The employment of MALDI-ToF in the chemical analysis of fingermarks.

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Abstract

Background and objectives: Many endogenous and exogenous molecules with different chemical nature including lipids, vitamins, amino acids polymers and other small molecules have been detected by Matrixassisted laser desorption/ionization (MALDI). Many studies were done to detect proteins in body fluids; however, few focused on detecting proteins in sweat. Recently peptides and small proteins in finger marks were detected in laboratory experiments including dermcidin and dermcidin-derived peptides. The aim of this study is to confirm the identity of dermcidin and other peptide species that can be found within fingermarks.

Methodology: This study was performed with the employment of MALDI following two constitutive enzymatic digestions by trypsin and carboxypeptidase respectively. Bioinformatics tools were employed to identify and verify peptide maps. This work is important to both forensic and biomedical science fields. These sciences are interested in dermcidin and dermcidin-derived peptides. Also this study established the protocols with a high potential for many other detectable peptides.

Results: In this work, the detection and identification of dermcidin and dermcidin-derived molecules and possibly dual specificity phosphatase (DUSP27_ HUMAN, formerly known as DUPD1) has been achieved. This has been done by using enzymatic digestions while the expected fragments obtained with MALDI and confirmed by the employment of bioinformatics tools.

Discussion and Conclusions: The species of dermcidin and dermcidin tryptic digested peptides have been successfully identified directly in fingermark. Extra efforts are recommended to identify and confirm other peptides detected in fingermarks.

Introduction

Dermcidin is an antimicrobial agent that is excreted by keratinocytes, neutrophils, sebocytes or sweat glands and excreted with sweat to the outer skin [1]. In the same context, the antimicrobial

peptide dermcidin was detected in a healthy volunteer eccrine sweat[2]. The major function of this peptide is to keep the human skin healthy. This is achieved by moderating the innate immune system guarding mechanism and also by possessing a wide range of antimicrobial activity, which acts to keep the skin intact from possible pathogens. In addition, extra information regarding the name, position and the amino acid sequences of dermcidin and dermcidin-derived peptides was delivered[3].

By a study, scientists hypothesized that dermcidin and its derivatives proteins and peptides can be detected in human fingermarks. This can be explained as the sweat carrying these peptides is transferred to the fingers and ultimately can be transferred to other surfaces through fingermarks[4]. Recently, these peptides were tentatively identified in fingermarks by the work of Francese's group using Matrix-assisted laser desorption/ionization time of flight mass spectrometry MALDI-TOF MS[5].

MALDI has been considered as fast soft-ionization technique originally reported for the ionization of high molecular weight molecules such as peptides and proteins[6,7,8,9,10]. In addition to peptides and proteins, many more molecules can be analyzed namely, polymers, oligonucleotides and lipids. This versatility has led MALDI to contribute to a high number of lifescience fields, such biomedical, pharmaceutical and chemical sciences[11].

Many researches were performed to optimize a MALDI method to detect and identify the proteins and peptides analytes. Accordingly, some chemists utilised fluorescence- or radio- labelling. Other chemists prefer enzymatic digestions to confirm the identity of the peptide(s) of interest[12].

This study has particularly focused on the confirmation of the identified dermcidin, dermcidin-derived peptides and, if possible, other available peptides. This can be achieved by using in-situ proteomics which involves the combination of classical protein chemistry techniques and mass spectrometry. These in-situ proteomics can be used directly on fingermarks to identify and verify the peptides and to produce peptide maps. Bioinformatic tools have also been employed to aid peptide identification.

The aim of this study is to verify the identified peptide molecules (i.e. dermcidin and dermcidin-derived peptides) that have been detected in other laboratories[5] as well as attempting to identify other possible peptides employing both traditional medical chemistry and instrumentation (i.e. MALDI). Bioinformatics are utilised to assign peptide maps. The importance of this work is to detect and verify the chemical structure of dermcidin in fingermarks. These peptides are of potential interest for both forensic, medicinal chemistry, and pharmaceutical science fields. In this work, the confirmation of the identified dermcidin and dermcidin-derived molecules and possibly Dual specificity phosphatase (DUSP27_HUMAN, formerly known as DUPD1) has been

performed by utilising two constitutive enzymatic digestions (trypsin and carboxypeptidase Y respectively) where the expected fragments were confirmed by the employment of bioinformatics. Further work is needed to identify and confirm other potential detectable peptides in fingermarks.

Materials and Methods:

Materials:

Trypsin (Porcine type) was purchased from Promega (Southampton,UK), Carboxypeptidase Y, α-Cyano-4-hydroxycinnamic acid, TLC Aluminium sheets, Methanol, Octyl β-D-glucopyranoside, Ammonium acetate, Ammonium bicarbonate, Trifluoroacetic acid (TFA), Chloroform, Acetonitrile and Ferulic acid were purchased from Sigma-Aldrich (Poole, UK), Dermcidin, DCD - 1L was purchased from ABgent (San Diego, US), double-sided Conductive Carbon Tape was purchased from TAAB Laboratories Equipment Ltd, (Berks, England).

Methods:

1. Sample preparation:

1.1. Aluminium sheet preparation:

Silica was removed from a 25cm X 25cm TLC Aluminium sheets by sonication in 50:50 ethanol: water solution. Acetone was subsequently employed to wipe the sheets gently, and render them to be cleaned. The Aluminium sheets were then cut into 2.0 cm X 2.5 cm and these small aluminium sheets were kept in a clean petri dish, covered and sealed to exclude any contamination. Prior to fingermark deposition, the strips were washed with 50:50 ethanol: water and then wiped and cleaned with acetone.

1.2. Fingermark preparation:

For the eccrine fingermark, the hands were washed with 50:50 ethanol: deionized water, air-dried (these two steps were done routinely for all types of fingermarks preparations). Then the hand was putted inside new/clean plastic bag and sealed well and left to induce sweating; after 10 minutes the fingermark was laid directly on a pre-prepared aluminium strip. While in the ungroomed fingermark, the hands were rubbed for few seconds and the fingermark was then laid on the aluminium strip. The third type of fingermark that was prepared was the groomed fingermark in which the fingers were rubbed against nose or forehead and the fingermarks were laid on the aluminium strip using medium pressure.

