GLUCOSE-6-PHOSPHATE DEHYDROGENASE ISOENZYMES

AMONG FIVE SPECIES OF <u>CANDIDA</u>

by

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ABSTRACT

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Polyacrylamide gel electrophoresis was performed with subsequent chemical staining to elucidate isoenzymes of the common enzyme glucose-6-phosphate dehydrogenase in five species of Candida. Band differences and scanning densitometric patterns demonstrated reproducible isoenzyme patterns within individual species and variances in patterns among different species. These findings suggest that this characteristic in combination with other morphological and physiological characteristics may be of value in the systematics of the taxonomy among these species.

ACKNOWLEDGMENTS

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER I

Introduction

The genus Candida is most often classified as a
yeast. The term yeast has no taxonomic significance
and is simply a morphologic form of a fungus. Due to
the lack of a perfect form in this genus, it is considered
a Deuteromycetes or Fungi Imperfecti (Rippon, 1982).

Morphological investigations are of less import-
ance than physiologicial characteristics in identification
of these organisms. Physiological traits which have been
emphasized for identification and classification include
carbohydrate fermentation and assimilation, nitrogen
utilization and production of enzymes (Rippon, 1982).

Although at least 200 species of yeast have been
described and probably thousands exist, only a few
species have been associated with the production of disease
in man. Within these medically important species, frequently,
however, the same yeasts are described as new species be-
cause of their propensity to infect many different body
areas. In order to prevent these misidentifications of
clinical isolates, better methods of identification and
taxonomy need to be developed (Rippon, 1982).

The concept of species is difficult to apply to
asporogenous (imperfect) yeast due to their lack of

natural crossing, however, recent studies have attempted
to relate and identify species as well as strains by
new methods. (Shepherd, 1985).

The biology of C. albicans shows many similar-
ities to the saprophytic ascosporogenous yeasts such as
Saccharomyces cervisiae (Shepherd, 1985). Since a common
generic name does not necessarily imply descent from a
common ancestor, it is probable that the genus Candida
may include a number of species that are imperfect forms
of Ascomycetes. As examples, C. pseudotropicalis is
possibly related to Kluyveromyces fragilis and C. guillier-
mondii is probably related to Pichia guilliermondii.
This is supported by the fact that there is no coherent
relationship in the molecular biology within the species
(Riggsby, 1985) (Shepherd, 1985).

Despite the medical importance of yeasts, partic-
ularily Candida species, few investigators have studied
them biochemically (Montes and Wilborn, 1985) (Shepherd,
1985). Areas which have been recently investigated in
order to better classify yeast, emphasize their natural
relationships to perfect genera and include cell wall
analysis of monosaccharides, DNA homology and immuno-
electrophoretic studies of cell antigens (Montrocher and
Claisse, 1984) (Riggsby, 1985).

DNA studies have shown homology between Candida
albicans and Candida stellatoidea with little relationship

to other species. DNA reassociation studies have established and confirmed perfect forms of several Candida species (Riggsby, 1985).

Antigenically, C.albicans, C. stellatoidea and C. tropicalis show 85-100% similarity while C. parapsilosis and C. guilliermondii show 50-70% similarity and C. krusei shows only 30% similarity (Riggsby, 1985). C. guilliermondii and C. krusei show very distinct antigenic uniqueness according to the current literature (Lyon and Domer, 1985).

Cell wall analysis of levels of monosaccharides, mannose and glucose, reveal the general trend of decreasing similarity in the following order: C. albicans, C. stellatoidea, C. tropicalis, C. parapsilosis, C. guilliermondii and C. krusei (Lyon and Domer, 1985).

DNA homology studies, however, do not always support these relationships. C. albicans and C. tropicalis, which show high antigenic correlations and many physiological similarities, have a low DNA homology (Riggsby, 1985).

The more frequently studied biochemical characteristics,such as production of enzymes, are receiving renewed attention. Current literature provides data and support for the existence of multimolecular forms of various dehydrogenase enzymes of C. albicans and the spectra of these isoenzymes are well defined for each growth phase (Tinsberg Suleinanov and Olifson, 1980).

It has been shown that the most complex isoenzyme spectrum of the dehydrogenases occurs in the logarithmic

growth phase (48 hrs) and that the enzyme glucose-6-phosphate
dehydrogenase has the greatest number of zones of activity
since, at log phase, within a growing population of cells,
the hexose monophosphate pathway predominates. At log phase,
G-6-PDH shows seven zones of activity for C. albicans
(Tinsberg, Suleinanov and Olifson, 1980). Earlier studies
have identified only two dimers of G-6-PDH (Kato, Sahm,
Schuette and Wagner, 1979).

It has also been reported that electrophoretic
relative mobilities of G-6-PDH and MDH vary between related
species and even strains. Species of Debaryomyces are
distinguishable by their electrophoretic patterns of glucose-
6-phosphate dehydrogenase and malate dehydrogenase (Nakase
and Suzuki, 1985).

The levels of G-6-PDH do not appear to reflect
activity alterations based on nitrogen sources supplied,
while carbon source variations may affect the levels (Hirai,
Shiotani, Tanaka and Fukui, 1976). This seems reasonable
in light of the role of G-6-PDH in metabolic pathways
concerned with an increase in cellular membranes and
conversion of carbon (as carbohydrates) to lipids (Evans
and Ratledge, 1983). Not only does the concentration
of G-6-PDH increase as the growth curve progresses, but
the number of isoenzymes also increases (Evans and
Ratledge, 1983) (Tinsberg, Suleinanov and Olifson, 1980).
Again this is reasonable due to an increased rate of metabo-

lism at peak replication times.

As indicated, the enzyme G-6-PDH is involved in the hexose monophosphate shunt and the pathway begins with the oxidation of glucose-6-phosphate to 6-phospho-gluconate by glucose-6-phosphate dehydrogenase, a NADP+ linked enzyme. The process ends with production of CO_2 and reduction of NADP+ to NADPH (Dyson, 1979).

The reaction appears as below (Dyson, 1979);

$$\text{G-6-P} + 12\text{NADP+} + 7H_2O \longrightarrow 6CO_2 + 12\text{NADPH} + 12 \text{ H+} + P_i$$
$$\text{G-6-PDH}$$
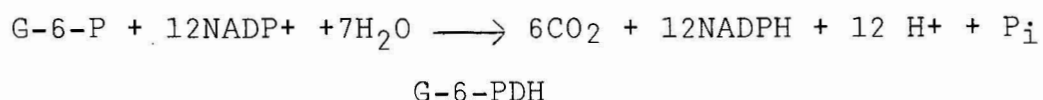
The primary purpose of this pathway is to generate NADPH which is used for lipid (fatty acid and steroid) synthesis, to maintain membrane components in their reduced state and presumably to provide additional reducing power for mitochondrial electron transport. An additional function of the pathway is to provide pentoses for DNA replication (Dyson, 1979) (Hirai, Shiotani, Tanaka and Fukui, 1976).

The purpose of this study is to demonstrate the isoenzyme patterns of G-6-PDH in five species of Candida. The existence of different isoenzyme patterns might be useful for identification purposes, as an adjunct to tax-onomic placement and expanding the enzymology of this group.

CHAPTER II

Materials and Methods

Organisms

Five clinically significant species of Candida
were used in the isoenzyme separation of G-6-PDH. Sub-
cultures were made of each species on Sabouroud's dextrose
agar (Emmon's modification) from stock cultures. After
forty-eight hours, species identification was made by
API 20C methodology (Ayerst Laboratories, 1982). See
Figure 1 for API 20C procedure.

Each species was again subcultured and grown
to a standard forty-eight hour growth phase at a constant
temperature of $30^{\circ}C$, $\pm$ $5^{\circ}C$.

Approximately ten colonies of each species were
then emulsified in individual 2 ml aliquots of .05M phosphate
buffer, pH 7.5. In order to obtain cell lysis sufficient
for enzyme analysis, each emulsion was transferred to a
mortar containing sterile sand. Yeast emulsion was then
ground with a pestle for two minutes and checked micro-
scopically for lysis. If lysis was not sufficient (greater
than 50% of the cells), grinding was repeated.

When all samples were adequately lysed, they were
centrifuged at 10,000 rpm for ten minutes at $4^{\circ}C$. The
supernatant served as the source of G-6-PDH enzyme.

Electrophoresis

Polyacrylamide gel electrophoresis was performed on each species in two separate trials to identify isoenzyme bands.

The medium used was a 7% gel containing 3.41 grams gel, .092 grams BIS, 15 μl Temed crosslinker, 49 ml gel buffer and .5 ml ammonium persulfate. Gel buffer was a .37M Tris-HCL, pH 8.9. The tank buffer contained .005M Tris-glycine buffer, pH 8.3.

For each run, six gels were placed in the buffer filled tank and equilibrated at 2 mv/gel for 15 minutes.

Samples were mixed in porcelain plate wells. Twenty drops of each species sample aliquot were mixed with a drop of bromphenol blue and .5M sucrose. A glucose-6-phosphate dehydrogenase control from Saccharo-myces cervisiae (Sigma Chemical Co.) was reconstituted with buffer and treated the same as the Candida samples.

Once loaded onto the gels, the voltage was set at 12 mv/gel to initiate the run and tighten the bands. Voltage was then increased to 24 mv/gel to complete phoresis. Time of phoresis varied slightly between runs but was always less than one hour.

All sample preparation, loading and phoresis was conducted at 0°C.

Staining

Sixty ml of gel buffer with 150 mg D-glucose-6-

phosphate, 30 mg MTT, 30 mg β-TPN and 20 mg PMS were
mixed for the staining process (Beutler and Morrison,
1968) (Shaw and Barto, 1965). Gels were removed from
tubes with water, placed into test tubes containing the
reaction mixture and incubated at 37°C for ten minutes.
Gels were then fixed in a 7% glacial acetic acid, 7% abso-
lute methanol fixative.

Scanning

Gels were analyzed on a Varian Scanning Densitometer.
Each gel was placed in a cuvette and scanned at a speed of
5 cm/min with a wavelength of 628 mμ, slit of 0.4.

CHAPTER III

Results

Organism Identification

Table I shows API 20C results on the yeast isolates.
The Analytical Profile Index 20C method allows identificat-
ion of yeast based on biochemical assimilation. Wells on
the API 20C incubation tray are inoculated with a suspension
of the organism in question. After 72 hours incubation,
all wells are compared with a positive and a negative growth
well and the turbidity (growth) recorded (Ayerst Laboratories,
Inc., 1982).

