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APPENDICES

APPENDIX A

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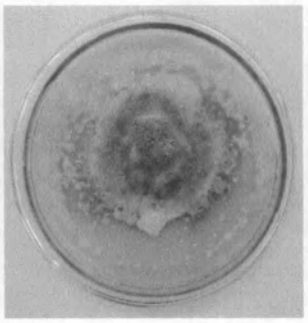

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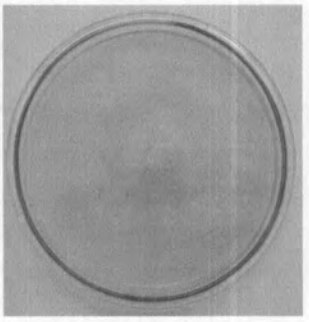

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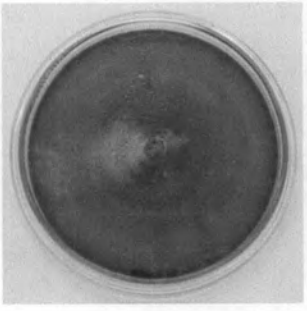

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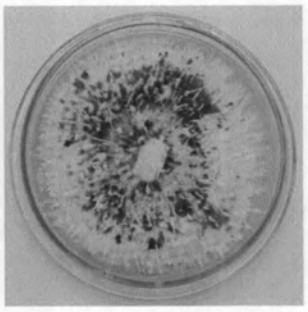

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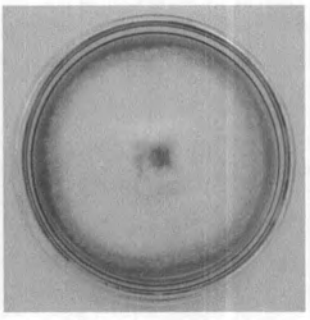

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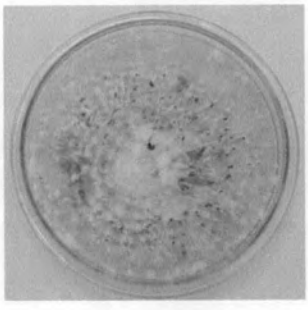

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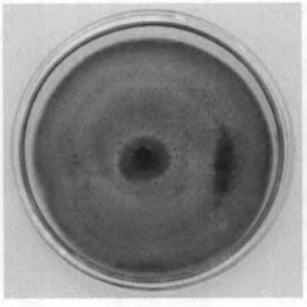

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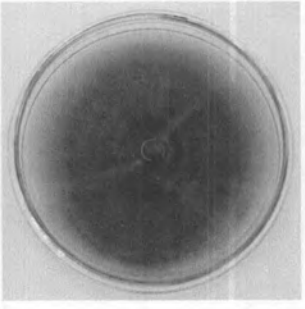

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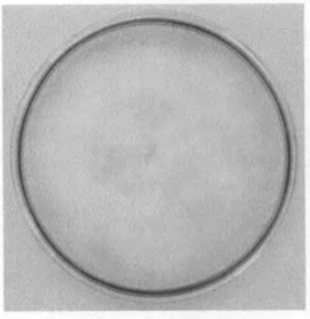

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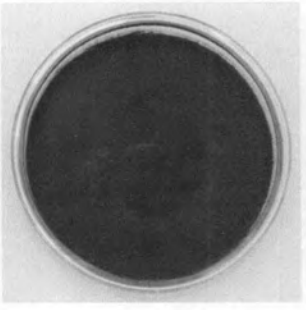

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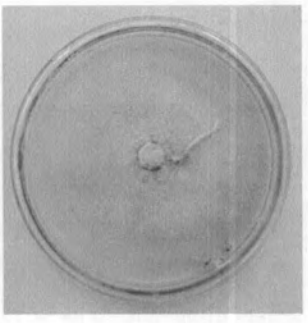

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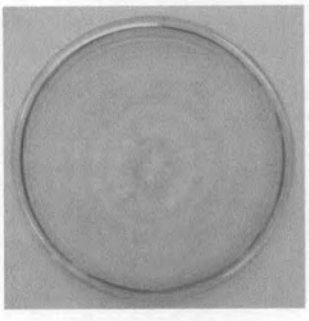

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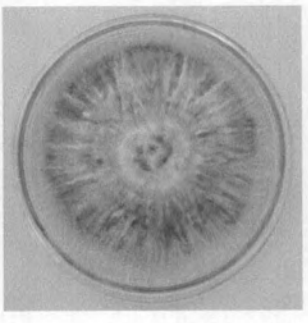

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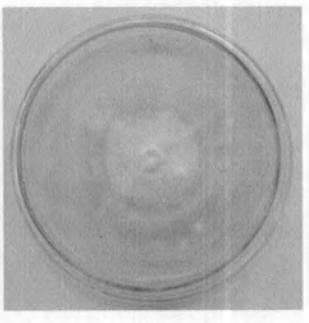

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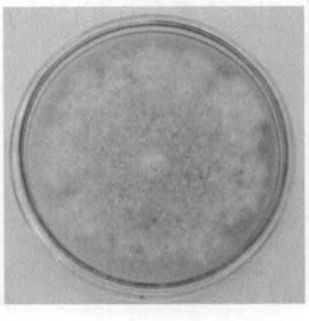

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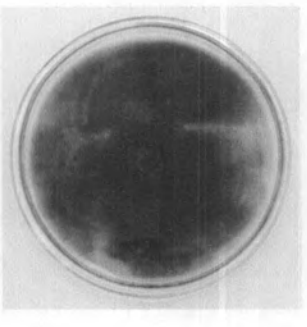

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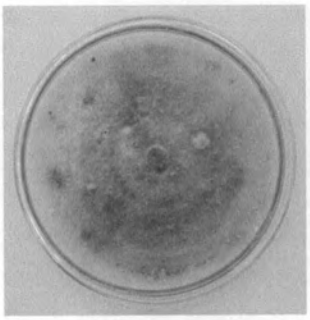

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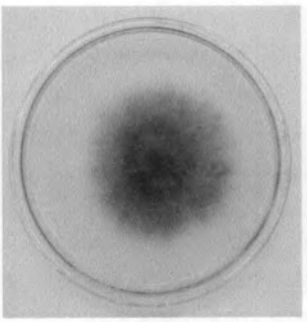

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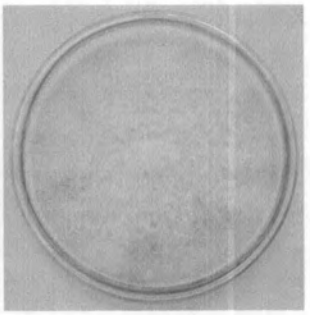

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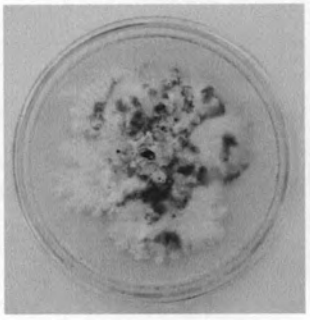

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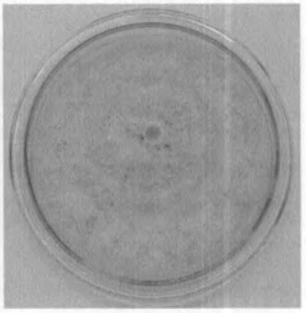

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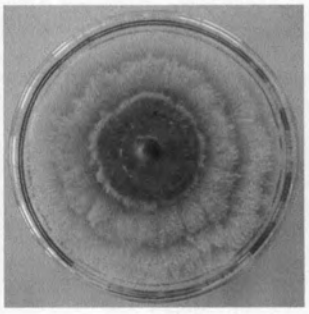

EF25

APPENDIX B

1. MEDIA

The media were sterile by autoclaving at 121°C, 15 lb/in^2 for 15 minute

1.1 Potato dextrose agar (PDA)

(a) Potatoes, peeled and diced	200.0	g
(b) Dextrose	20.0	g
(c) Agar	15.0	g
(d) Distilled water	1000	ml

Boil 200 g of peels, diced potatoes for 30 minute in 1000 ml distilled water. Filter, and adjust the filtrate to 1000 ml. Add the dextrose and agar and dissolve by steaming and sterilize by autoclaving at 121°C for 15 min.

1.2 Malt extract agar (MEA)

(a) Malt extracts	20.0	g
(b) Glucose	20.0	g
(c) Peptone	1.0	g
(d) Agar	15.0	g
(e) Distilled water	1000	ml

1.3 Starch agar

(a) Soluble starch	20.0	g
(b) KH_2PO_4	2.0	g
(c) NH_4NO_3	6.0	g
(d) $MgSO_4.7H_2O$	0.1	g
(e) $FeSO_4.7H_2O$	0.01	g
(f) Agar	12.0	g
(g) Distilled water	1000	ml

1.4 Starch broth

(a) Soluble starch	20.0	g
(b) KH_2PO_4	2.0	g
(c) NH_4NO_3	6.0	g
(d) $MgSO_4.7H_2O$	0.1	g
(e) $FeSO_4.7H_2O$	0.01	g
(f) Distilled water	1000	ml

2. Iodine solution

(a) I_2	0.1%
(b) KI	1%

3. Sodium Dodecyl Sulfate – Polyacrylamide Gel Electrophoresis (SDS-PAGE)

3.1 Working Solutions

3.1.1 Solution A (Acrylamide Stock Solution),

(a) 29.2 g acrylamide

(b) 0.8 g bis-acrylamide

(c) Add distilled water to make 100 ml and stir until completely dissolved. Work under hood and keep acrylamide solution covered with Paraflim until acrylamide powder is completely dissolved. Can be stored for months in the refrigerator.