1.3 Fingermark sample preparation for MALDI-TOF MS analysis:

The aluminium strip with eccrine fingermark was then washed with ammonium acetate. Fingermarks were washed by using one of the ammonium acetate solutions that were prepared in three different concentrations (50, 100 and 150 mM). Furthermore, different washing methods

were tried; first of all the emerging which was done by submerging the aluminium strip in 10-mL of ammonium acetate solution for 30 seconds (results are not shown), the second was by pipetting 0.5 mL of the ammonium acetate solution 2-3 times and the third was by soaking the aluminium strip with 0.5 mL of ammonium acetate solution two times for 5 minutes each. In contrast the ungroomed fingermark was ready for analysis as there was no requirement for any washing process. However, the groomed fingermarks required different washing protocol to optimize the findings.

The fingermark on the aluminium strip was washed with 70% ethanol (HPLC graded ethanol in deionized water), the washing process was done by using a 10 ml-beaker filled with the 70% ethanol where the aluminium strip with the fingermark was merged for 30 seconds with an angle of 45^{o} and then emerged in another 10 ml-beaker filled with chloroform (HPLC graded) for 10 seconds and then left to air-dry at room temperature. For the all three mentioned fingermark types, the washed and dried aluminium strip containing the fingermark on it was stocked and well fixed on MALDI plate using double sided connective carbon tape. The best yield was observed with groomed fingermarks in terms of both good results and time reduction, so a decision was made to stick the groomed fingermarks preparation for the next enzymatic digestion steps.

2. Enzymatic digestion methods:

2.1. Tryptic digestion method:

A 20 µg Trypsin sealed-vial was reconstituted with 1 mL of 0.5% β-octyl-D-glucopyranoside and 40 mM ammonium bicarbonate (ammonium hydrogen carbonate) solution, and diluted down to make 10 millilitres of 2 µg/ mL with the same solvent composition and 10 aliquots (1mL each) were made and frozen at $-80^{o}C$ and when needed to be used, they were defrosted (one at a time). The prepared 1mL aliquot of trypsin solution was defrosted to apply to two different tried methods. The first was trypsin spraying method and the second was trypsin spotting method. In the trypsin spraying method, latent fingermarks were sprayed using a 'Suncollect' auto-spraying instrument. This auto-sprayer was purchased from Sunchrom GmbH (Friedrichsdorf, Germany). Fingermarks were sprayed with 5 layers of either a 0.02 µg/mL, 0.2µg/mL, 2µg/mL or 20µg/mL trypsin solution. The First layer was applied at a rate of 1.5µL/min and the 4 subsequent layers at 2µL/min. Fingermarks were then incubated for a period of either 3 hours or 24 hours before being sprayed or spotted with 5mg/mL α-CHCA solution (25:25:50) ACN/EtOH/0.5% TFA. The spraying was performed with 10 layers of a 5mg/mL α-CHCA solution. The first and second layer was applied at a rate of 5µL/min and 7µL/min respectively and all subsequent layers were deposited at a rate of 8µL/min. After spraying, two different incubation times were used; 3 and 24 hours. In trypsin spotting method, 1 µL-spots were spotted on different areas of the prepared

fingermark. In general, 5-6 spots were applied in each fingermark. After spotting, the sample was seated on a polystyrene pad and putted in a coupling jar filled with 40 mL of 50:50 methanol: water solution. The coupling jar then sealed and wrapped with parafilm and transferred to incubator to be incubated at 37^{o}C and 5% CO_2 for different incubation times; 1, 2, 3 and 24 hours were tried.

2.2. Carboxypeptidase Y digestion method:

A 1.1 mg-sealed vial was reconstituted with 1.1 mL of ultrapure water. The resultant 1 mg/mL solution was kept as a stock solution (kept at -20^{o}C). A diluted volume of 100 µL with 10 µg/mL was prepared. As a solvent, a solution of 0.5% β-Octyl-D-glucopyranoside and 40 mM ammonium bicarbonate (ammonium hydrogen carbonate), was used. The 10 µg/mL carboxypeptidase Y solution used after for digestion by spotting 3-4 of 0.5µL spots over most of the previous tryptic spots (leaving 2-3 trypsin-only spots to be used for comparison reasons). The carboxypeptidase spots were applied for 20, 30, and 60 minutes digestion whereas in the constitutive digestion the total time for tryptic digestion was always 3 hours. After the addition of the carboxypeptidase spots, the jar was sealed again and re-wrapped with a parafilm tape and re-incubated for the remaining time to achieve the total incubation period of 3 hours for both enzymes.

3. Matrix preparation:

Two different matrices, α-Cyano-4-hydroxycinnamic acid (CHCA) and Ferulic acid (FA) in three different concentrations (2, 5, and 10 mg/mL) were tried. The solvent system was initially 70/30 of ACN/0.5% TFA (results are not shown) then it was optimized to include ethanol: [ACN/ ethanol/0.5% TFA, 25/25/50]. 5 mg of CHCA was weighed and transferred to 1.5-mL eppendorf tube, 250 µL of each of ACN and EtOH were added and vortex for 1 minute then 500 µL of 0.5% TFA and vortex for few seconds, after preparation the matrix was kept in fridge at 4^{o}C. In general, the matrix was prepared twice weekly.

4. MALDI procedures:

4.1. MALDI plate preparation:

After the digestion was accomplished, two protocols of matrix application have been done; spraying protocol (used only for some of the tryptic sprayed fingermarks) and spotting protocol (used for the rest of work). Usually equal volumes of matrix with the previous spots volumes were used (i.e. 0.5 µL for the 0.5 µL and 1 µL for the 1µL …. etc.). The matrix was spotted over all the previous spotted areas with extra 2-3 spots onto fingermark area only (for comparison reasons). The spots were left to be air-dried and then loaded to the MALDI instrument for analysis.

4.2. Instrument settings

Positive, negative, reflector and linear modes were tried. Two mass spectrometers were used namely: Voyager -DE STR and Voyager -DE PRO.

4.2.1. Voyager-DE STR

Mass spectrometric analyses were conducted using an Applied Biosystems MALDI TOF Voyager DE-STR mass spectrometer (Foster City, CA) with serial number of 4286 was equipped with a 355 nm Nd-YAG solid state laser operating at a repetition rate of 20 Hz. Full scan mass spectra in the m/z range 500-5000 Daltons were recorded in positive linear delayed mode and 100 shots were accumulated per spectrum. The accelerating voltage was set at 25,000 Volts, the grid voltage was set at 93% and the delay time was 150 ns. The acquisition control was manual while the guide wire was 0.15%. The calibrant used was a mixture of eight different proteins summarized in Table 2.

