than the most prominent polypeptides secreted later (10 hr). These results point towards the possibility of either a differential gene expression or a selective translation including post translational modifications of certain messages or both.

The data are preliminary but it is clearly shown that certain polypeptides could be predominantly found at a specific position in the fermentor in a given growth phase. Such a situation is likely to be significant in large production fermentors. It appears likely that bubble fractionation of the proteins is a major contributor to the polypeptide separation as a function of the fermentor height. Here the bubbles are generated within the system by

Table 2 *A molecular weight (Mr) profile of yeast secretory proteins in samples taken from top, middle and bottom of the fermentor during various stages of the growth*

Sampling time (hr)	Sampling position	Number of polypeptides in Mr(kD) range			Mr(kD) of three prominent polypeptides*
		122–138	54–75	17.5–39	
4.5	Top	3	4	4	28,39,62.5
	Middle	3	4	5	39,28,62.5
	Bottom	1	4	5	39,28,54
7.0	Top	3	4	4	28,39,138
	Middle	3	4	4	39,28,62.5
	Bottom	3	4	5	39,62.5,24
10.0	Top	5	3	5	138,75,122
	Middle	4	4	4	138,54,75
	Bottom	4	4	5	138,122,54

* In order of abundance (relative concentrations).

Table 3 *A molecular weight (Mr) profile of yeast secretory proteins in samples taken from top, middle and bottom positions of the fermentor at the 7 hr sampling time*

Mr (kD)		Protein (relative concentration)		
		Bottom	Middle	Top
24	17.5-39 kD range	Medium/low	Medium	Medium
28		Low/medium	High	High
39		Medium/low	High	High
54	54–75 kD range	Low	Medium	Medium
62.5		Medium/low	High	Medium
75		Low	Medium	Medium
138	122–138 kD range	Low	Medium	High

This is an elaboration of the data in table 2 at the 7 hr sampling time as taken from figure 2. Major peak analysis.

the carbon dioxide evolving from the yeast cells. Back mixing from the top position to the middle position (due to a low length/diameter ratio of the tall 1 L beaker:about 2/1) seems to reduce the protein level at the top of the fermentor.

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NEWS

PROVING RESEARCH SAFETY

..."The burden of proof for control of research appears to be changing. Increasingly, the individual researcher or research facility must prove that the research is safe rather than the regulator prove that it is unsafe. A shift in responsibility is clearly occurring in the case of restrictions on scientific and technical communication. Under schemes proposed in 1980 by the American Council on Education, for example, cryptology researchers were asked to carry the burden of deciding which papers to submit to the Natl. Security Agency for review. A similar shift in the burden of responsibility occurred in 1980 changes in Natl. Science Foundation (NSF) grant policy, which made the grantee responsible for notifying NSF if, in the course of an NSF supported project, 'information or materials are developed which may affect the defense and security of the US'. If a fundamental constitutional right is involved, then in the past the courts have placed the burden of proof on the government to show a compelling need to infringe. But some legal scholars argue that the situation is now muddied because it is increasingly difficult to distinguish between pure speech and 'impure' special action."

[(US Congress, Office of Technology Assessment in *The Regulatory Environment for Science—A Technical Memorandum*, Washington, DC: US Government Printing Office, Feb. 86). Reproduced with permission from Press Digest, *Current Contents*®, No. 26, June 30, 1986, p. 10. (Published by the Institute for Scientific Information®, Philadelphia, PA, USA)].