3.1.2 Solution B (4x Separating Gel Buffer), 100ml

(a) 75 ml 2 M Tris-HCl (pH8.8)

(b) 4 ml 10% SDS

(c) 21 ml H_2O

Stable for months in the refrigerator.

3.1.3 Soluion C (4x Stacking Gel BufFer), 100 ml

(a) 50 ml 1 M Tris-HCl (pH 6.8)

(b) 4 ml 10% SDS

(c) 46 ml H_2O

Stable for months in the refrigerator.

3.1.4 10% Ammonium persulfate (APS), 5ml

(a) 0.5 g ammonium persulfate

(b) 5 ml H_2O

Stable for months in a capped tube in the refrigerator.

3.1.5 Electrophoresis Buffer, 1 liter (pH8.3)

(a) 3 g Tris

(b) 14.4 g glycine

(c) 1 g SDS

(d) H_2O to make a 1 liter

3.1.6 5x Sample Buffer, 10 ml

(a) 0.6 ml 1 M Tris-HCl (pH 6.8)

(b) 5 ml 50% glycerol

(c) 2 ml 10 % SDS

(d) 0.5 ml 2-mercaptoethanol

(e) 1 ml 1% bromophenol blue

(f) 0.9 ml H_2O

Stable for weeks in the refrigerator or for months at $-20^{\circ}C$

	Separating gel (10 ml)	Stacking gel (4 ml)
Solution A	5	0.67
Solution B	2.5	-
Solution C	-	1.0
10% APS	50	30
TEMED	5	5
H_2O	2.5	2.3

3.2 Staining solution

3.2.1 Coomasie gel stain, 1 liter

(a) 1.0 g Coomasie Blue R-250

(b) 450 ml methanol

(c) 100 ml glacial acetic acid

(d) 450 ml H_2O

3.2.2Coomasie Gel Destain, 1 liter

(e) 100 methanol

(f) 100 ml glacial acetic acid

(g) 800 H_2O

4. Native-Polyacrylamide Gel Electrophoresis (Native-PAGE)

4.1 Working Solutions

4.1.1 Solution A (Acrylamide Stock Solution)

(a) 30 g acrylamide

(b) 0.8 g bis-acrylamide

(c) Add distilled water to make 100 ml and stir until completely dissolved. Work under hood and keep acrylamide solution covered with Paraflim until acrylamide powder is completely dissolved. Can be stored for months in the refrigerator.

4.1.2 Solution B (4x Separating Gel Buffer), pH 8.8

(a) 18.2 g Tris-HCl

(b) 100 ml H_2O

4.1.3 Soluion C (4x Stacking Gel BufFer), (pH 6.8)

(a) 6.0 g Tris-HCl

(b) 100 ml H_2O

4.1.4 10% Ammonium persulfate (APS)

(a) 0.5 g ammonium persulfate

(b) 5 ml H_2O

4.1.5 Electrophoresis Buffer, 1 liter (pH8.3)

(a) 3 g Tris

(b) 14.4 g glycine

(c) 1 g SDS

(d) H_2O to make a 1 liter

4.1.6 5x Sample Buffer

(a) 3.1 ml 1 M Tris-HCl (pH 6.8)

(b) 5 ml glycerol

(c) 0.5 ml 1% bromophenol blue

(d) 1.4 ml H_2O

	Separating gel (10 ml)	Stacking gel (4 ml)
Solution A	5	0.67
Solution B	2.5	-
Solution C	-	1.0
10% APS	50	30
TEMED	5	5
H_2O	2.5	2.3

4.2 Staining solution

4.2.1 Coomasie gel stain, 1 liter

(a) 1.0 g Coomasie Blue R-250

(b) 450 ml methanol

(c) 100 ml glacial acetic acid

(d) 450 ml H_2O

4.2.2 Coomasie Gel Destain, 1 liter

(a) 100 methanol

(b) 100 ml glacial acetic acid

(c) 800 H_2O

5. Bradford solution

5.1 Bradford Stock Solution

(a) 100 ml 95% ethanol

(b) 200 ml 88% phosphoric acid

(c) 350 mg Serva Blue G

Stable indefinite at room temperature

5.2 Bradford Working Buffer

(d) 425 ml distilled water

(e) 15 ml 95% ethanol

(f) 30 ml 88% phosphoric acid

(g) 30 ml Bradford Stock solution

Filter through Whatman No.1 paper, store at room temperature in brown glass bottle. Usable for several weeks, but may need to be refiltered.

6. DNS Reagent

6.1 Dinirosalicylic Acid Reagent solution, 1%

(a) 10 g Dinirosalicylic acid

(b) 2g Phenol

(c) 0.5 g Sodium sulfite

(d) 10 g Sodium hydroxide

(e) 1 liter distilled water

6.2 Potassium sodium tartrate solution, 40%

7. Reagent solution for extract DNA

7.1 Washing buffer 200 ml		
(a) Polyvinyl pyrolidone	2	g
(b) Ascorbic acid	1.76	g
(c) Tris-HCl	20	ml
(d) 2-mercaptoethanol	4	ml
7.2 2x CTAB lysis buffer 200 ml		
(a) Cetyl trimethyl ammonium bromide (CTAB)	4	g
(b) EDTA	8	ml
(c) Tris-HCl	20	ml
(d) NaCl	16.36	g
(e) 2-mercaptoethanol	1	ml
7.3 Chroroform/isoamyl alcohol		
(a) Chloroform	192	ml
(b) Isoamyl alcohol	8	ml
7.4 TE buffer		
(a) Tris-HCl	10	mM
(b) EDTA	1	mM