Table 2. This is used standard calibrant peptide mixture, showing their names, charges and their average masses in ionized form. This covers mass to charge range of 905.05-5734.59 Daltons.

No.	Peptide name	Charge	($M H^+$) Average
1	des-Arg^1-Bradykinin	+1	905.05
2	Angiotensin I	+1	1297.51
3	Glu^1-Fibrinopeptide B	+1	1571.61
4	Neurotensin	+1	1673.96
5	ACTH (clip 1-17)	+1	2094.46
6	ACTH (clip 18-39)	+1	2466.72
7	ACTH (clip 7-38)	+1	3660.19
8	Insulin (bovine)	+1	5734.59

4.2.2. Voyager-DE PRO

MALDI analyses were conducted using Perspective Biosystems MALDI Voyager DE-PRO Biospectrometry Workstation. Solid state nitrogen laser with a wavelength of 2.0 A^0 (Angstroms). Full scan mass spectra in the m/z range 500-3000 Daltons were recorded in positive reflector delayed mode and 100 shots were accumulated per spectrum. The accelerating voltage was set at 25,000 Volts, the grid voltage was set at 93% and the delay time was 150 ns. The acquisition control was manual while the guide wire was 0.15%. With the 1.30-m effective path length, reflector mode. (Very poor results were obtained with the reflector mode and hence are not shown in this study).

Results

Dermcidin (DCD-1L, *m/z* = 4819.47 Da) was a specific target to seek its peak in the fingermarks. The overall aim of this project was pursued through an experimental step-wise approach rationale and results will be presented accordingly.

1. MALDI MS *Matrix* selection:

As the ungroomed fingermark is considered faster in preparation than both groomed and eccrine fingermarks, it has been used for method optimization for the matrix selection. Two matrices were considered namely α-Cyano-4-hydroxycinnamic acid (α -CHCA) and ferulic acid (FA). α- CHCA performed better than Ferulic acid (FA) to detect DCD-1L in fingermarks. Also, positive mode for both organic acids worked better than negative mode as shown in details in figures 1-4 in Appendix A. Among the three concentrations of CHCA used, the 5 mg/mL was better than the 2 and 10 mg/mL concentrations and the solvent system used for the CHCA matrix (ACN/ ethanol/0.5% trifluoroacetic acid, 25/25/50) has been found to be the best amongst all the compositions tested (data not shown).

2. Fingermarks types selection:

After selection of the matrix system, the selection of one of the three different types of fingermarks was tried, namely; groomed, ungroomed and eccrine fingermarks types. The following observations were obtained with the three kinds of fingermarks: the first one is the *ungroomed* fingermarks. The peptides detection was good in respect to groomed and eccrine fingermarks. The MALDI spectra of the ungroomed fingermark can be seen individually in figure 5 appendix A and also shown in comparison with other two types of fingermarks in figure 1. The second type of fingermarks is the *groomed* fingermarks which was better than the ungroomed as the peaks were more intense (explained in fig. 6 appendix A) and the time used was the same of the ungroomed plus few seconds for grooming and approximately 1 minute for fingermark washing procedure. The washing protocol itself has an influence on the groomed fingermark, the washed fingermarks with both ethanol and chloroform gave better results as shown in figure 7 appendix A. The third and last fingermarks type used in this work is the *eccrine* fingermarks. The dermcidin and its derivatives peptides were detected, after method optimisation regarding the concentration of ammonium acetate used in washing, the time of washing and the washing protocol. The eccrine fingermark washed with 100mM ammonium acetate by soaking for 10 minute (figure 12 appendix A) was the best among all the observed data as shown in figures 8-12 in appendix A.

In general, the best selected fingermarks type was the ungroomed fingermarks with a good yield of the peptides of interest and a reasonably time consumed for the sample preparation as can be seen in figure 1 below.

3. Enzymatic digestion:

This is further divided to standard enzymatic digestions and in situ enzymatic digestions:

3.1. A. *Standard enzymatic digestion*

Trypsin cleaves peptide bonds at the C-terminal end of the amino acids lysine (K) and arginine (R), except those are next to proline (P). However, there is no single proline amino acid residue present in DCD-1L. By the ease of using the bioinformatic tool online (http://www.expasy.org/) the tryptic digestion can be predicted. The identification process employed in this project encompasses the theoretical digestion of dermcidin with trypsin enzyme allowing the maximum number missed cleaved bonds (to mimic the real *in situ* digestion). As explained the cleaving should occur on the C-terminal of lysine (***K***) or arginine (R) amino acids. The detailed theoretical peptide mass list of the DCD-1L sequence [SSLLE***K***GLDGA***KK***AVGGLG***K***LG***K***DAVEDLESVG***K***GAVHDV***K***DVLDSVL] is shown in table 3.