Assimilation patterns are compared to a computer-
ized data base derived from type and neotype cultures and
strains from various collections. Assimilations are com-
pared to expected assimilations for a given organism. The
degree of correlation provides assurance of correctness of
identification (Ayerst Laboratories, Inc., 1982).

Table I outlines the assimilation patterns obtained
and the degree of correlation. Excellent identification is
seen with C. albicans, C. parapsilosis and C. tropicalis and
indicates that the pattern obtained has a high frequency of
occurrence for that organism. The patterns of C. guillier-
mondii and C. krusei offer good likelihood with stated fre-

quencies.

PAGE Reaction

The staining results of the runs are seen in
Fig. 14 as diagrams of the actual gels. This was done
in order to facilitate visualization of the bands. Stain
intensities differ and are consistent with varying enzyme
activity.

Scan

Figures 2 through 13 show densitometric scans of
the control and five species of Candida. A diagram of the
bands is included below each graph to enable better com-
parison. Gain settings were adjusted in order to accommo-
date different staining intensities of individual gels and
to facilitate the inclusion and visualization of all band
peaks on the scan.

Saccharomyces cervisiae controls reveal five
distinct bands. In trial #1 (Fig. 2) band 2 has three
sub bands, a, b, and c, which are not detected in trial
#2 (Fig.3). The mobilities of bands 1 and 2 in run #1
are greater, making them more separated from bands 3, 4,
and 5 than they are in run #2. In both runs, band 2 is
the most intensely staining band.

Candida albicans shows the greatest number of bands
of the Candida species examined. In run #1 (Fig. 4), the
7th band appears to have three sub bands, a, b, and c.

For the same species in a second run (Fig. 5),only
six bands are evident with two sub bands, a and b, in
band 4 and two sub bands, a and b, in band 5. In both
runs, the first band is considerably ahead of and
distinct from the others. All band intensities are
approximately the same.

Candida parapsilosis has six bands. All bands
are close together with band 1 being the broadest and
most intensely staining in both runs. Band 6 also stains
very intensely. In run #1 (Fig. 6), bands 2 and 3 are
very close as are bands 4 and 5. In run #2 (Fig. 7),
separations are approximately equal and more distinct.
Band 6 is very close to the application point in both
runs.

Five bands are observed in Candida guilliermondii.
In both runs, band 1 is much more rapid in its mobility,
being distinctly separate from the others. In run #1
(Fig. 8) band 2 is also distinctly separated from 3,4,
and 5. However, in run #2 (Fig. 9), bands 2 and 3 are
close together but clearly separated from bands 4 and 5.
In both runs, band 4 and 5 are very broad and band 4
shows an increase in intensity from its most rapidly
migrating end to its more slowly migrating end.

Candida krusei also shows five bands. In both
runs, (Fig. 10 and 11) band 1 is very broad and has three
sub bands, a, b, and c. All bands in this species are

very close together.

Two distinct bands are seen in _Candida tropicalis_. In both runs (Fig. 12 and 13), band 1 has three sub bands a, b, and c, while band 2 has five sub bands, a, b, c, d, and e. Both bands are close together and migrate little from the point of application.

api 20C ™

REFERENCE NUMBER	PATIENT	SEX	AGE	SOURCE/SITE
1				Stock

DATE: 6/86   DEPT./SERVICE: PHYSICIAN:

	O	GLU	GLY	2KG	ARA	XYL	ADO	XLT	GAL	INO	SOR	MDG	NAG	CEL	LAC	MAL	SAC	TRE	MLZ	RAF
24 H	−	+	−	+	−	+	−	−	+	−	−	−	+	−	−	+	+	−	−	−
48 H	−	+	−	+	−	+	−	−	+	−	+	−	+	−		+	+	−	−	−
72 H								+			+					+	+	+		

Profile Number C- 2   5   6   2   1   7   0

ADDITIONAL INFORMATION: Excellent identification

IDENTIFICATION: Candida albicans

MICROSCOPIC MORPHOLOGY:

00 42 009   12/78/Y-5

---

api 20C ™

REFERENCE NUMBER	PATIENT	SEX	AGE	SOURCE/SITE
2				Stock

DATE: 6/86   DEPT./SERVICE: PHYSICIAN:

	O	GLU	GLY	2KG	ARA	XYL	ADO	XLT	GAL	INO	SOR	MDG	NAG	CEL	LAC	MAL	SAC	TRE	MLZ	RAF
24 H	−	+	−	+	−	+	+	−	+	−	−	+	+	−	−	+	+	+	+	−
48 H	−	+	−	+	−	+	+	−	+	−	+	+	+	−	−	+	+	+	+	−
72 H											+	+	+							

Profile Number C- 2   5   5   6   1   7   1

ADDITIONAL INFORMATION: Excellent identification

IDENTIFICATION: Candida tropicalis

MICROSCOPIC MORPHOLOGY:

00 42 009   12/78/Y-5

---

api 20C ™

REFERENCE NUMBER	PATIENT	SEX	AGE	SOURCE/SITE
3				Stock

DATE: 6/86   DEPT./SERVICE: PHYSICIAN:

	O	GLU	GLY	2KG	ARA	XYL	ADO	XLT	GAL	INO	SOR	MDG	NAG	CEL	LAC	MAL	SAC	TRE	MLZ	RAF
24 H	−	+	+	+	−	+	−	−	+	−	−	+	+	+	−	+	+	−	+	+
48 H	−	+	+	+	+	+	+	+	+	−	+	+	+	+	−	+	+	+	+	+
72 H																				

Profile Number C- 6   7   7   6   3   7   3

ADDITIONAL INFORMATION: Good likelihood   Freq. 1/1

IDENTIFICATION: Candida guilliermondii

MICROSCOPIC MORPHOLOGY:

00 42 009   12/78/Y-5

Table I

api 20C™

REFERENCE NUMBER	PATIENT	SEX	AGE	SOURCE/SITE
4				Stock

DATE	DEPT./SERVICE	PHYSICIAN
6/86		

	O	GLU	GLY	2KG	ARA	XYL	ADO	XLT	GAL	INO	SOR	MDG	NAG	CEL	LAC	MAL	SAC	TRE	MLZ	RAF
24 H	−	+	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−
48 H	−	+	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−
72 H																				

Profile Number C- 6   0   0   0   1   0   0

ADDITIONAL INFORMATION

Good likelihood Freq. 1/2

IDENTIFICATION

Candida krusei

MICROSCOPIC MORPHOLOGY

00 42 009   12/78/Y-5

---

api 20C™

REFERENCE NUMBER	PATIENT	SEX	AGE	SOURCE/SITE
5				Stock

DATE	DEPT./SERVICE	PHYSICIAN
6/86		

	O	GLU	GLY	2KG	ARA	XYL	ADO	XLT	GAL	INO	SOR	MDG	NAG	CEL	LAC	MAL	SAC	TRE	MLZ	RAF
24 H	−	+	−	+	+	+	+	−	+	−	+	−	+	−	−	+	+	−	+	−
48 H	−	+	+	+	+	+	+	−	+	−	+	+	+	−	−	+	+	+	+	−
72 H																				

Profile Number C- 6   7   5   6   1   7   1

ADDITIONAL INFORMATION

Excellent identification

IDENTIFICATION

Candida parapsilosis

MICROSCOPIC MORPHOLOGY

00 42 009   12/78/Y-6

Table I   (cont'd)

Figure 1

API 20C Clinical Yeast
Identification System Procedure

API 20C CLINICAL YEAST SYSTEM

Purpose

API 20C is a microtube method using 19 assimilation tests
for the identification of most clinically significant
yeasts and yeast-like organisms.

Materials

        Sterile wooden applicator sticks
        Sterile Pasteur pipettes, 5 ml.
        Squeeze bottle with tap water
        Incubation tray and lid
        API 20C strip
        Basal medium

Storage

The API 20C strips and basal medium should be stored at
2-8°C until used.

Procedure

        1. Preparation of strips
            a. Set up an incubation tray and lid
            b. Dispense 10 ml. of water into the
               incubation tray (use squeeze bottle).
            c. Place one strip in each incubation
               tray.

        2. Preparation of yeast suspension
            a. Allow the ampule of basal medium to
               reach room temperature before use.
            b. Remove the plastic cap and heat the
               ampule at 100°C. Allow the ampule to
               remain at that temperature for 5 minutes
               after the medium appears to melt to assure
               complete liquefaction.
            c. Remove ampule from heat and allow to cool
               for 10-20 minutes.
            d. Replace the plastic cap on the ampule.
               Snap the top off by applying thumb pressure
               at the base of the flattened side of the
               plastic cap.
            e. Prepare a yeast suspension in the API Basal
               Medium from a fresh culture of 48-72 hours

FIGURE 1

growth from Sabouroud's dextrose agar
or tryptic soy agar with 5% sheep blood.
The yeast suspension is standardized to
a density just below 1+ on a Wickerham
Card (lines appear clearly distinguished
but have rough edges).

3. Inoculation of the strip
    a. With a sterile Pasteur pipette, inoculate
       each cupule by placing the pipette tip
       against the side of the cupule. The sus-
       pension in the cupule should be level
       rather than concave or convex to avoid
       misreading of the test. Place a lid on
       the incubation tray.

4. Incubation of the strips
    a. Incubate the strip at $30^{\circ}C$ for 72 hours.

5. Reading the strips
    a. Reactions are read and recorded at 24,
       48 and 72 hours incubation.
    b. The 0 cupule serves as a negative control.
       Cupules showing turbidity heavier than the
       0 cupule are positive. Positive reactions
       should compare to the turbidity in the GLU
       cupule which serves as a positive growth
       control.

6. Identification of the organisms
    a. Identification of the organisms can be
       made with the API 20C Analytical Profile
       Index.

7. Disposal
    a. All contaminated trays, lids and strips
       are placed in a biohazard container to
       be autoclaved.

Reference: Analytabe Products, API 20C Clinical Yeast
           System, 1982.