APPENDIX C

Figure 1 Q Sepharose ion exchange chromatogram of crude protein from EF6 strain

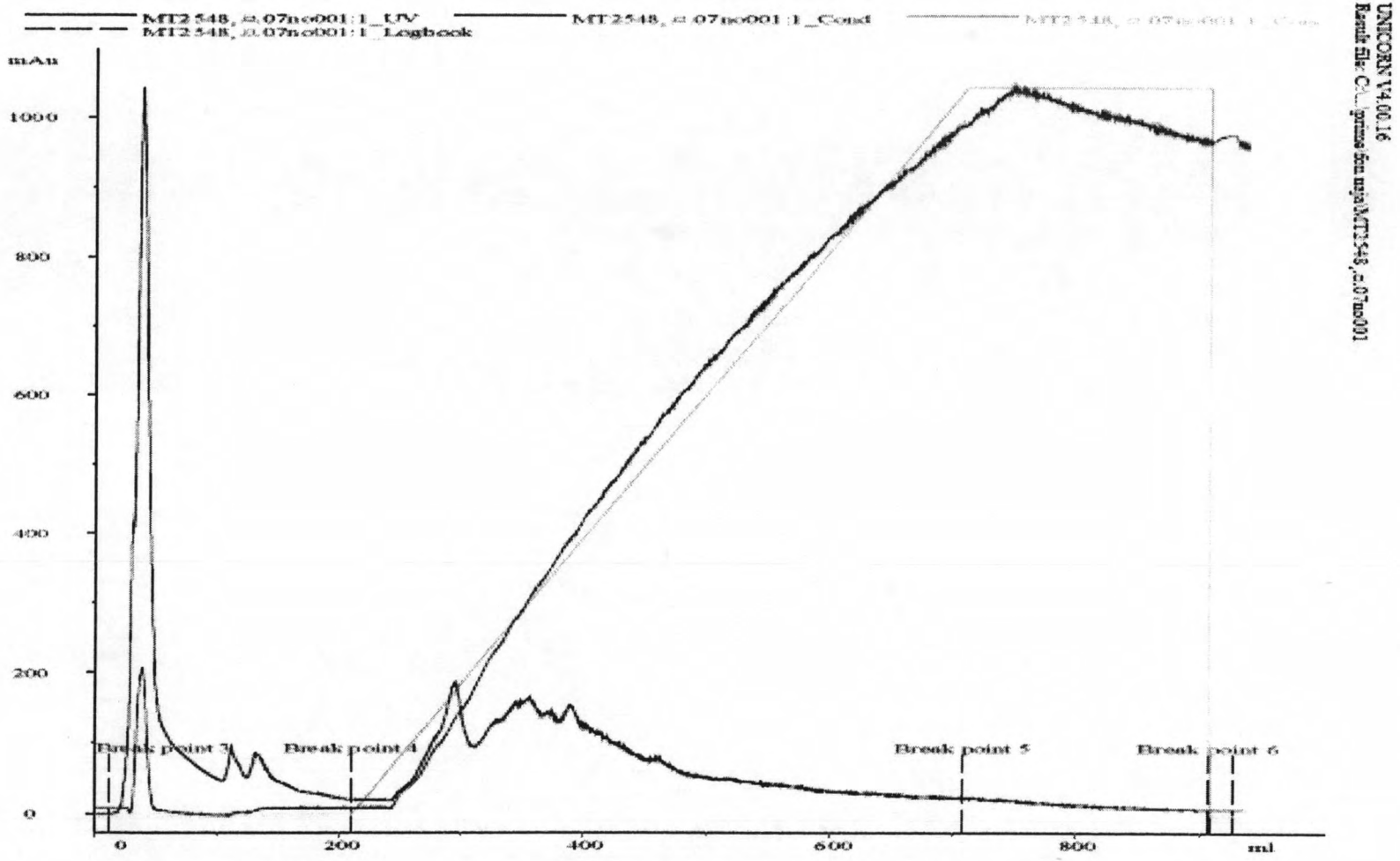

Figure 2 Q Sepharose ion exchange chromatogram of crude protein from EF6 strain

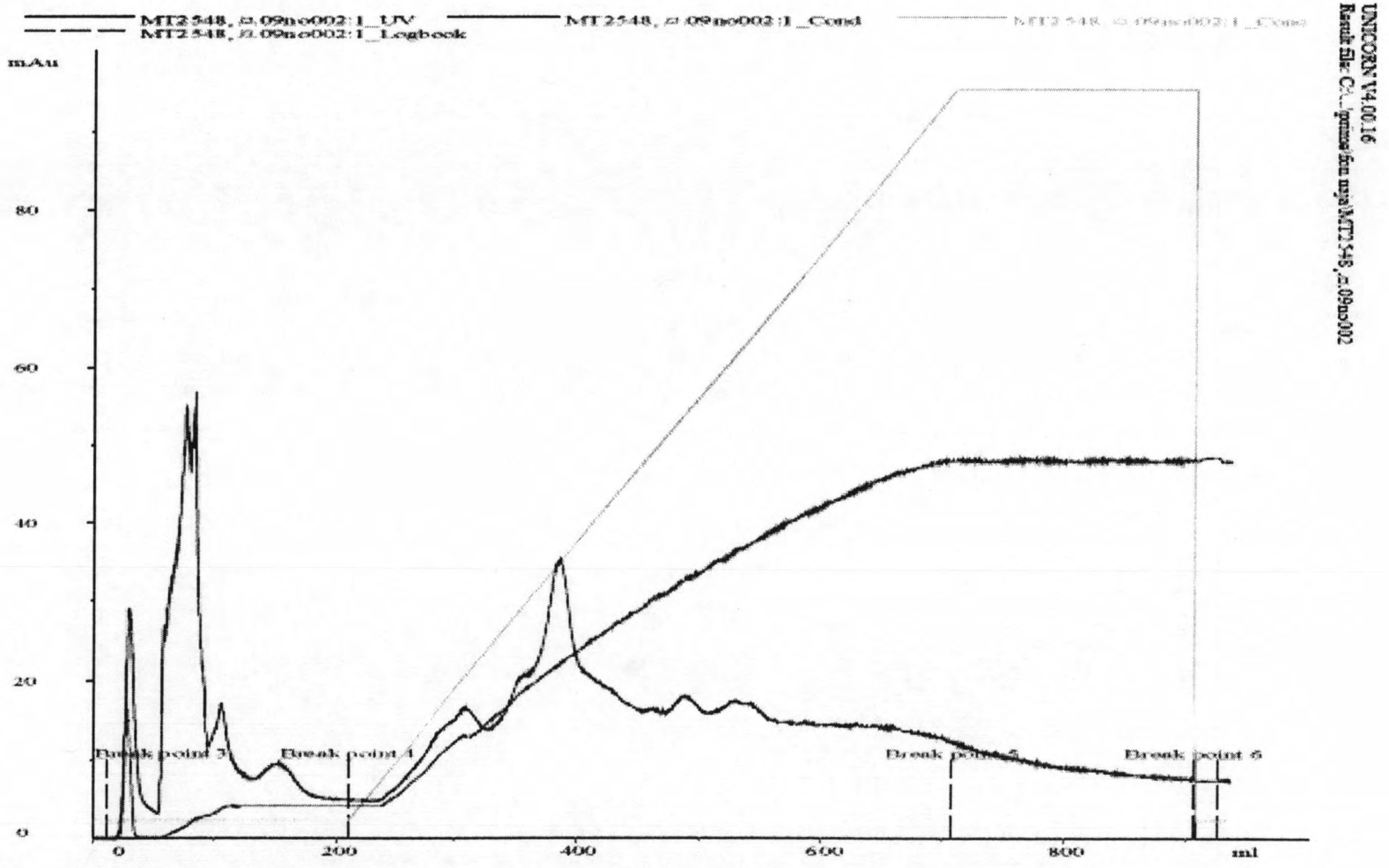

Figure 3 Q Sepharose ion exchange chromatogram of crude protein from EF6 strain

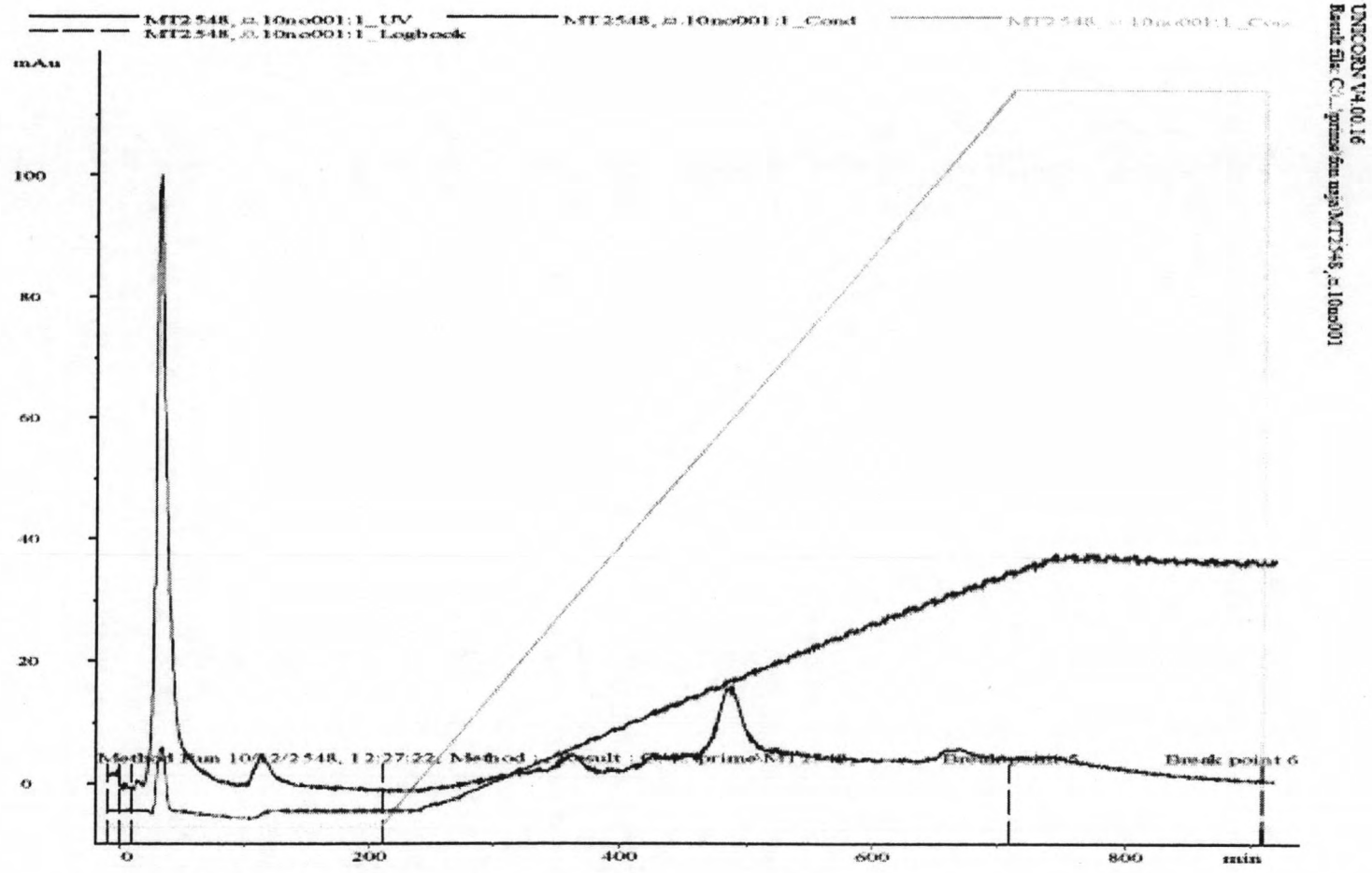

Figure 4 Q Sepharose ion exchange chromatogram of crude protein from EF6 strain