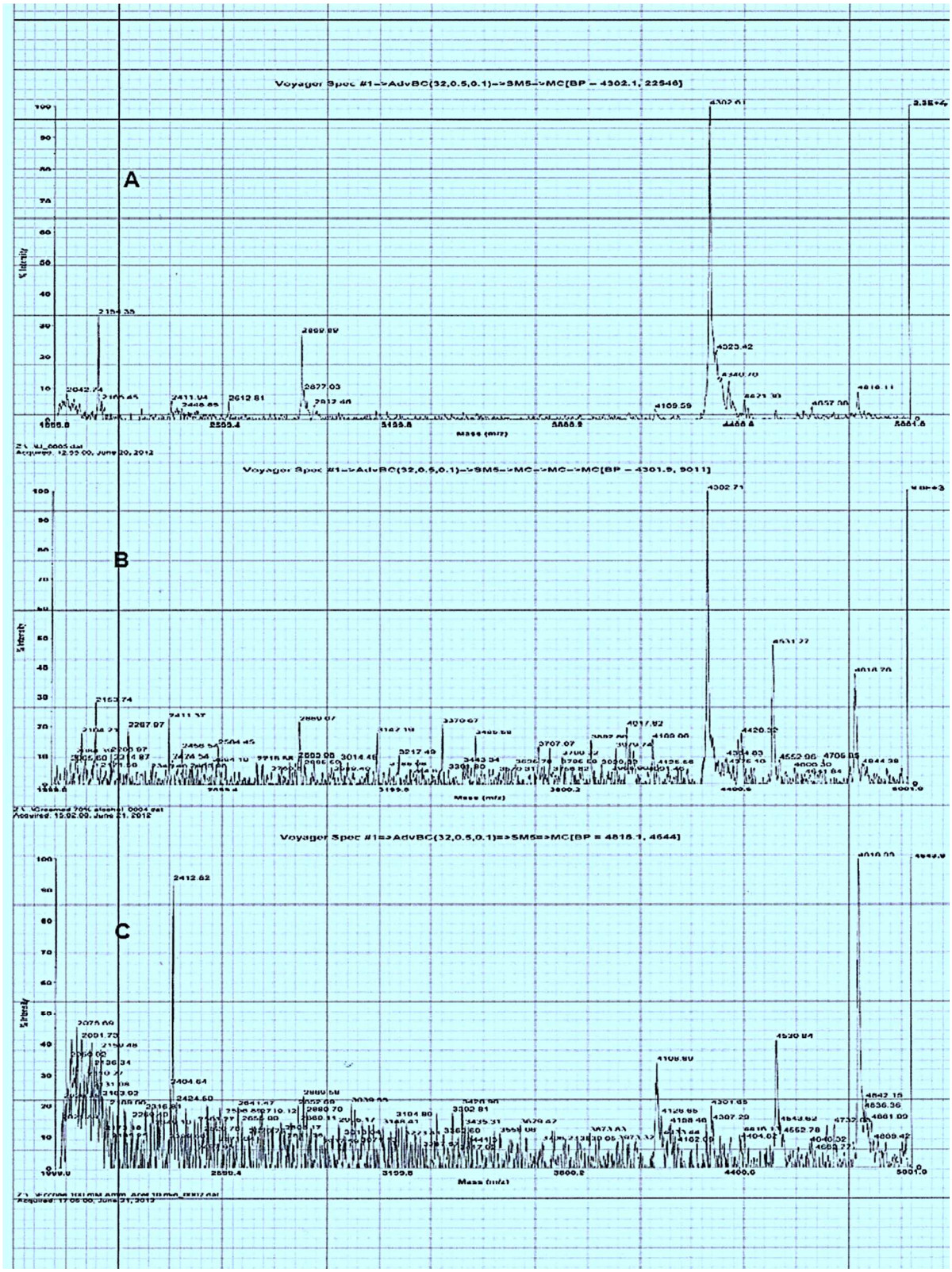


Fig. 1 MALDI-TOF mass spectrum which is compares the three types of the fingermarks of:

A: ungroomed fingermarks.

B: groomed fingermarks.

C: eccrine fingermarks.

Table 3 shows the theoretical expected cleavages of DCD-1L at the C-terminal of the lysine (K) amino acids allowing maximum missed cleavages. The chosen mass range was from 500 to 5000 Daltons (peptides with masses less than 500 Da are not shown) as the study focuses on this range. While in this work the star (*) labelled sequences were observed.

No.	Amino acid sequences within the peptides	*m/z* [M+
1.	SSLLEKGLDGAKKAVGGLGKLGKDAVEDLESVGKGAVHDVKDVLDSVL*	4819.47
2.	GLDGAKKAVGGLGKLGKDAVEDLESVGKGAVHDVKDVLDSVL*	4161.70
3.	SSLLEKGLDGAKKAVGGLGKLGKDAVEDLESVGKGAVHDVK	4077.63
4.	KAVGGLGKLGKDAVEDLESVGKGAVHDVKDVLDSVL*	3620.10
5.	AVGGLGKLGKDAVEDLESVGKGAVHDVKDVLDSVL*	3491.93
6.	GLDGAKKAVGGLGKLGKDAVEDLESVGKGAVHDVK	3419.86
7.	SSLLEKGLDGAKKAVGGLGKLGKDAVEDLESVGK*	3370.84
8.	LGKDAVEDLESVGKGAVHDVKDVLDSVL*	2909.24
9.	KAVGGLGKLGKDAVEDLESVGKGAVHDVK*	2878.26
10.	AVGGLGKLGKDAVEDLESVGKGAVHDVK	2750.09
11.	GLDGAKKAVGGLGKLGKDAVEDLESVGK	2713.07
12.	DAVEDLESVGKGAVHDVKDVLDSVL*	2610.86
13.	SSLLEKGLDGAKKAVGGLGKLGK*	2227.62
14.	KAVGGLGKLGKDAVEDLESVGK	2171.47
15.	LGKDAVEDLESVGKGAVHDVK	2167.40
16.	AVGGLGKLGKDAVEDLESVGK*	2043.30
17.	SSLLEKGLDGAKKAVGGLGK *	1929.24
18.	DAVEDLESVGKGAVHDVK	1869.02
19.	GLDGAKKAVGGLGKLGK	1569.85
20.	GAVHDVKDVLDSVL*	1467.64
21.	LGKDAVEDLESVGK	1460.61

22.	SSLLEKGLDGAKK *	1346.55
23.	GLDGAKKAVGGLGK	1271.47
24.	SSLLEKGLDGAK	1218.38
25.	DAVEDLESVGK	1162.23
26.	KAVGGLGKLGK	1028.25
27.	AVGGLGKLGK	900.08
28.	DVLDSVL	760.85
29.	KAVGGLGK	729.87
30.	GAVHDVK	725.80
31.	GLDGAKK	688.78
32.	SSLLEK *	676.78
33.	AVGGLGK*	601.70
34.	GLDGAK	600.61

Trypsin was spotted with DCD-1L standards as a kind of improving methodology. To do so, different trypsin spotting procedures were done on fingermarks (almost with duplicates). Multiple concentrations of both trypsin and DCD-1L standards have been tried. The tested trypsin concentrations were 2, 0.2, and 0.02 µg/mL whereas the DCD-1L standard concentrations were 10, 1, and 0.1 µg/mL. Each trypsin concentration was used to digest the three different concentrations of dermcidin, resulting in a total of 9 MALDI MS spectra of DCD-1L tryptic digest (explained in details in the figures from 1A to 3C appendix B). These spectra can also be represented by chart with columns as shown in figure 2, where the peak intensity refers to that of the dermcidin cleaved peptide signal LGKDAVEDLESVGKGAVHDVKDVLDSVL at a m/z of 2909.24). Unsurprisingly, the resultant peak-intensities depend on both concentrations of DCD-1L and trypsin, as can be observed from the chart.