Figure 2

Scan Run #1 Control
Saccharomyces cervisiae

0.4 nm 1.0 min 1 cycles set 1 cycles complete 0.4 nm 1.0 min 1

Time (5 cm/min)
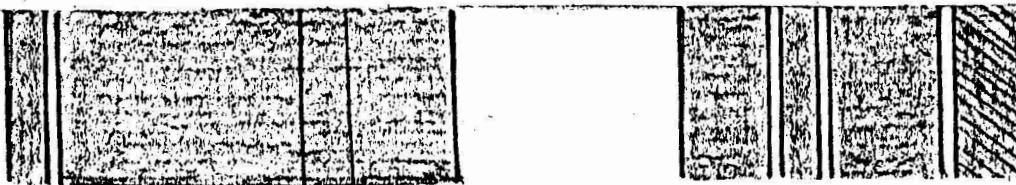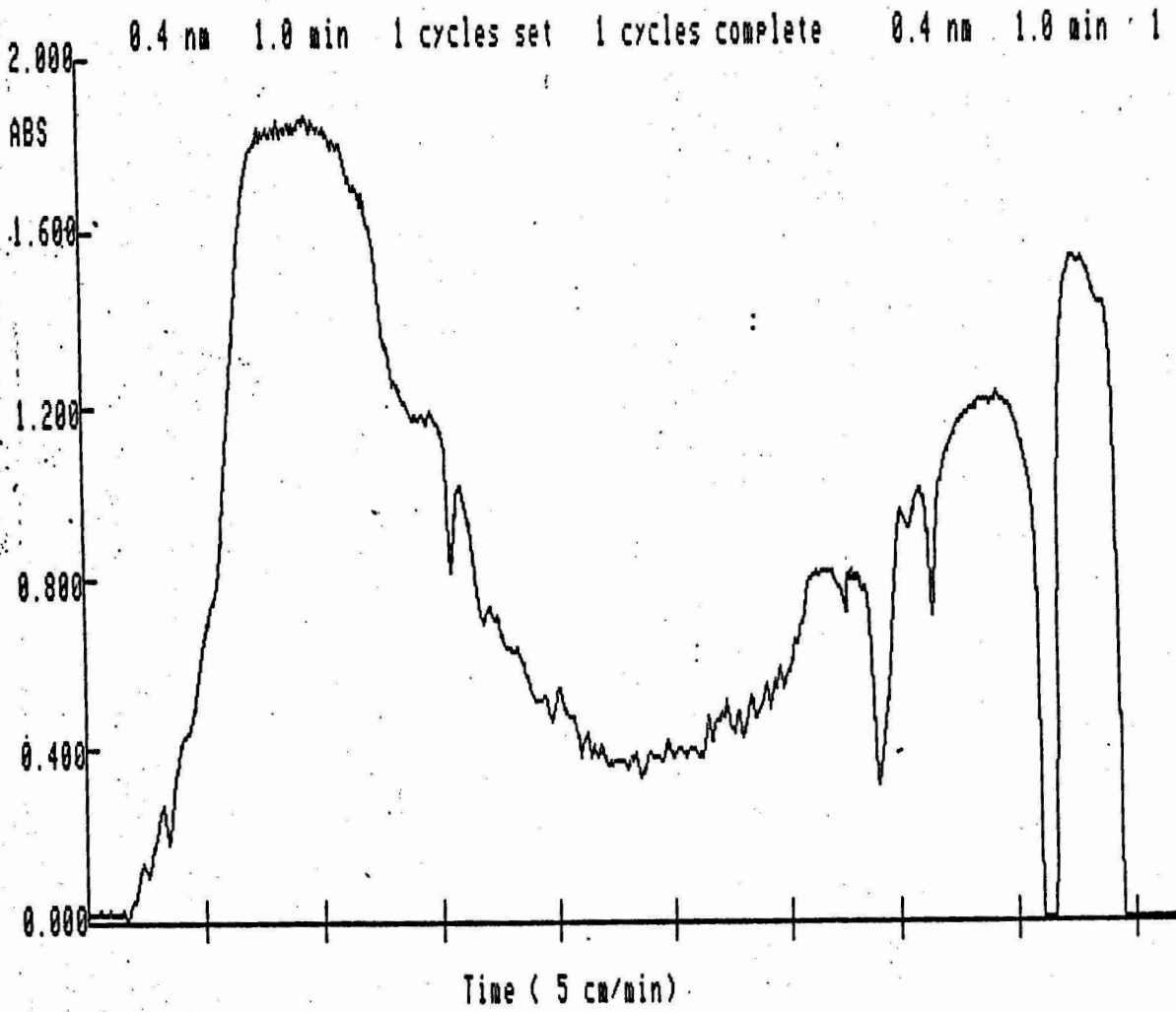
1   2a   2b 2c   3   4   5

Control

Run #1

FIGURE 2

Figure 3

Scan Run #2 Control
Saccharomyces cervisiae

18
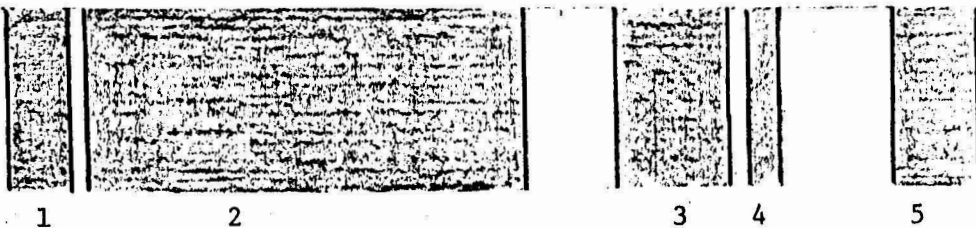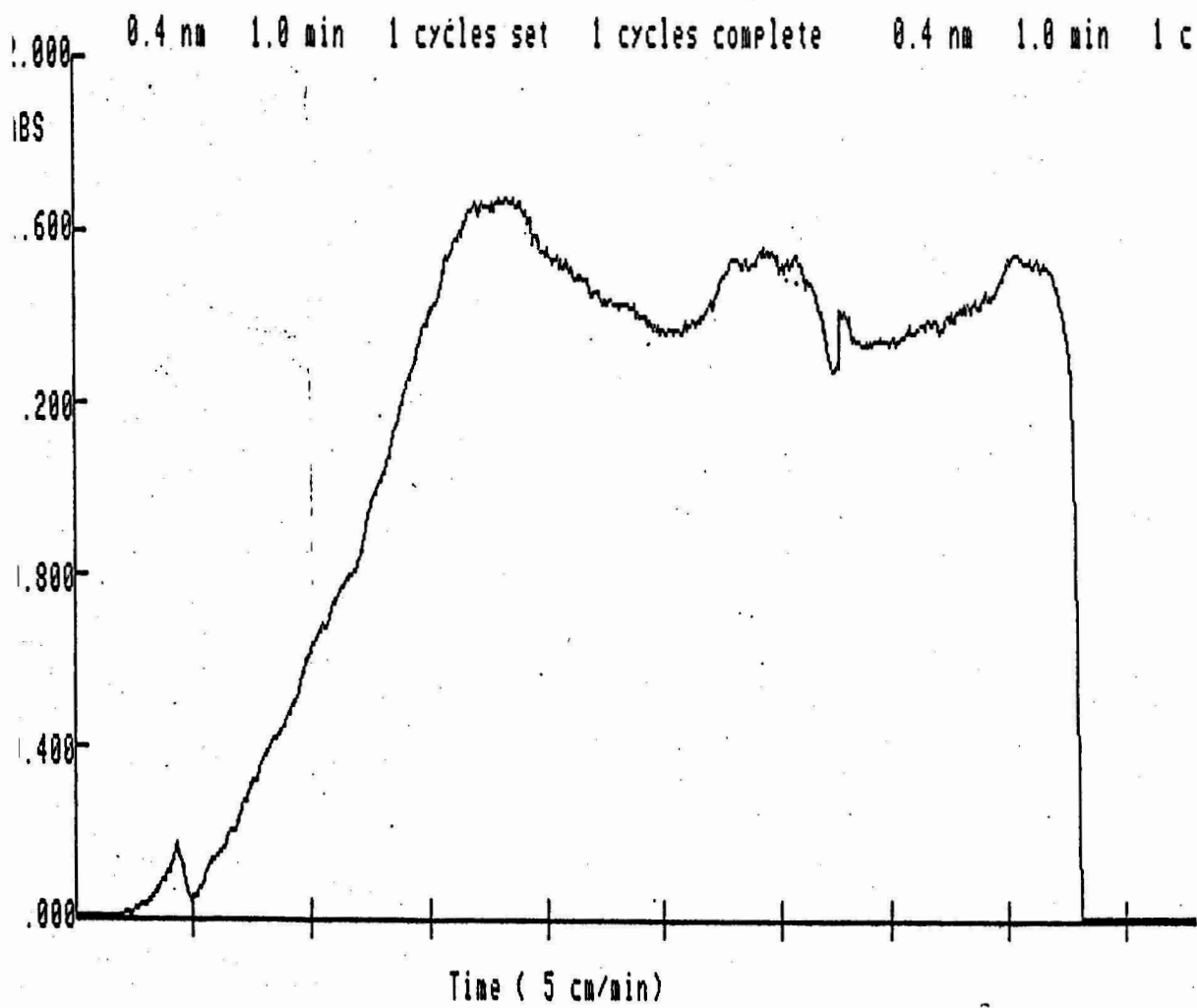
FIGURE 3