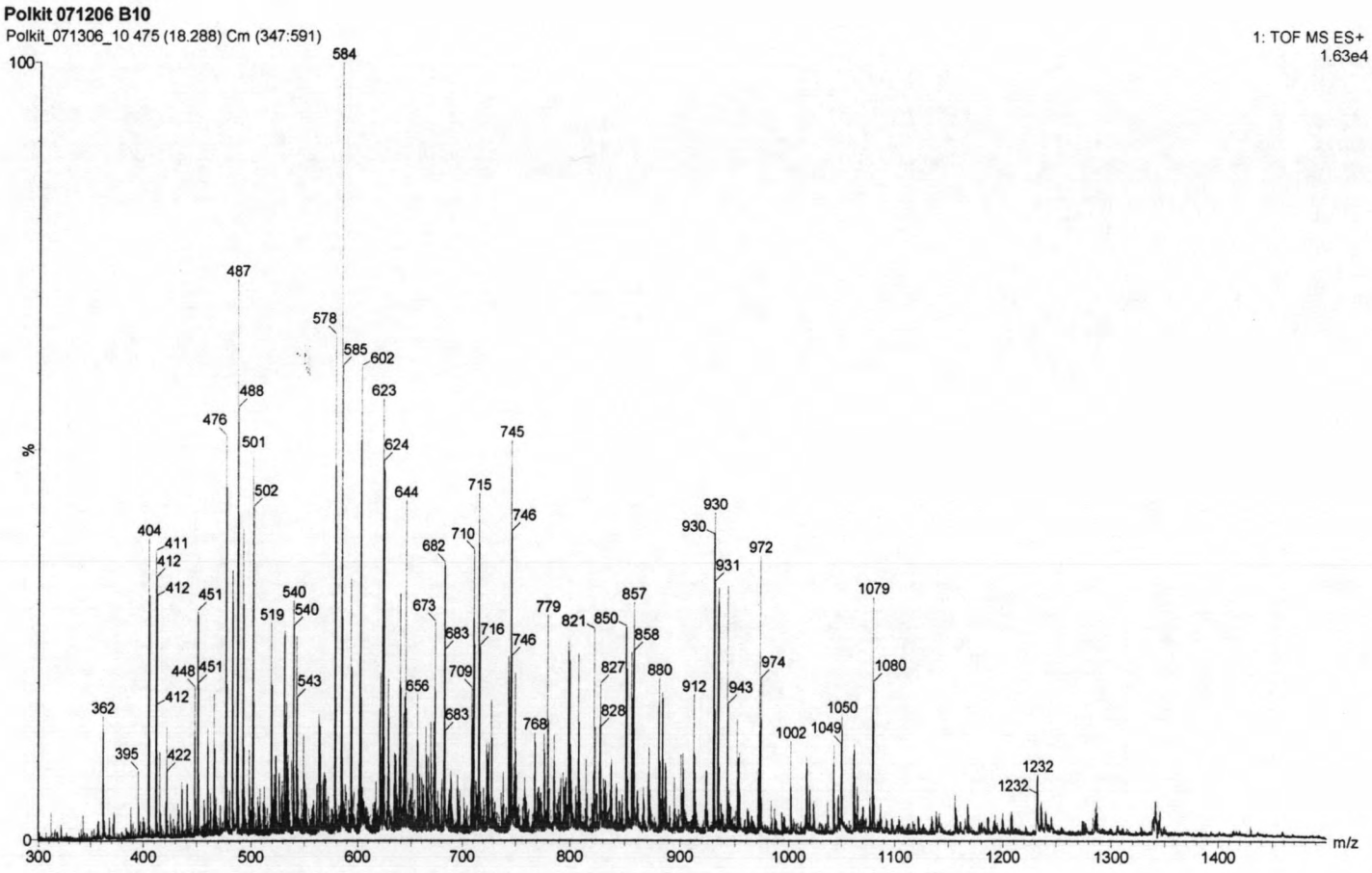

Figure 5 Mass spectrum of tryptic fragments of purified protein from EF6

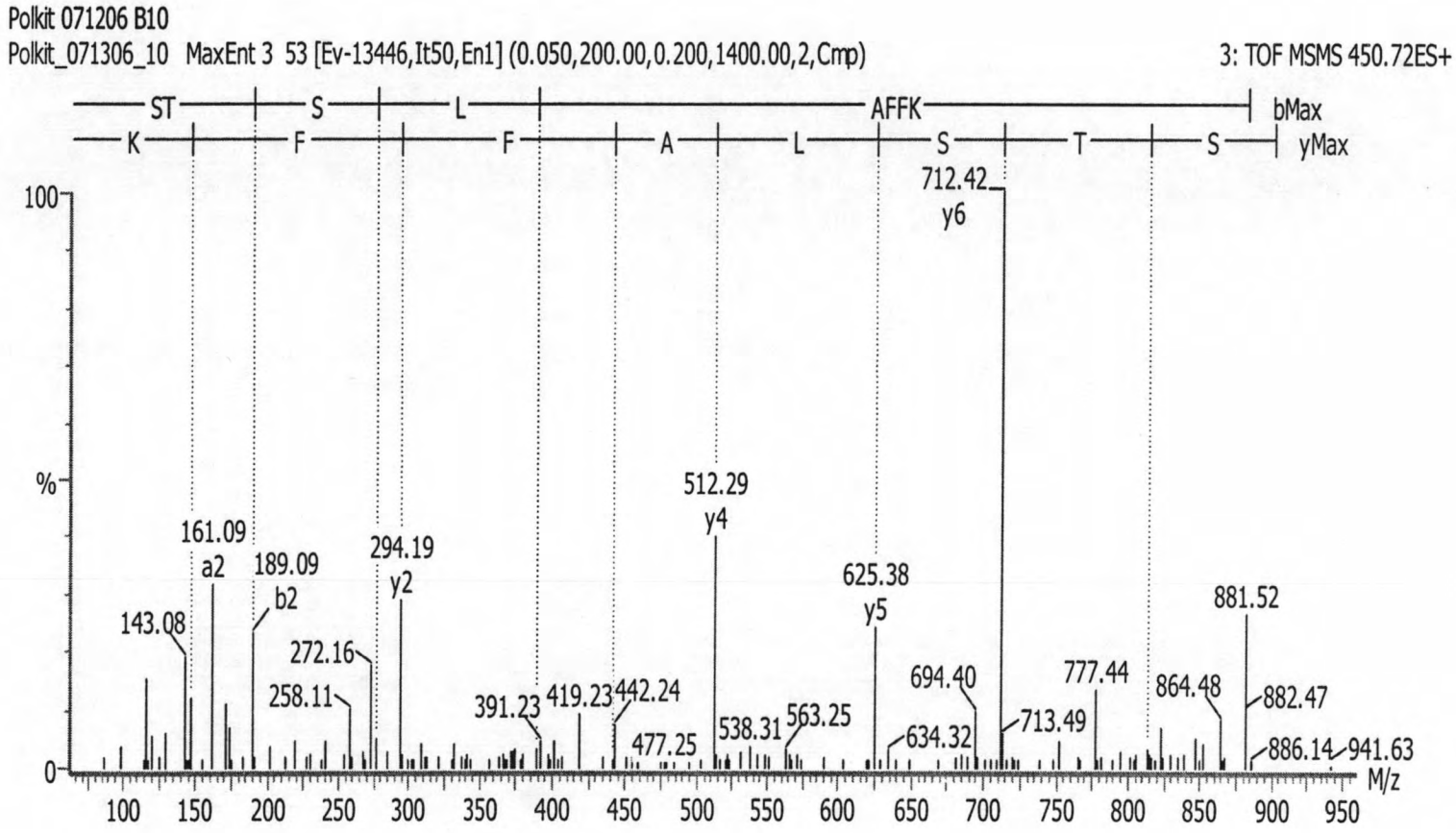

Figure 6 The product ion spectrum of peptide precursor *m/z* 450.72

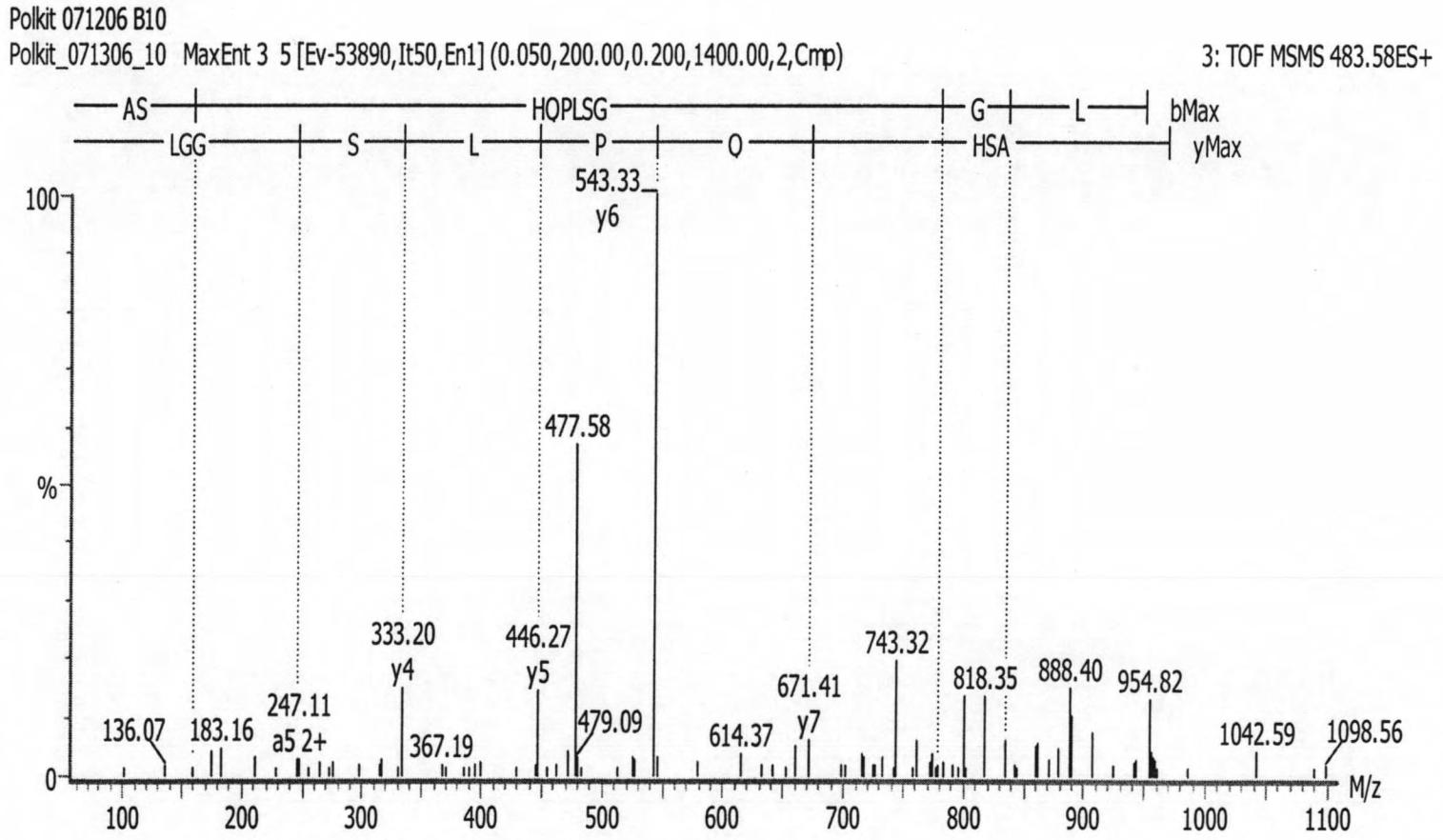

Figure 7 The product ion spectrum of peptide precursor *m/z* 483.58

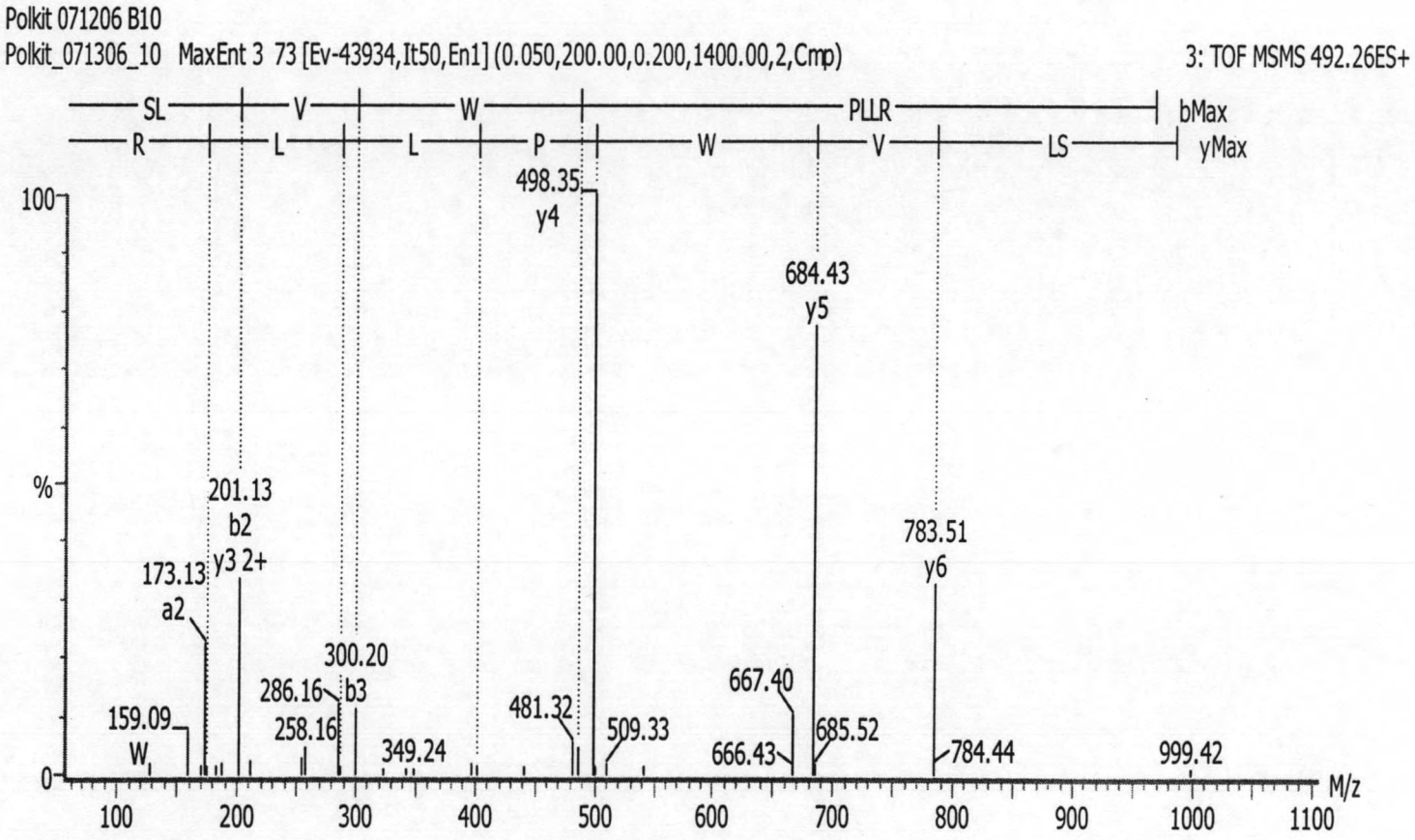