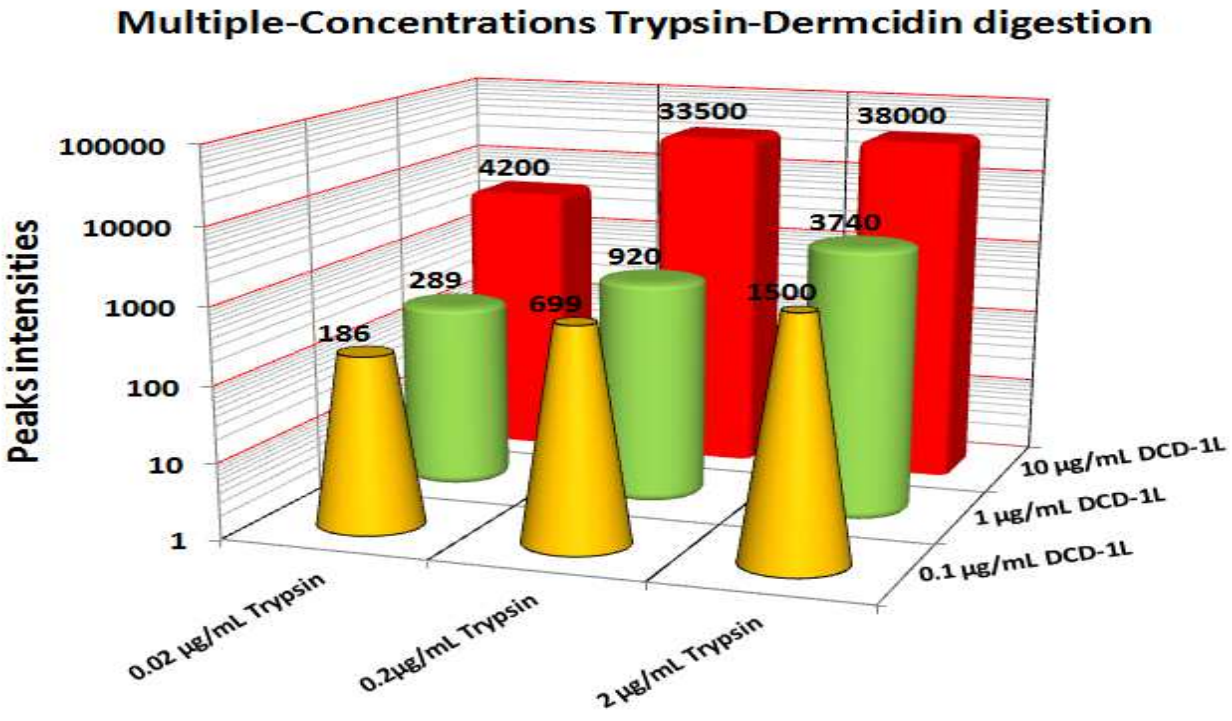


Fig. 2 Optimisation of trypsin digestion conditions on DCD-1L standard. This figure shows the differences in peak-intensities (peaks intensities are located on the top of each column). These peaks correlate to different concentrations of both trypsin and dermcidin. The peak used to measure the intensities has m/z ratio of 2909.24.

The best result was observed with the 0.2 µg/mL trypsin and 10 µg/mL DCD-1L (shown in figure 3) which yields the highest number of peptides covering the dermcidin amino acid sequence as shown in figure 4