Figure 4

Scan Run #1
Candida albicans
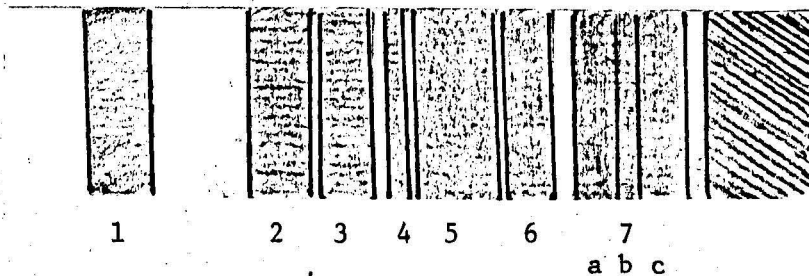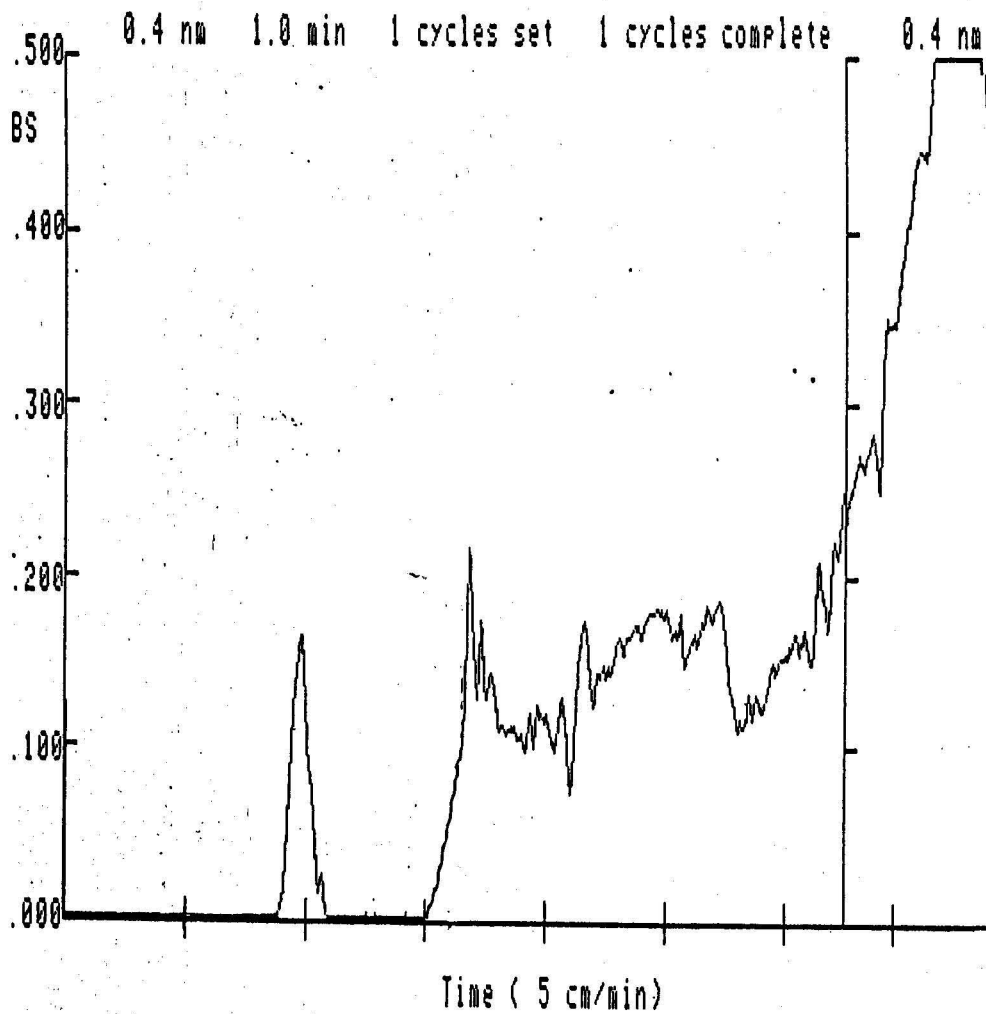
Candida albicans

Run #1

FIGURE 4

Figure 5

Scan Run #2
Candida albicans
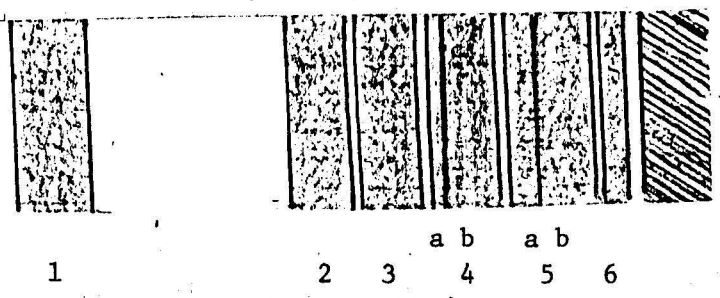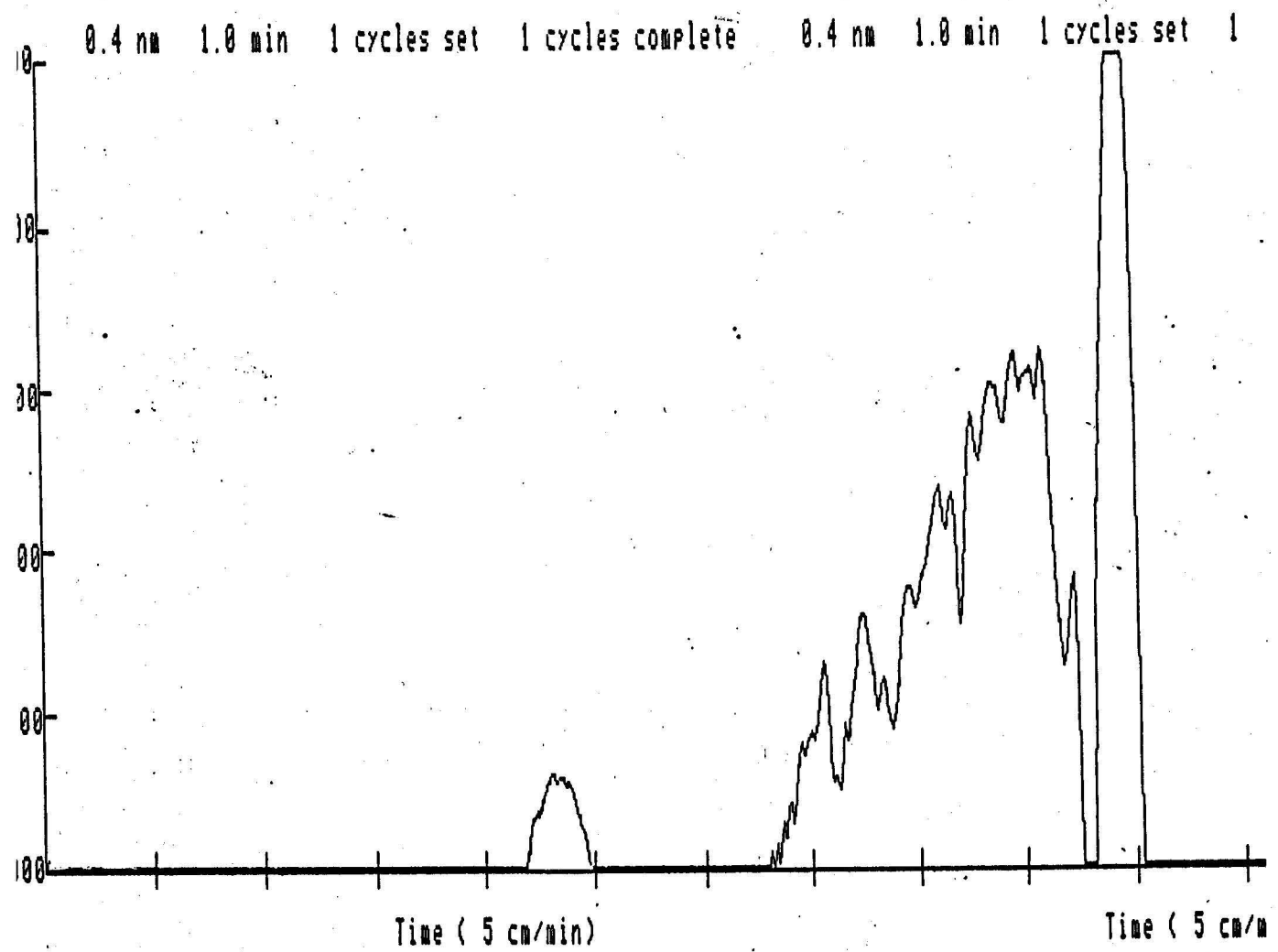
Candida albicans
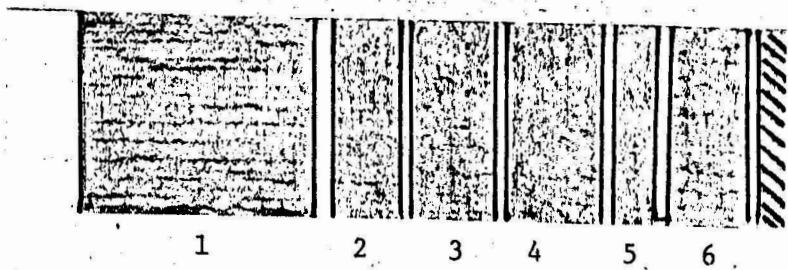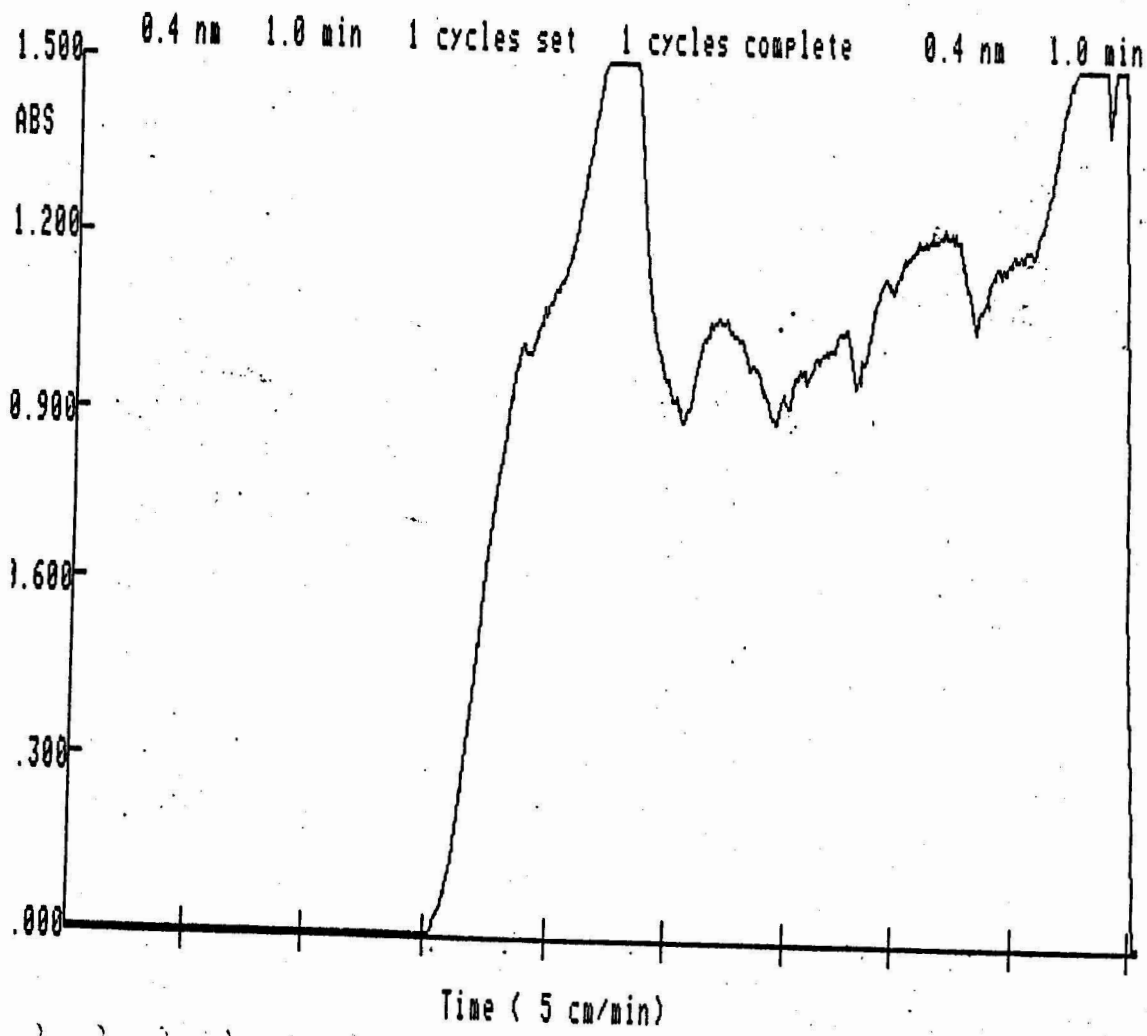
Run #2