Figure 8 The product ion spectrum of peptide precursor *m/z* 492.26

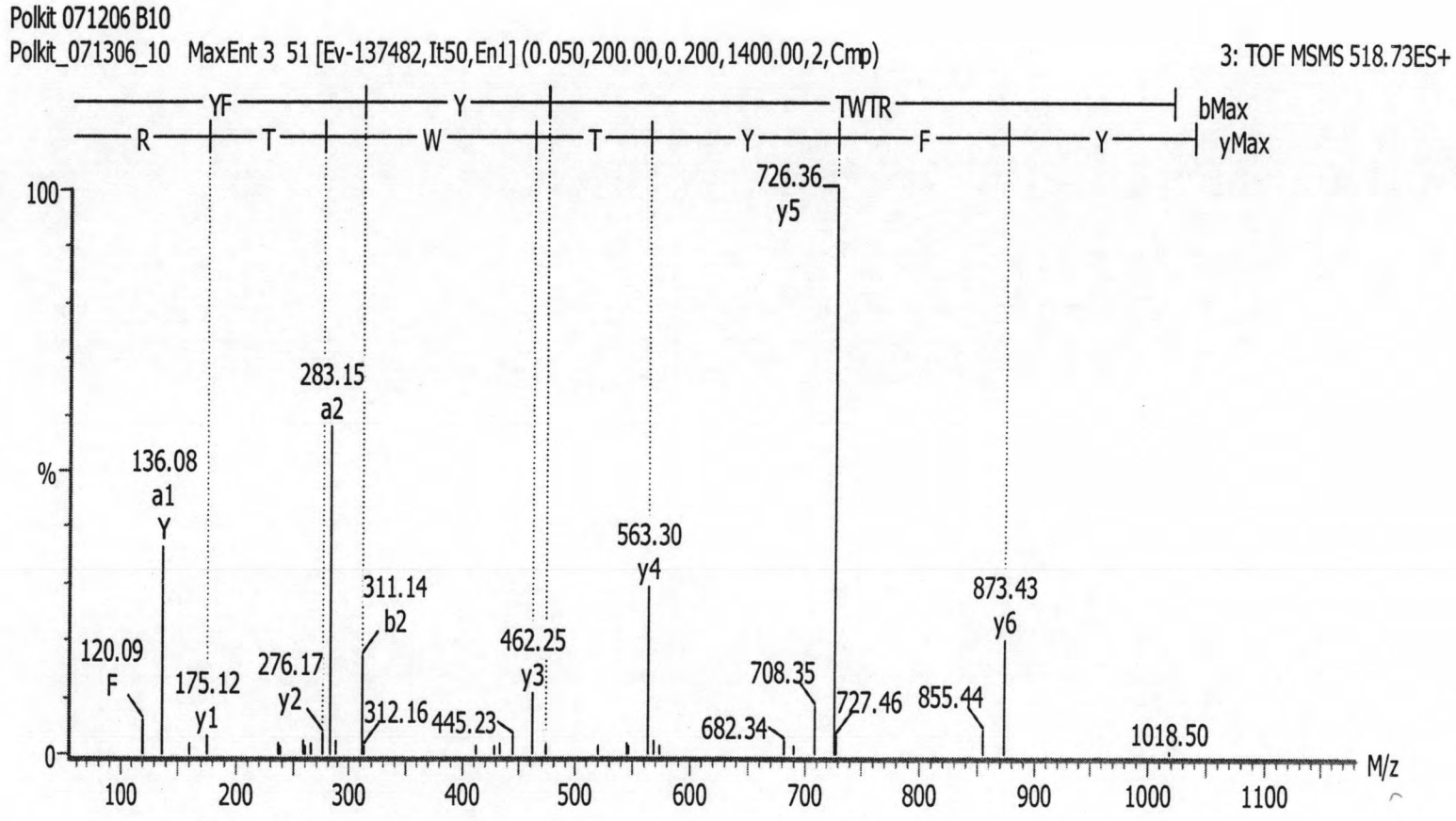

Figure 9 The product ion spectrum of peptide precursor *m/z* 518.73

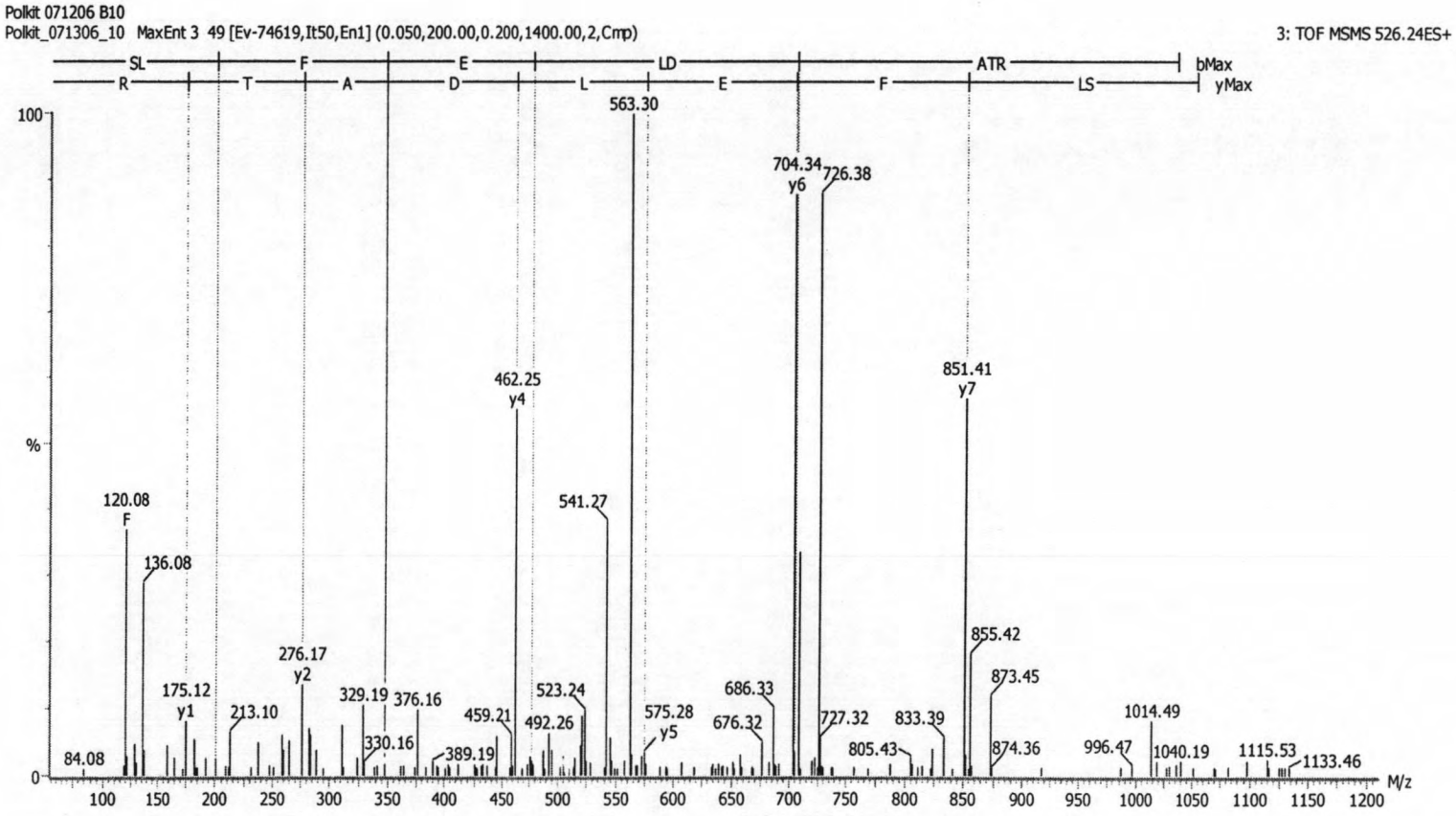

Figure 10 The product ion spectrum of peptide precursor *m/z* 4526.24

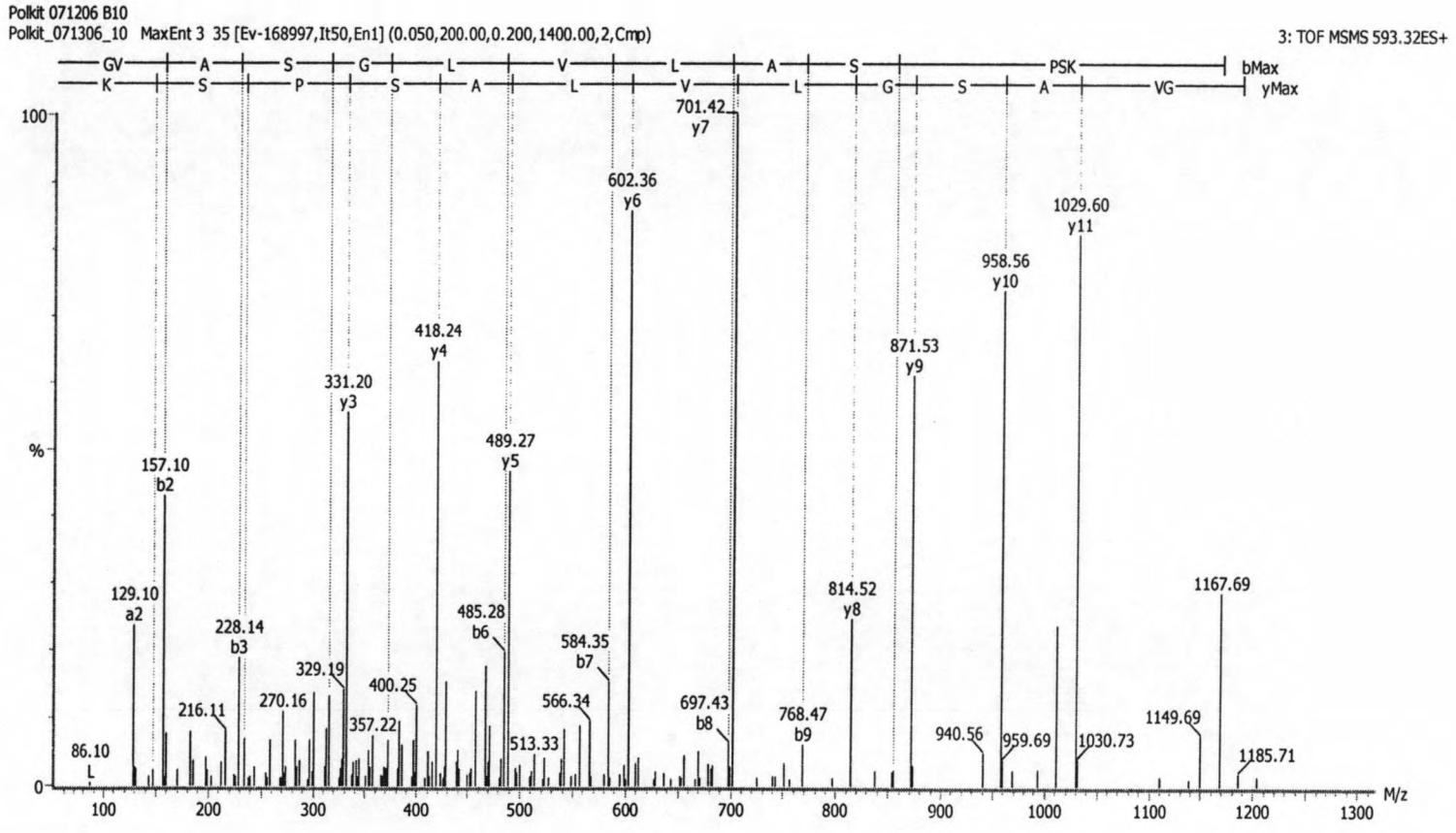

Figure 11 The product ion spectrum of peptide precursor *m/z* 593.32

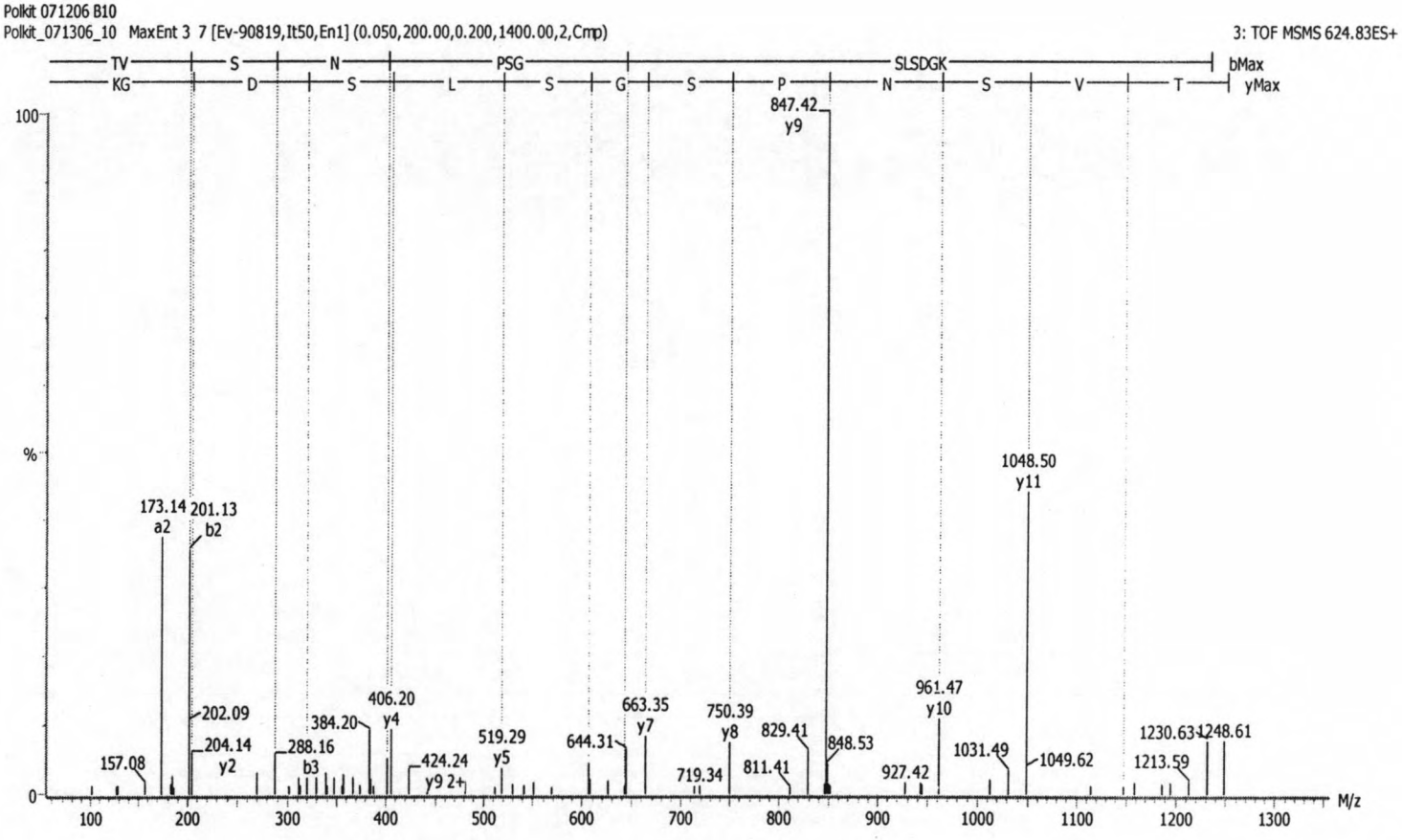