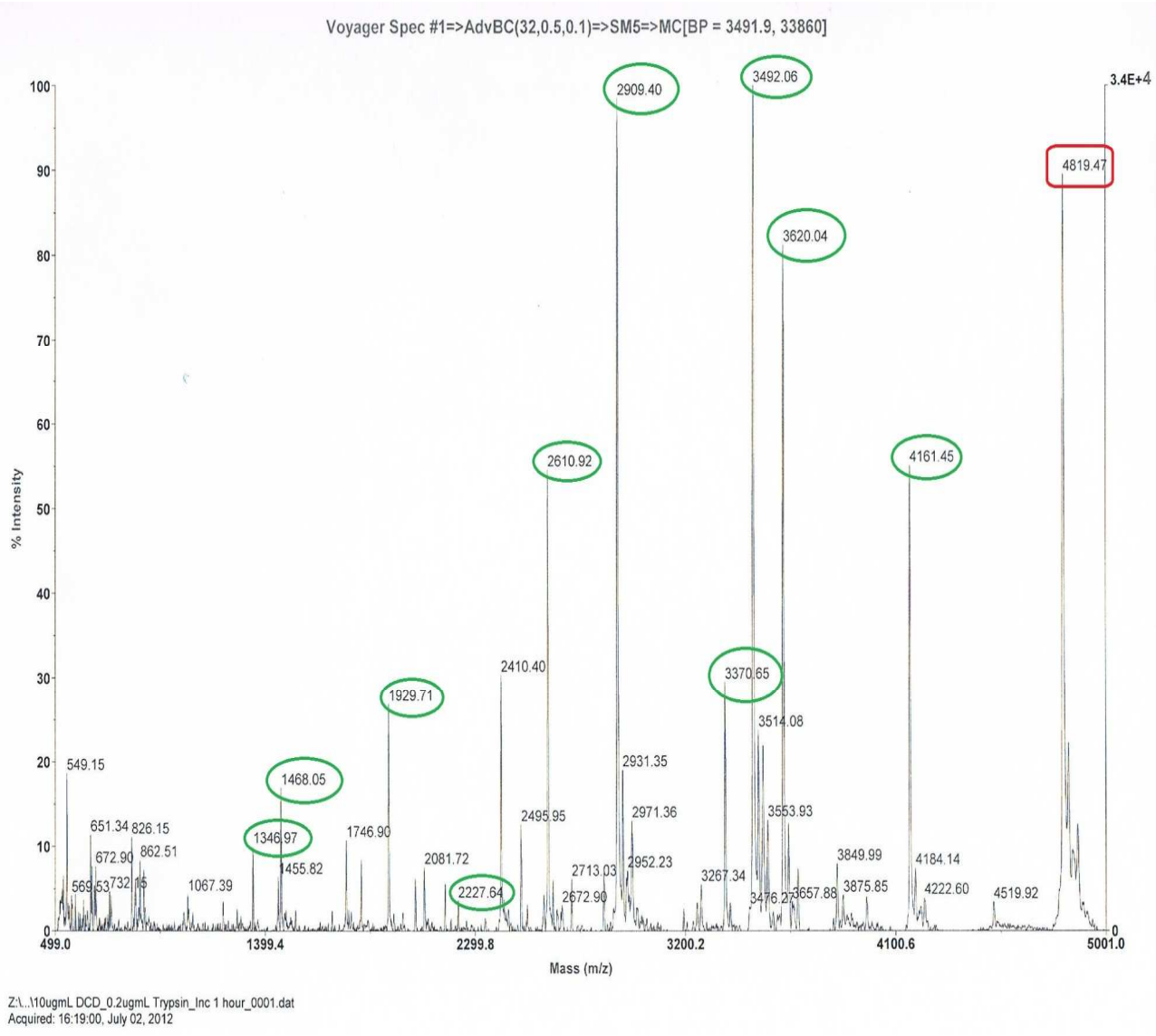


Fig. 3 MALDI-TOF mass spectrum which shows the digestion of 10 µg/mL of standard DCD-1L by 0.2 µg/mL trypsin. The digestion performed for 1 hour.

= DCD-1L the starting material (the substrate)

= The cleaved peptides peak.

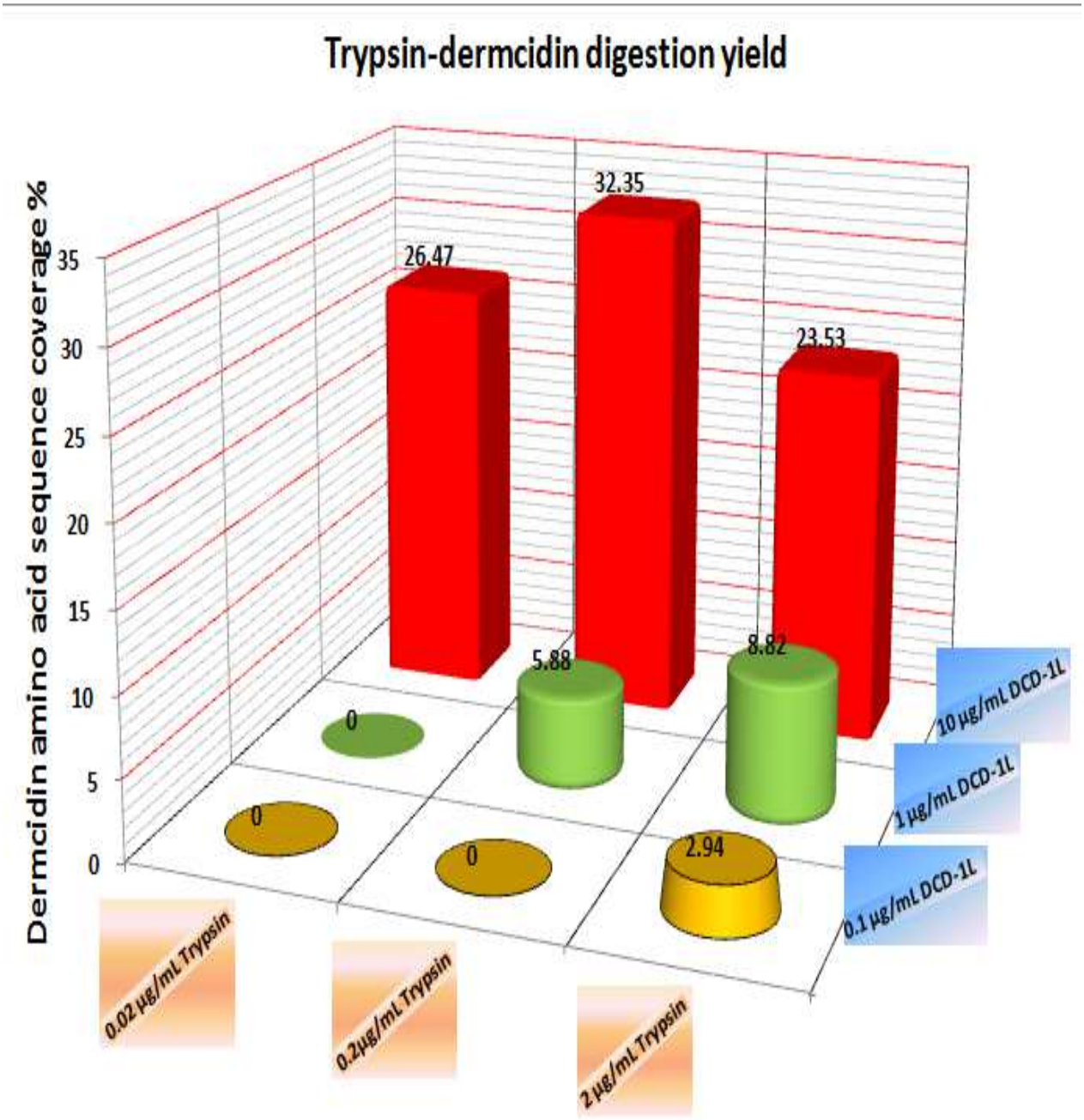


Fig. 4 represents the yield% of tryptic digestion of standard DCD-1L depending on the covered number of the amino acids sequences resulted from the digestion. The columns with zero values do not mean there is no yield, but it cannot be seen.

Moreover, the peaks resulted from the 1 hour incubation tryptic digestion under these conditions are listed in table 4. From which the accuracy can be calculated depending on the theoretically predicted peaks masses which are listed in table 3.

Table 4. This table shows a comparison between the peaks observed from tryptic digestion of standard dermcidin (shown in figure 1 appendix B) with the theoretical calculated peaks (shown in

table 3). The mass accuracy shows a fair proximity of the observed peaks with their corresponding theoretical peaks.