FIGURE 5

Figure 6

Scan Run #1
Candida parapsilosis
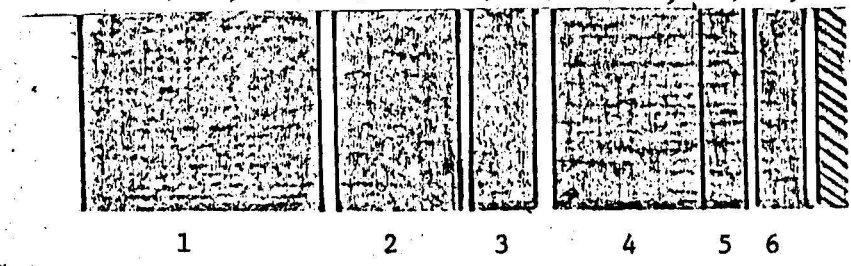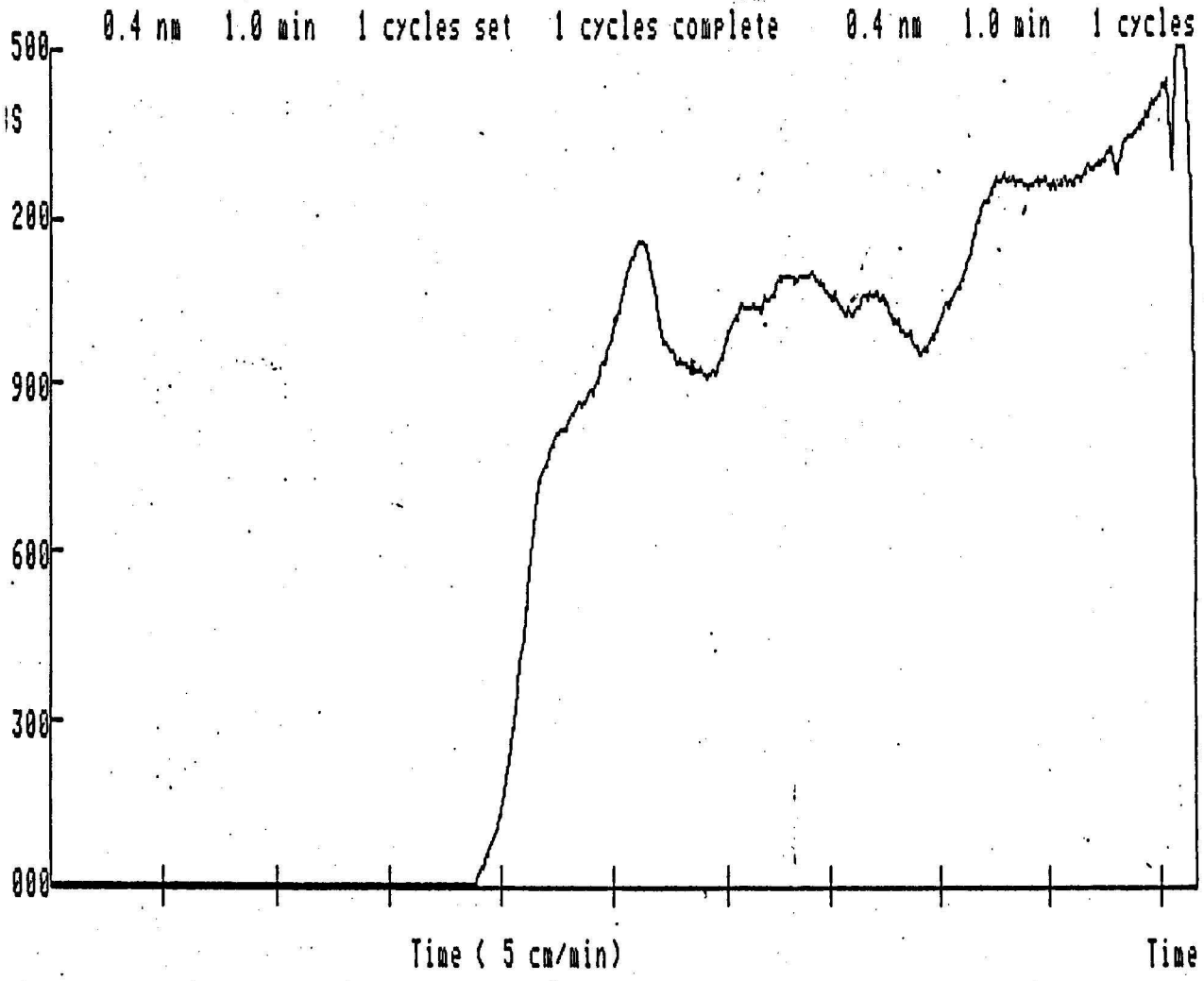
Candida parapsilosis

Run #1

FIGURE 6

Figure 7

Scan Run #2
<u>Candida</u> <u>parapsilosis</u>

Candida parapsilosis

Run #2

FIGURE 7

Figure 8

Scan Run #1
Candida guilliermondii
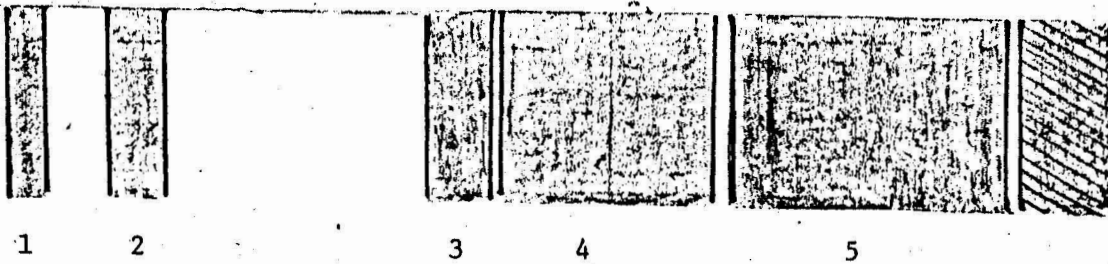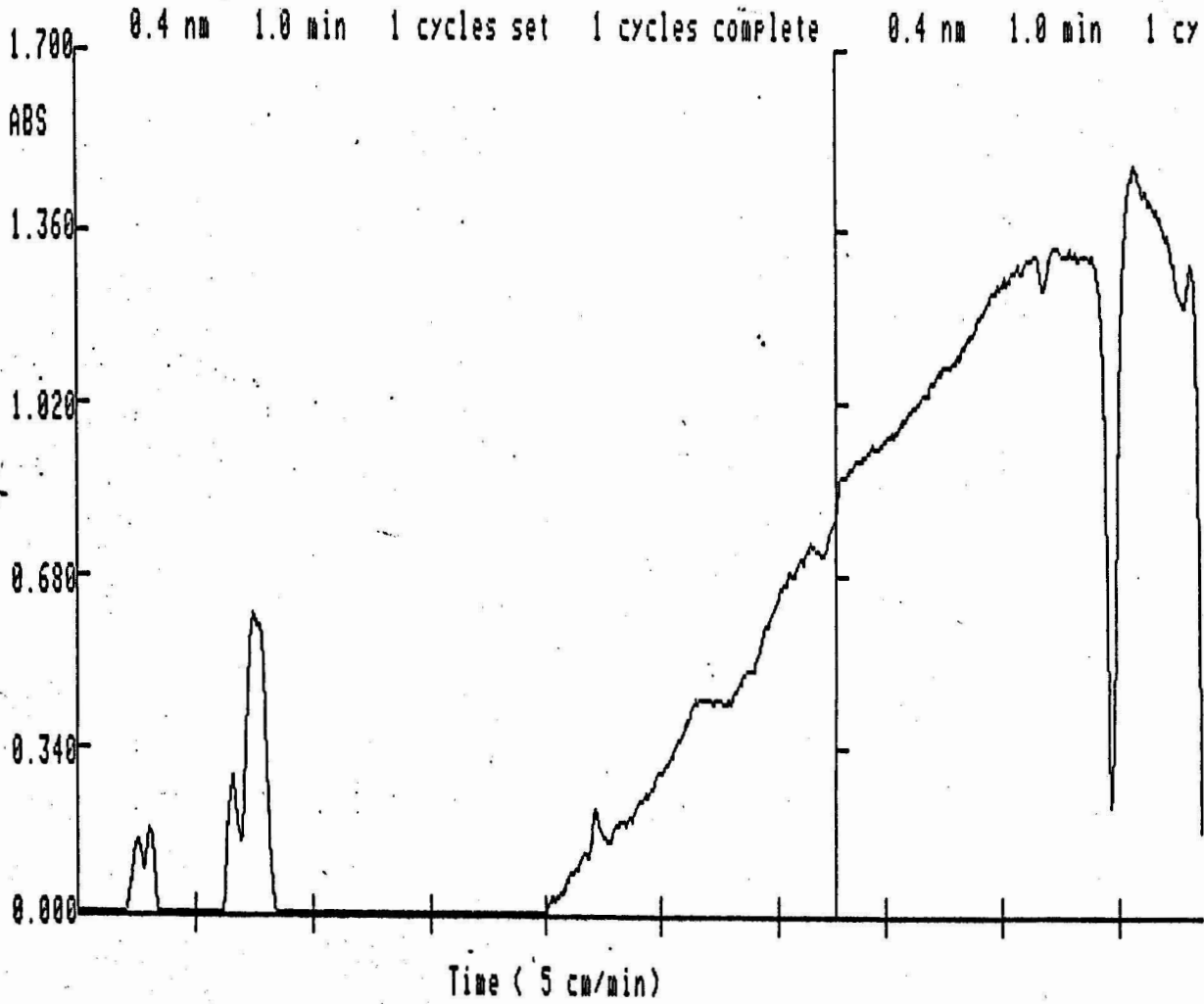
Candida guilliermondii