Figure 12 The product ion spectrum of peptide precursor m/z 624.83

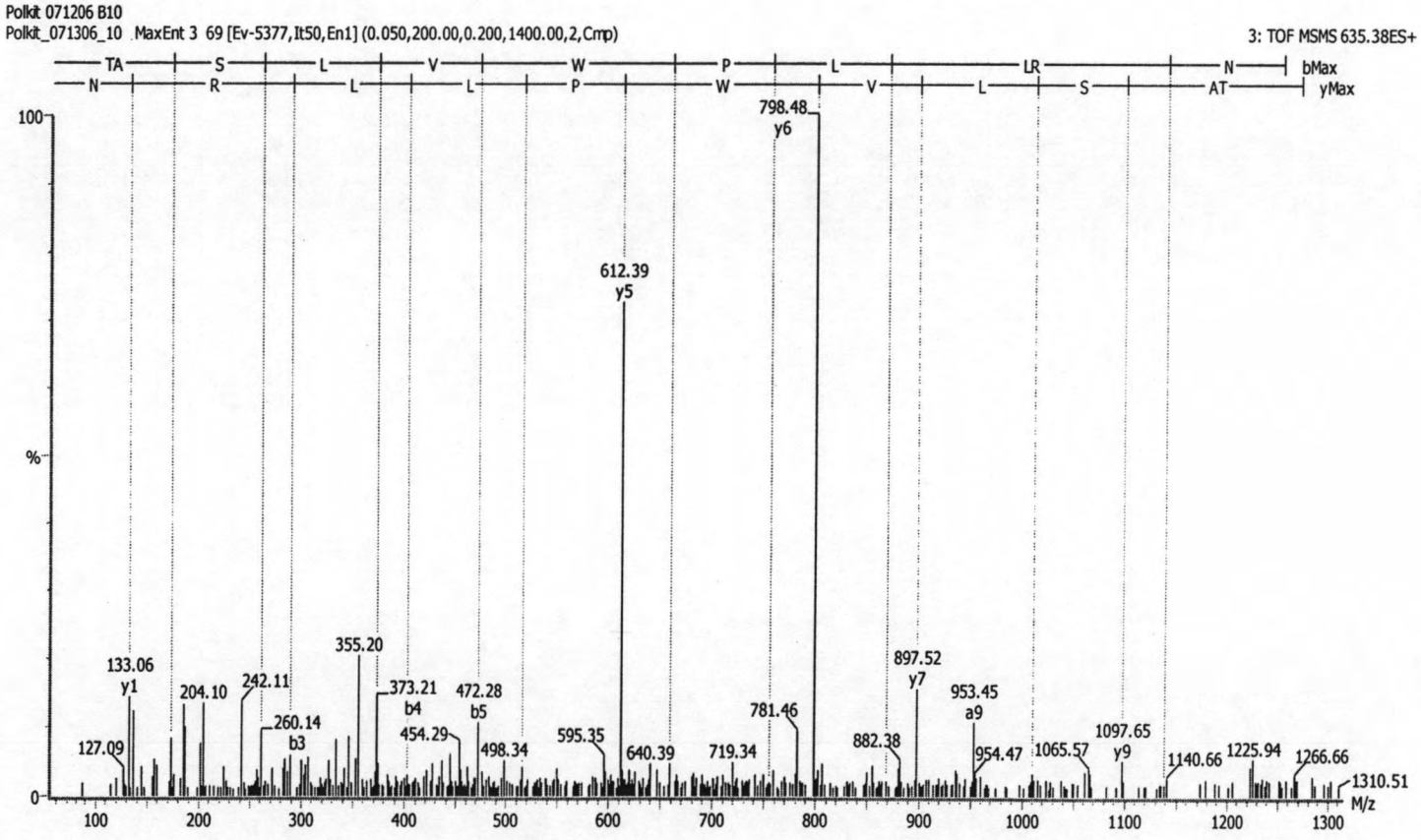

Figure13 The product ion spectrum of peptide precursor m/z 635.38

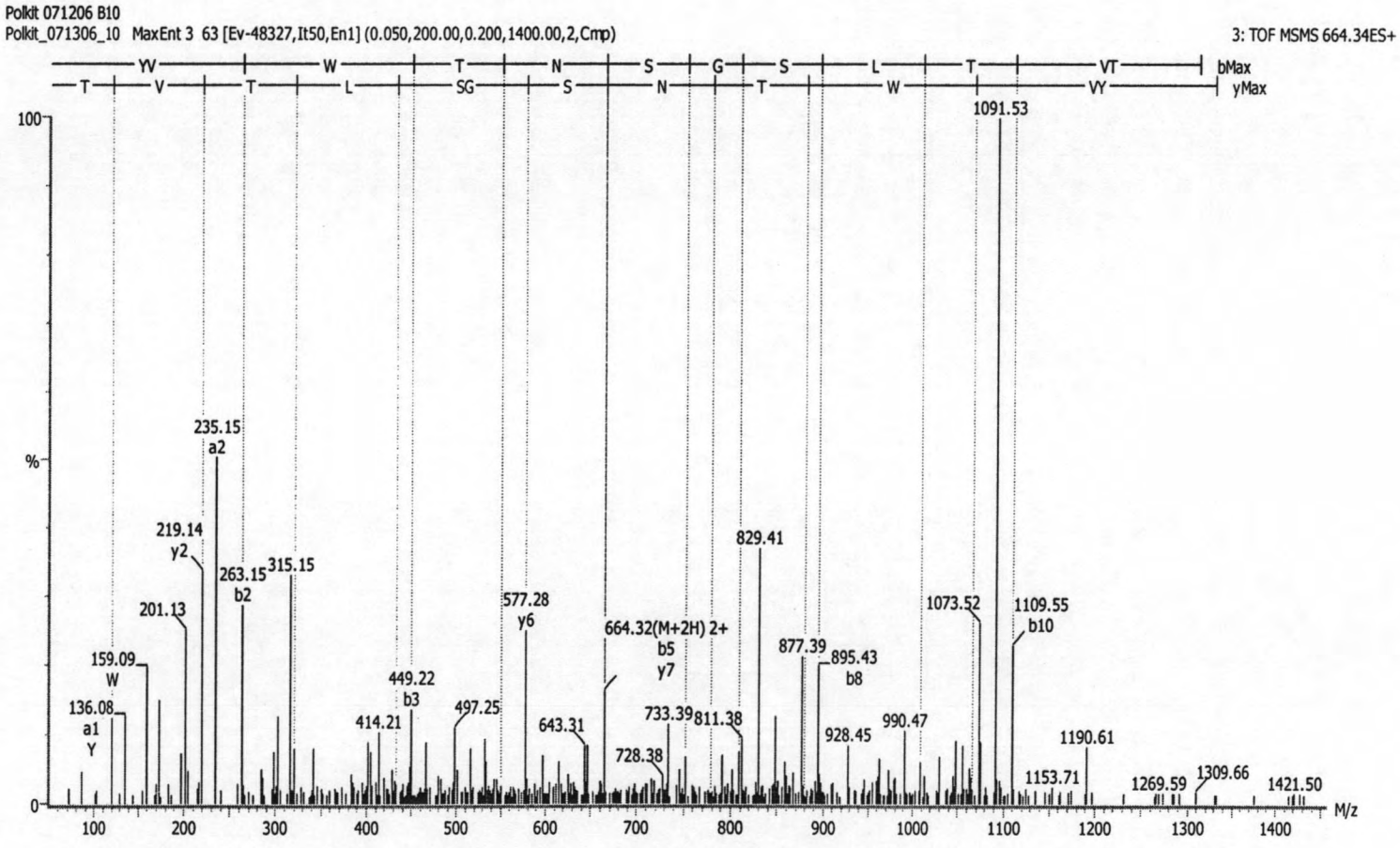

Figure 14 The product ion spectrum of peptide precursor m/z 664.34

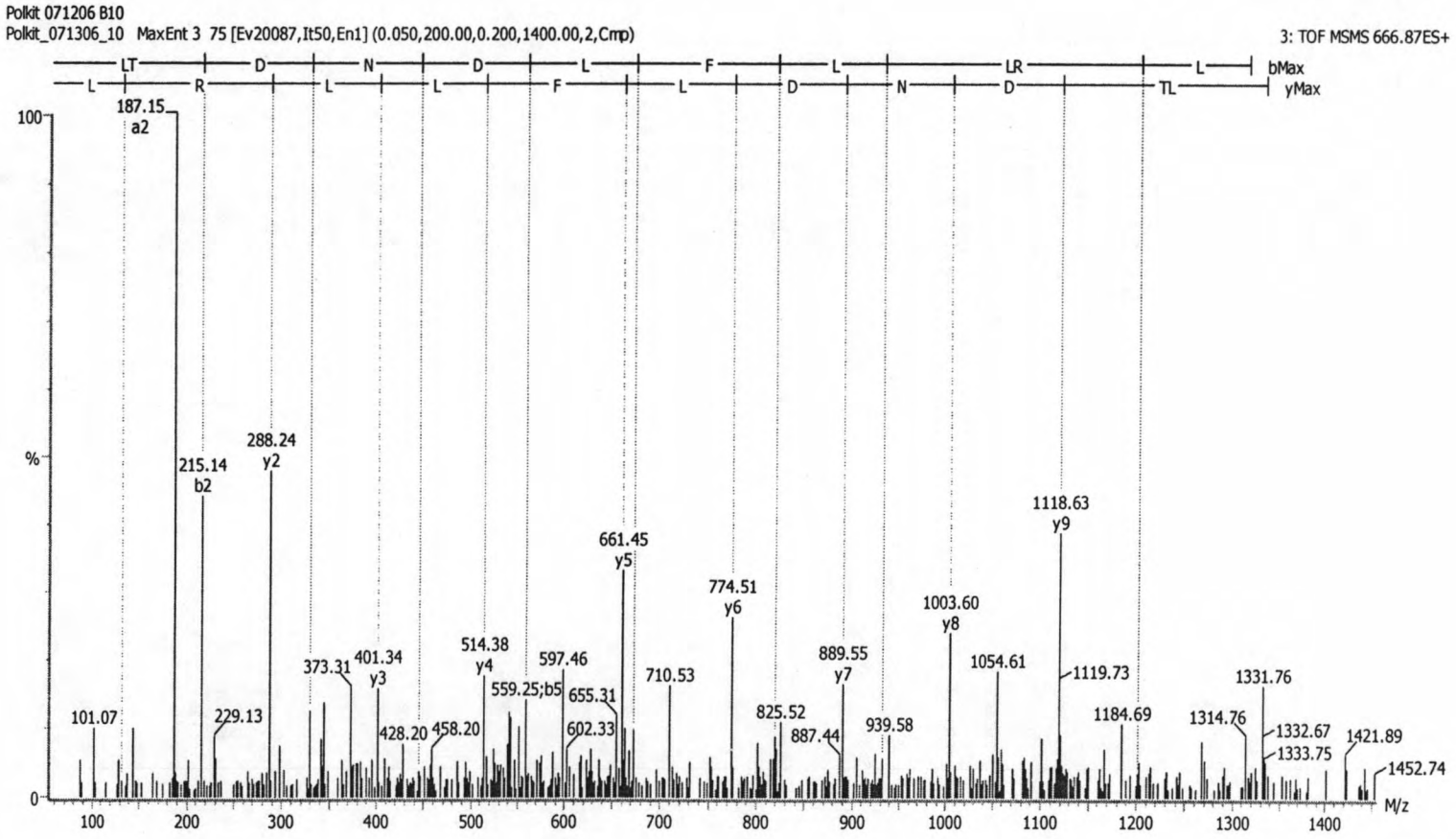

Figure 15 The product ion spectrum of peptide precursor m/z 666.87

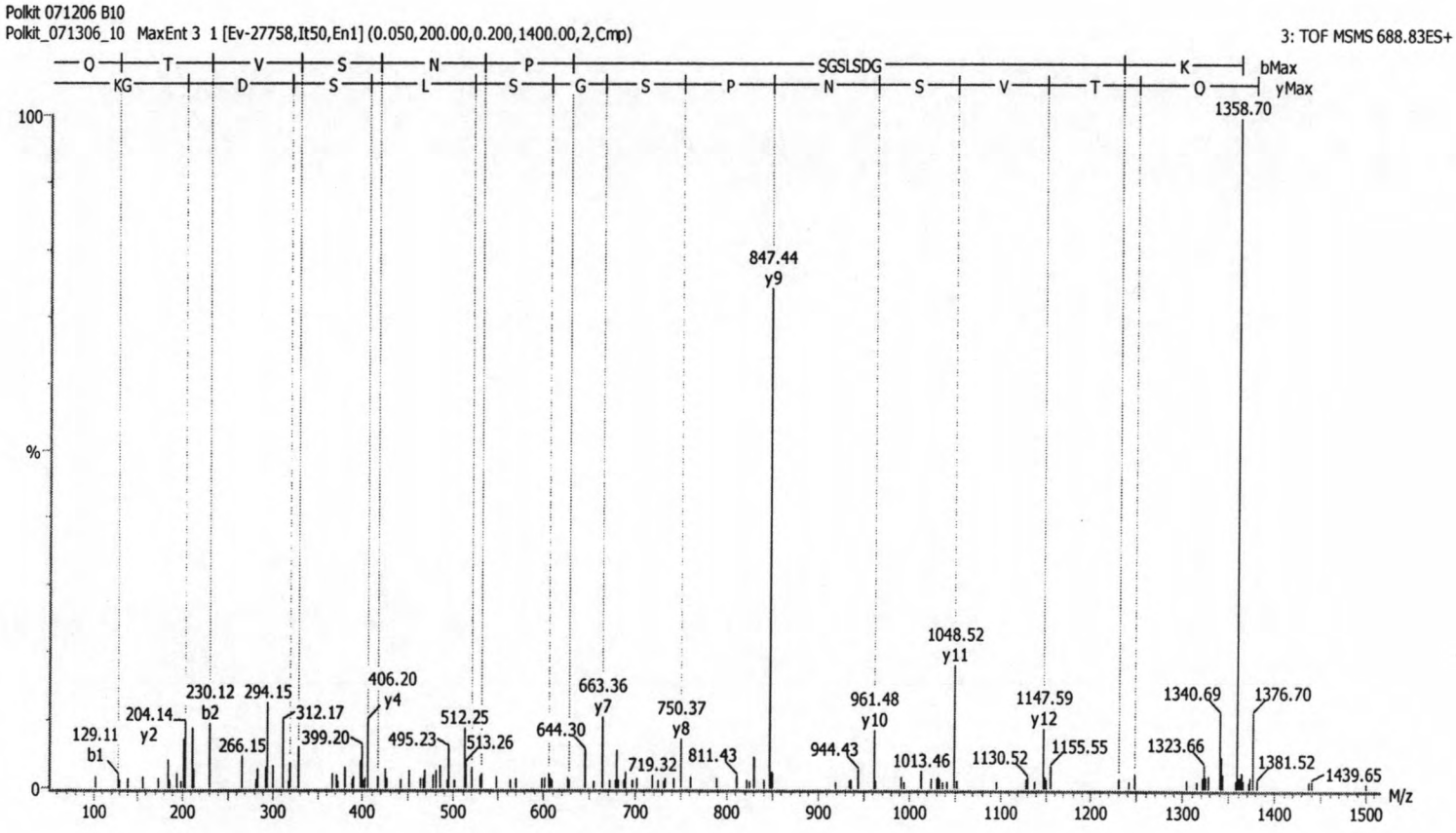