No.	*Observed peak masses (Da)*	*Theoretical peak masses (Da)*	*Mass accuracy %*
1	**4161.51**	**4161.70**	**99.99543**
2	**3619.93**	**3620.10**	**99.9953**
3	**3491.82**	**3491.93**	**99.99685**
4	**2909.50**	**2909.24**	**99.99106**
5	**2610.69**	**2610.86**	**99.99349**
6	**2043.14**	**2043.30**	**99.99217**
7	**1467.61**	**1467.64**	**99.99796**

3.1. B. *In situ enzymatic digestion*

- *Spotting method:*

Similar to the optimization of the tryptic digestion on the DCD-1L standard, *in situ* optimization was carried out to yield maximum peptide coverage of the endogenous DCD-1L and other peptides directly from fingermarks using the spotting method. Tested concentrations were 0.02, 0.2, 2 and 20 µg/mL of trypsin. By having a close look to the figures 4-8 appendix B, figure 8 shows the best digestion as expected, in which the used tryptic concentration was 20 µg/mL and 24 hrs incubation time performed. However, there is no big difference between figures 7 and 8 in appendix B, as in the former one the incubation time was 3 hrs instead of 24 hrs whereas the tryptic concentration still 20 µg/mL. In addition, the resultant spectra were converted to text files and processed with mMass software programme, peaks picking was used to create a list of peaks with good intensities as shown in figure 5 below.

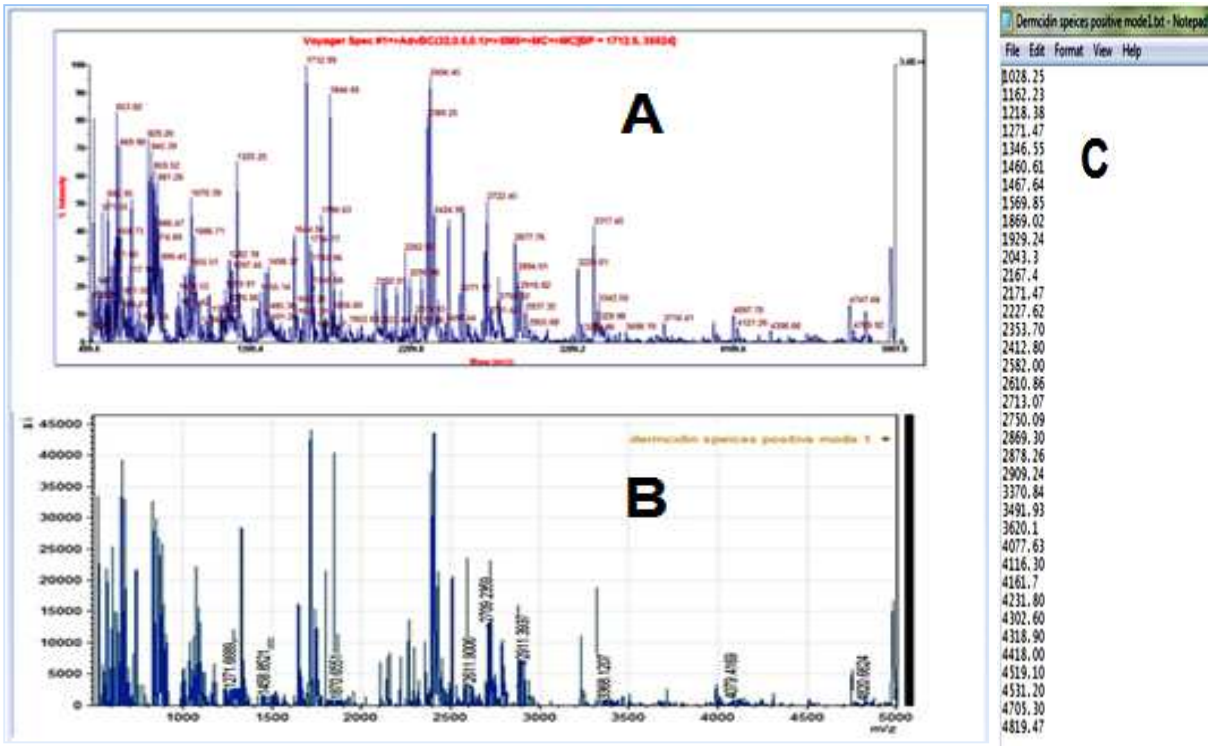


Fig. 5 this figure shows:

A: The original calibrated MALDI-TOF mass spectrum of a groomed fingermarks digested with 2µg/mL trypsin for 3hrs.

B: A spectrum created in mMass after converting the original spectrum to text file (text file not shown) and manually picking the peaks of interest.

C: The text file created from the assigned peaks from spectrum B without their intensities (m/z only).

This list ultimately converted to text file where the later was submitted online through Matrix Science website to Mascot search engine for peptide mass fingerprinting. Dermcidin has been confirmed to be in the tryptic digested fingermark. The peptide mass tolerance used was ±1.2 Da and the allowed missed cleavages were 2. The score for dermcidin is 89 and by knowing that the score of 56 is considered to be significant, so 89 would be highly significant and more reliable as can be seen in figure 6 below.

MATRIX SCIENCE **Mascot Search Results**

User : ZAID AL-OBAIDI
Email : zaid_alobaidi@yahoo.com
Search title : possible peptides in fingermarks
MS data file : Dermcidin speices positive model.txt
Database : SwissProt 2012_07 (536789 sequences; 190518892 residues)
Taxonomy : Homo sapiens (human) (20232 sequences)
Timestamp : 12 Aug 2012 at 10:26:04 GMT
Top Score : 89 for DCD_HUMAN, Dermcidin OS=Homo sapiens GN=DCD PE=1 SV=2

Mascot Score Histogram

Protein score is -10*Log(P), where P is the probability that the observed match is a random event.
Protein scores greater than 56 are significant (p<0.05).