Run #1

FIGURE 8

Figure 9

Scan Run #2
Candida guilliermondii
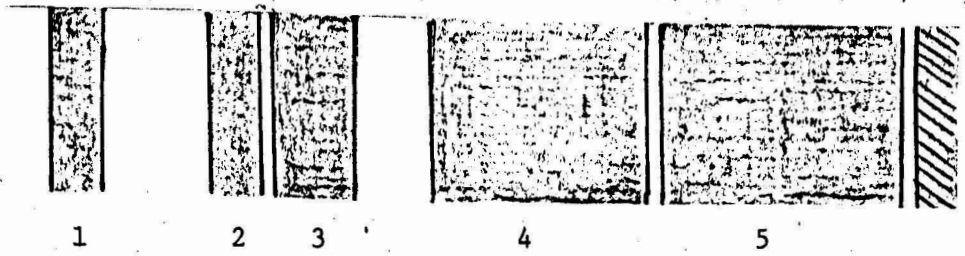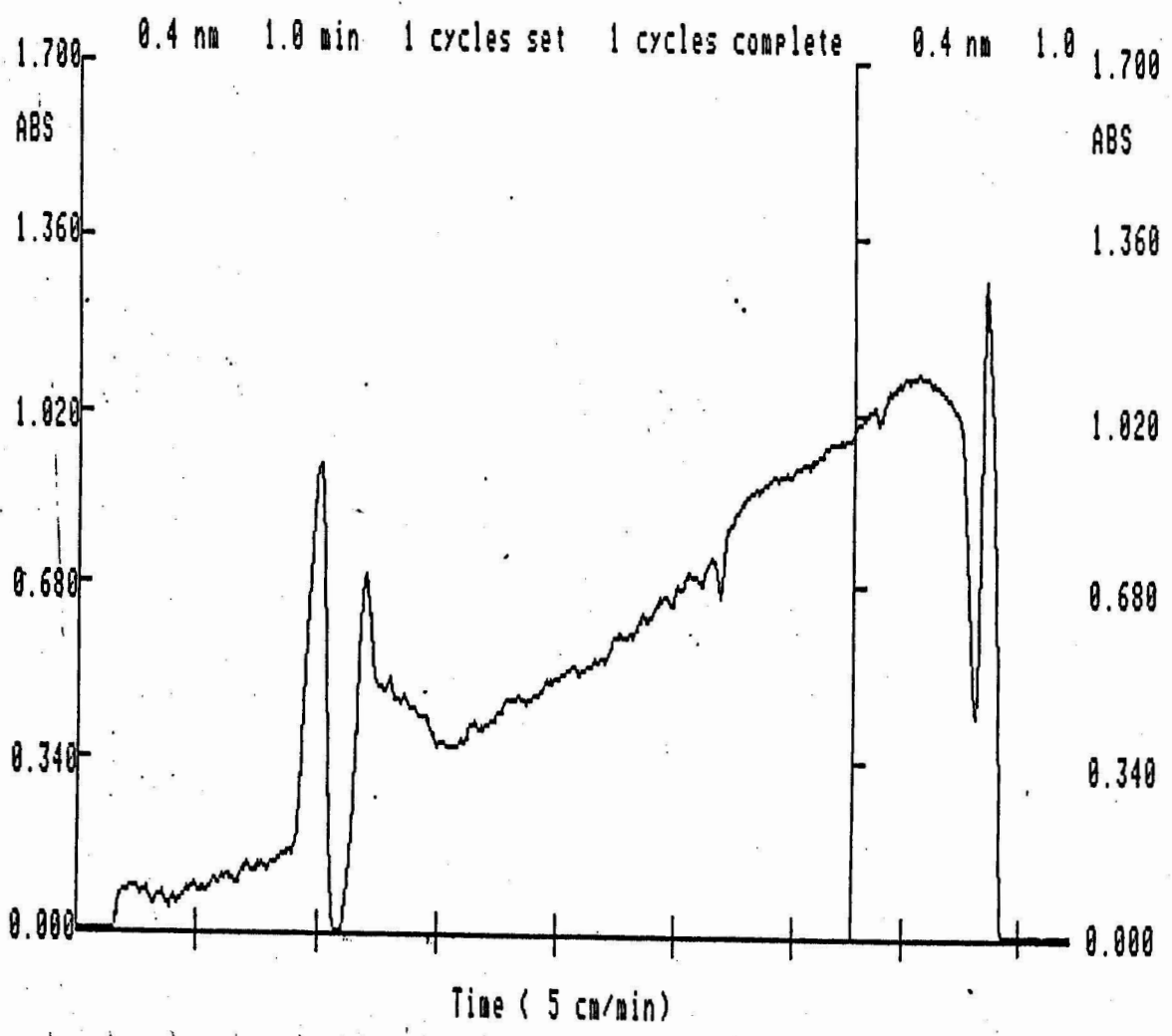
Candida guilliermondii

Run #2

FIGURE 9

Figure 10
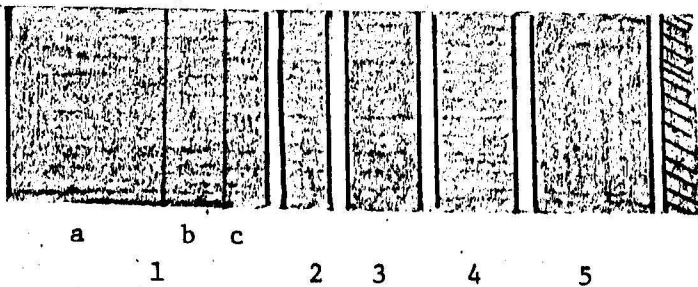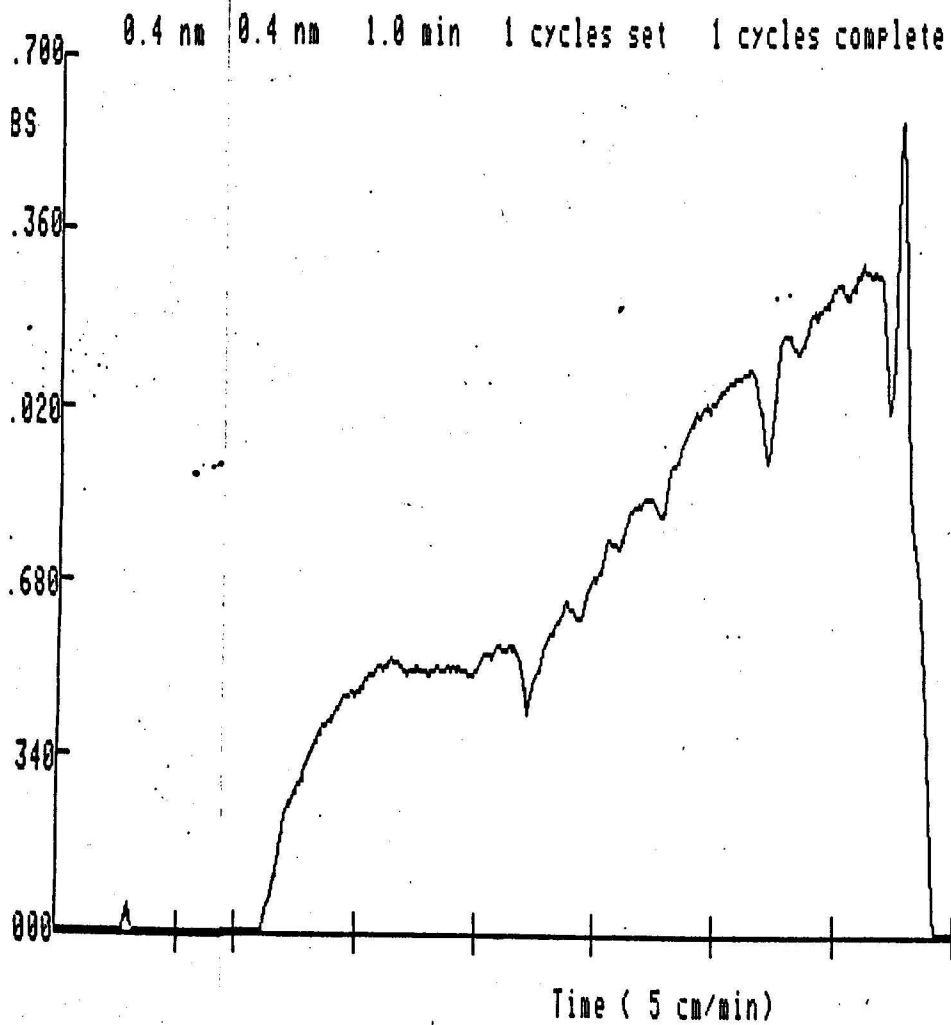
Scan Run #1
Candida krusei