Figure 16 The product ion spectrum of peptide precursor m/z 688.83

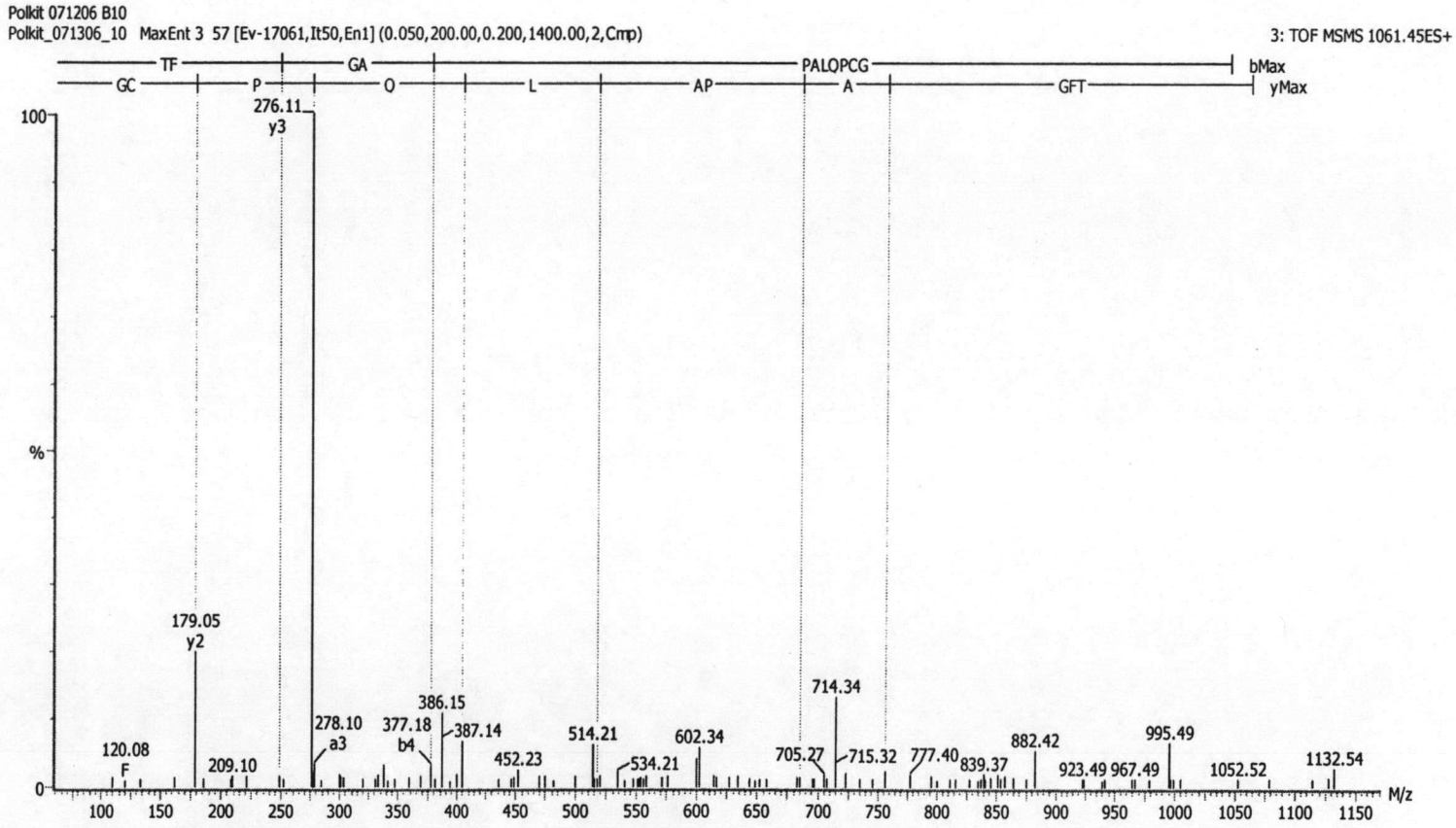

Figure 17 The product ion spectrum of peptide precursor m/z 1061.48

APPENDIX D

Amino Acid	3 letter-abbreviation	1 letter-abbreviation
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspatic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	G
Glycine	Gly	E
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Try	W
Tyrosine	Tyr	Y
Valine	Val	V

BIOGRAPHY

Miss Patcharaporn Tangngamsakul was born on August 22, 1982 in Bangkok province, Thailand. She graduated with Bachelor's degree of Science in Microbiology Department, from Faculty of Science, King Mongkut University of Technology Thonburi, Thailand in 2004. She continued the Master's degree of Science in Biotechnology, Faculty of Science, Chulalongkorn University.