0.4 nm   1.0 min   1 cycles set   1 cycles complete   0.4 nm   1.0 min

Time (5 cm/min)

a   b   c

1   2   3   4   5
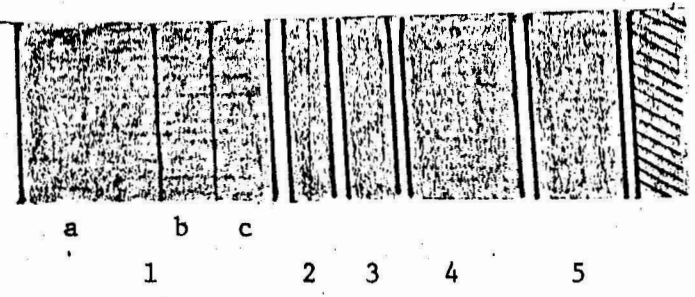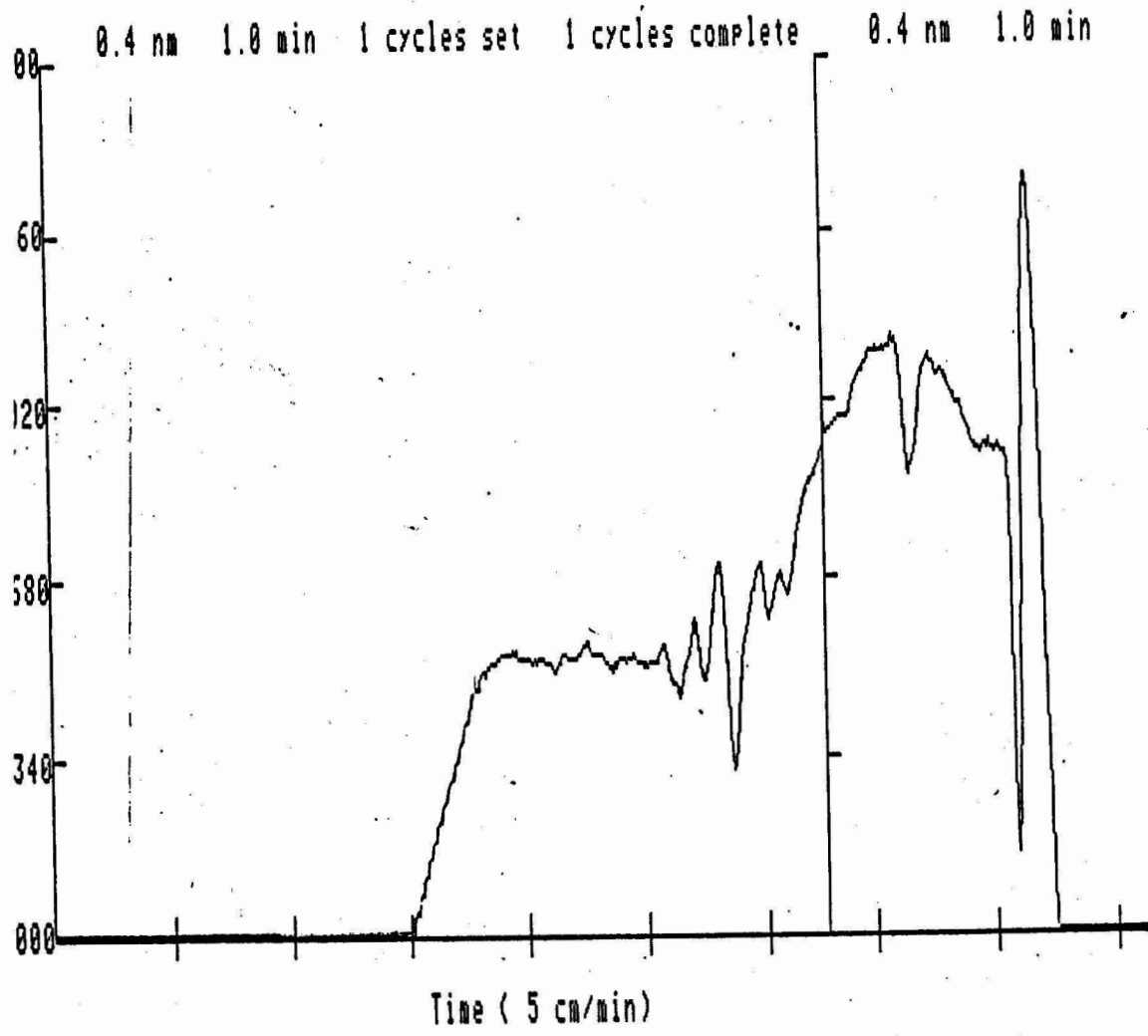
Candida krusei

Run #1

FIGURE 10

Figure 11

Scan Run #2
<u>Candida</u> <u>krusei</u>
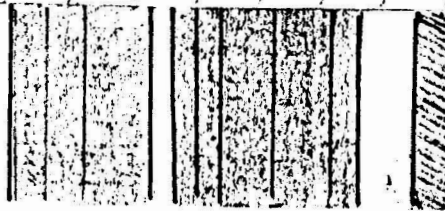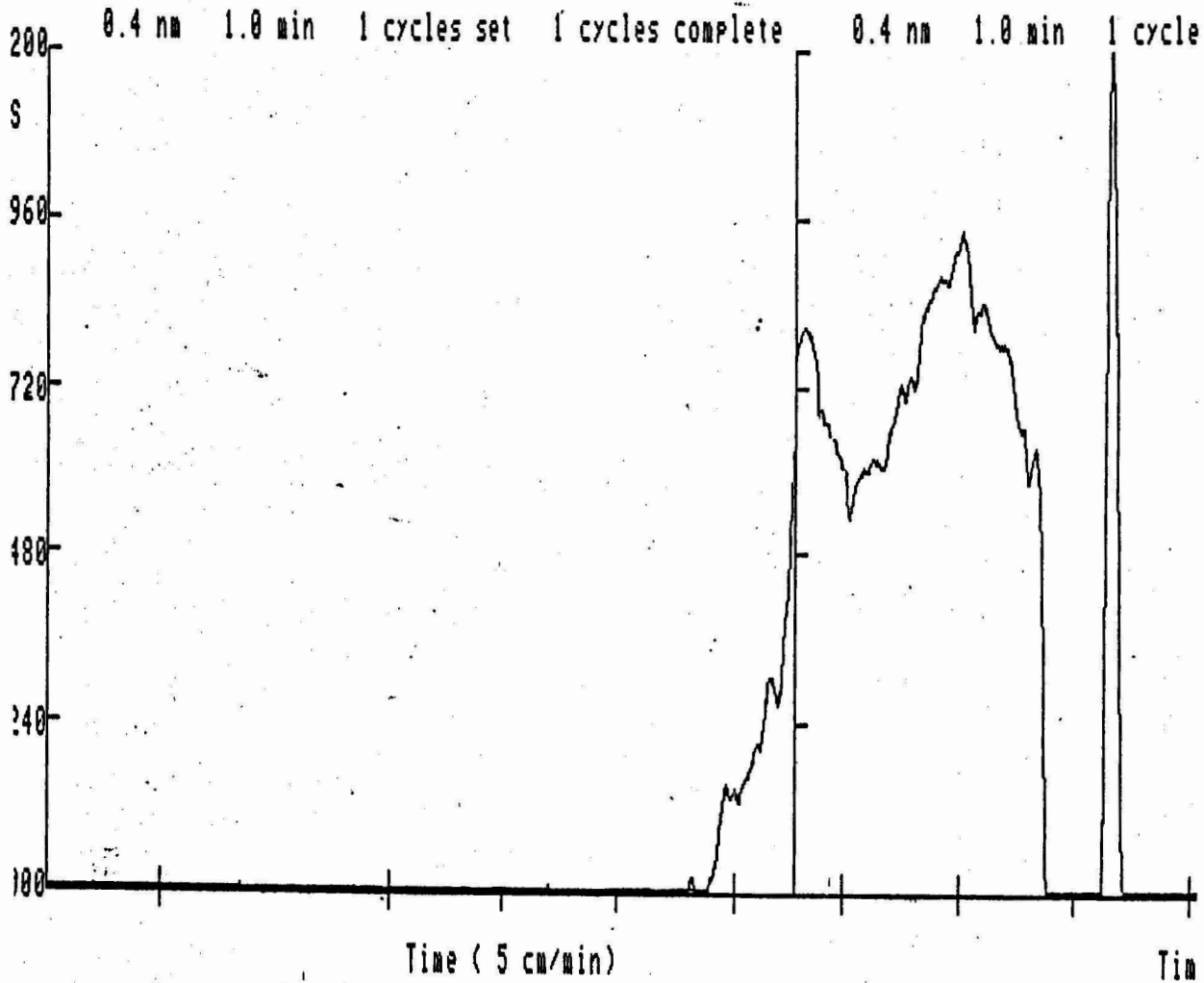
0.4 nm   0.4 nm   1.0 min   1 cycles set   1 cycles complete

Candida krusei

Run #2

FIGURE 11

Figure 12

Scan Run #1
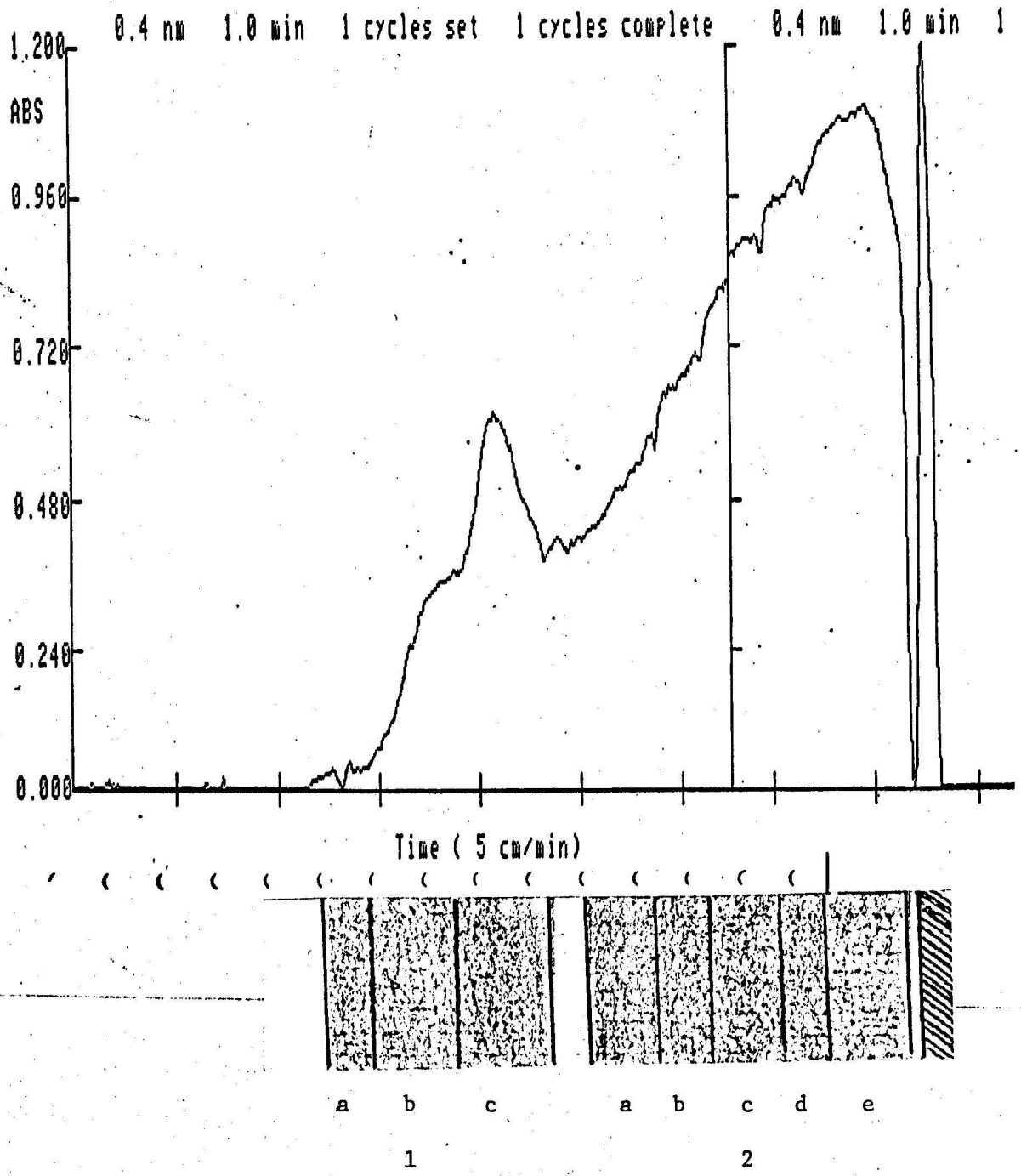Candida tropicalis

Candida tropicalis

Run #1

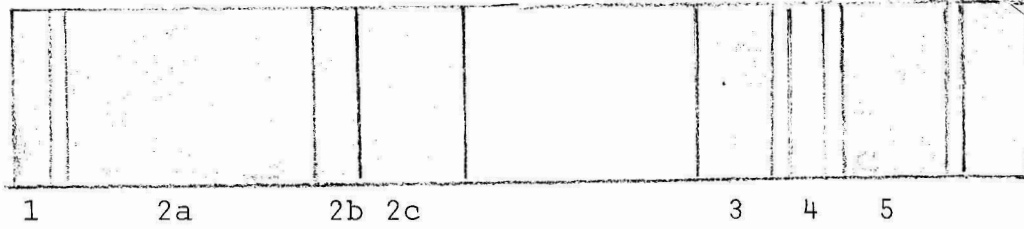FIGURE 12

Figure 13

Scan Run #2
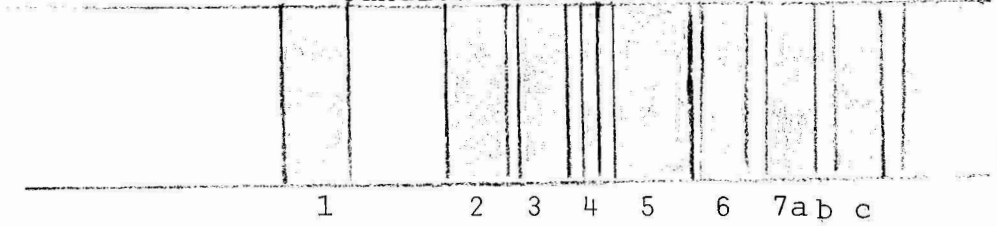Candida tropicalis

Candida tropicalis

Run #2

FIGURE 13

Figure 14

Comparison of scan patterns

Control

1   2a   2b 2c   3 4 5
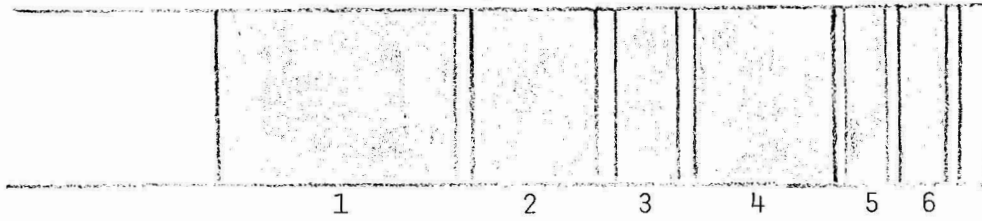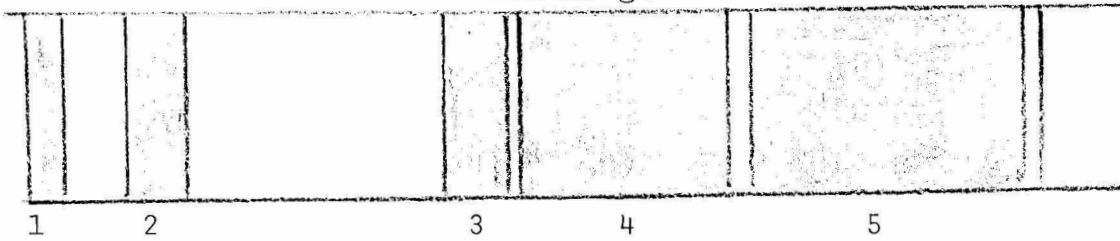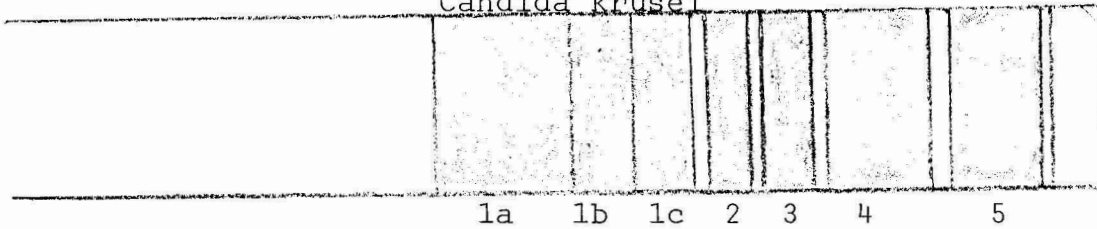
Candida albicans

1   2 3 4 5 6 7a b c

Candida parapsilosis

1   2   3   4   5 6

Candida guilliermondii

1   2   3   4   5

Candida krusei

1a   1b   1c 2 3 4   5

Candida tropicalis

1a b c 2a b c d e

FIGURE 14

CHAPTER IV

Discussion

Tinsberg et al. demonstrated that enzymes utiliz-
ing NAD and NADP as coenzymes have well defined iso-
enzyme spectra for each growth phase. They also describ-
ed seven multimolecular forms of the enzyme glucose-6-
phosphate dehydrogenase in Candida albicans at the log
phase.

The present study confirms the seven bands of
G6PDH for C. albicans at log phase. Additionally, this
research has established that in five species of Candida,
G6PDH can be separated into species specific isoenzyme
patterns by electrical mobilization in polyacrylamide gel.

Although many potential isoenzymes remain to be
investigated in Candida, G6PDH was selected for this study
because it would logically exhibit very high levels of
activity. Its metabolic role in both the glycolytic
pathway and the hexose monophosphate shunt characteristic
of rapid growth has been confirmed (Dyson, 1979). There-
fore, recovery in all species tested was likely. Logically
the log phase of growth was the time period selected for
analysis.

The evaluation of separate electrophoretic runs
of each species demonstrated a high level of reproducibility

and established an isoenzyme fingerprint for each individual species studied. Run #2 of C. albicans demonstrated six bands. However, bands 4 and 5 in run #2 showed sub bands while only band 7 showed sub bands in run #1. The difference in bands is probably the result of bridging in band 5 on run #2. This was not a significant factor as all other species, including the control, reproduced their patterns consistently on separate runs.

There is no apparent correlation between the number or location of electrophoretic bands and the API carbohydrate assimilation pattern. In fact, the species with the most nearly similar API profiles, such as C. albicans and C. tropicalis, have the most varied isoenzyme patterns. This would suggest that strains of a species with slightly different assimilation patterns would not necessarily have different isoenzyme patterns. Closely related species could be much better identified by electrophoretic means than by APIs.

All species show five to seven bands at log phase except C. tropicalis. This would imply that it is not diversification of this enzyme which affects pathogenicity. C. albicans and C. tropicalis are the most frequently encountered as human pathogens (Rippon, 1982) but possess seven and two bands respectively. This would suggest that the isoenzyme picture correlates more closely to DNA homology results which indicate C. albicans and C. tropicalis are

most dissimilar. The cell wall analysis and antigenic
studies which closely relate these two species, however,
would support the role of the cell wall in their path-
ogenicity.

Densitometric evaluation of all species separa-
tions demonstrated minor deflections in the bands and
these are designated as sub bands. The sub bands also
exhibited good reproducibility for each species. This
would indicate that finer resolution techniques,which
remain to be developed, would elucidate more bands, and
thus result in better speciation. Better speciation
could conceivably result in the development of criteria
for relative pathogenicity.

The distance between the first two bands and the
latter bands in the Saccharomyces control is seen only
in the C. guilliermondii pattern. Patterns such as this
may also be useful in identifying species interrelation-
ships.

The presence of diverse patterns of isoenzymes for
G6PDH would suggest that perhaps other enzymes would also
yield specific isoenzyme patterns or similarily interesting
results.

In human isoenzyme studies, various multimolecular
forms generally indicate varying tissues of origin. The
slightly different molecular structures observed are based
on different sites of production such as the heart and liver

components of lactic acid dehydrogenase. These var-
iations in structure are felt to be due to differing
environments and activities (Kaplan, 1984).

In an organism such as Candida, it is less
clear what the origins of these isoenzymes may be, or
their significance. The greater genetic load and greater
diversity of substrates available to meet metabolic needs
undoubtedly contribute to multiple molecular forms of the
enzyme. The variances in multimolecular forms is felt
to be related to the NADP dependent enzymes' adaptogenic
role in the cell (Tinsberg, 1985).

In order to make the data applicable to clinical
identification purposes, additional steps such as develop-
ment of monoclonal antibodies for individual isoenzyme
bands are needed.

Monoclonal antibody technology enables a very
specific and high affinity antibody to be produced to
a certain protein being assayed. Molecules of the protein
are injected into mice whose immune system then activates
a variety of antibody secreting lymphocytes. Each lymph-
ocyte secretes one molecular configuration of antibody.
Using tissue culture techniques, these lymphs can be grown
into separate cell colonies, or clones. Each clone then
produces a monoclonal antibody of uniform specificity and
affinity. The antibody can then be adapted to immuno-
fluorescent assay techniques or slide agglutination proced-

ures. Similarity in band specificity as seen through monoclonal antibodies could then support species relationships and taxonomic placement (Hybritech, 1983).

Areas requiring further evaluation include appearance of patterns on different strains of each species. This would particularly apply when slightly different biochemical pictures, i.e., assimilation patterns, occur. Also, further separation of sub bands by higher resolution techniques would be advantageous. And finally, comparisons of other genera of yeast are necessary to evaluate taxonomic potential of this approach.

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