Concise Protein Summary Report

Format As | Concise Protein Summary | Help
Significance threshold p< 0.05 | Max. number of hits AUTO
Re-Search All | Search Unmatched

1. DCD_HUMAN Mass: 11284 Score: 89 Expect: 2.4e-05 Matches: 11
Dermcidin OS=Homo sapiens GN=DCD PE=1 SV=2

2. AT7L3_HUMAN Mass: 38651 Score: 46 Expect: 0.52 Matches: 10
Ataxin-7-like protein 3 OS=Homo sapiens GN=ATXN7L3 PE=1 SV=1

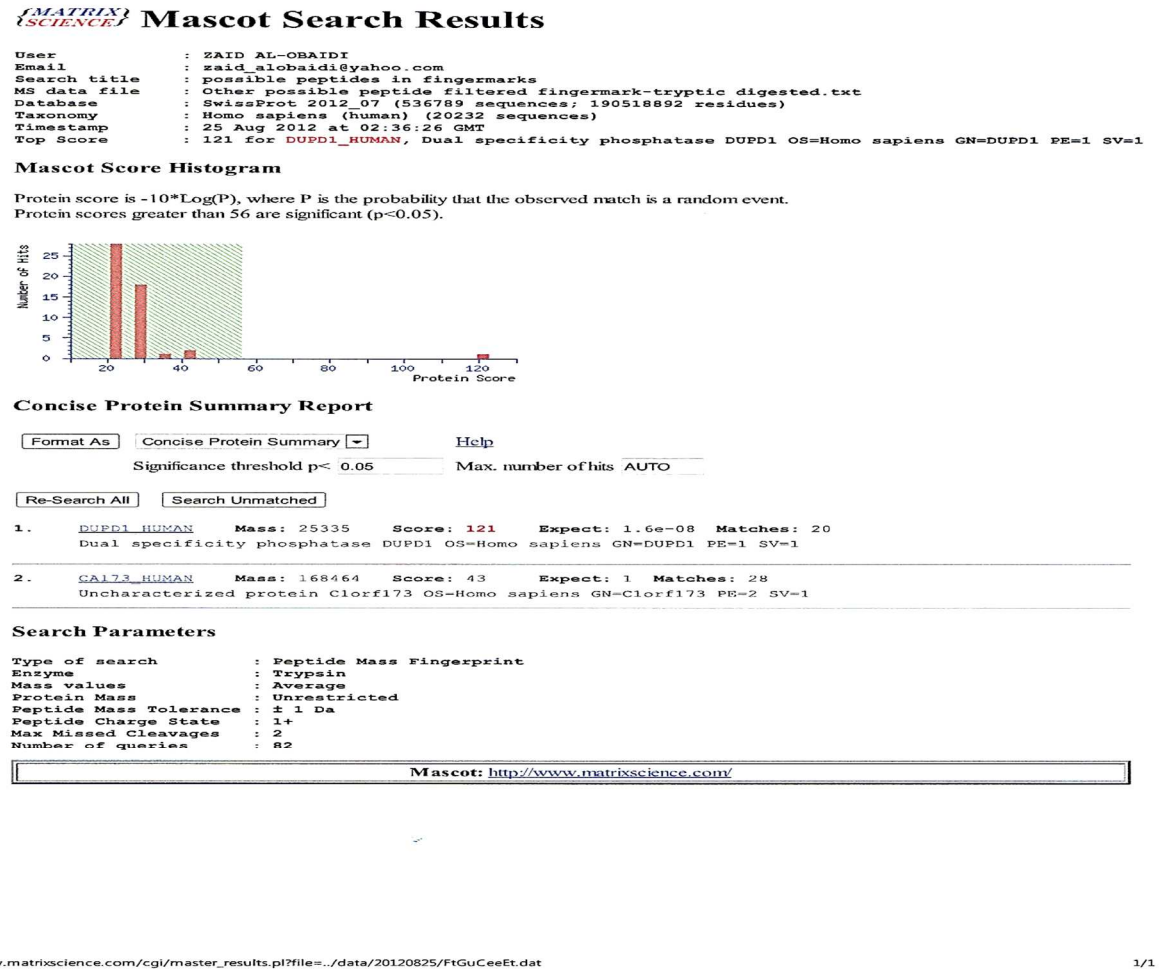
Search Parameters

Type of search : Peptide Mass Fingerprint
Enzyme : Trypsin
Mass values : Average
Protein Mass : Unrestricted
Peptide Mass Tolerance : ± 1.2 Da
Peptide Charge State : 1+
Max Missed Cleavages : 2
Number of queries : 37

www.matrixscience.com/cgi/master_results.pl?file=../data/20120812/FtGuSxYmt.dat 1/2

Fig. 6 represents Mascot research results were gained after a submission of a particularly pre-processed text file. The search confirms the existence of human dermcidin in fingermark. Can be seen also at: www.matrixscience.com/cgi/master_results.pl?file=../data/20120812/FtGuSxYmt.dat

This is not the full story; the Mascot has predicted a new peptide in fingermarks; it is ' Dual specificity phosphatase (DUSP27_HUMAN)'. This was done by filtering the data step by step, so the original spectrum was converted to text file. Then this text file was processed e.g. smoothing, also the peaks were picked as groups; so the peaks related to dermcidin are identified and well known and by excluding them, the resultant is the whole spectrum without dermcidin. This was applied for trypsin-autolysis peaks and then a contaminant peaks were detected (will be explained with details in figure 8). This led to the identification of the DUSP27_HUMAN enzyme with significantly high score as shown in figure 7.



MATRIX SCIENCE **Mascot Search Results**

User : ZAID AL-OBAIDI
Email : zaid_alobaidi@yahoo.com
Search title : possible peptides in fingermarks
MS data file : Other possible peptide filtered fingermark-tryptic digested.txt
Database : SwissProt 2012_07 (536789 sequences; 190518892 residues)
Taxonomy : Homo sapiens (human) (20232 sequences)
Timestamp : 25 Aug 2012 at 02:36:26 GMT
Top Score : 121 for DUPD1_HUMAN, Dual specificity phosphatase DUPD1 OS=Homo sapiens GN=DUPD1 PE=1 SV=1

Mascot Score Histogram

Protein score is -10*Log(P), where P is the probability that the observed match is a random event.
Protein scores greater than 56 are significant (p<0.05).

Number of Hits 0 5 10 15 20 25
20 40 60 80 100 120
Protein Score

Concise Protein Summary Report

Format As | Concise Protein Summary | Help
Significance threshold p< 0.05 | Max. number of hits AUTO
Re-Search All | Search Unmatched

1. DUPD1_HUMAN Mass: 25335 Score: 121 Expect: 1.6e-08 Matches: 20
Dual specificity phosphatase DUPD1 OS=Homo sapiens GN=DUPD1 PE=1 SV=1

2. CA173_HUMAN Mass: 168464 Score: 43 Expect: 1 Matches: 28
Uncharacterized protein C1orf173 OS=Homo sapiens GN=C1orf173 PE=2 SV=1

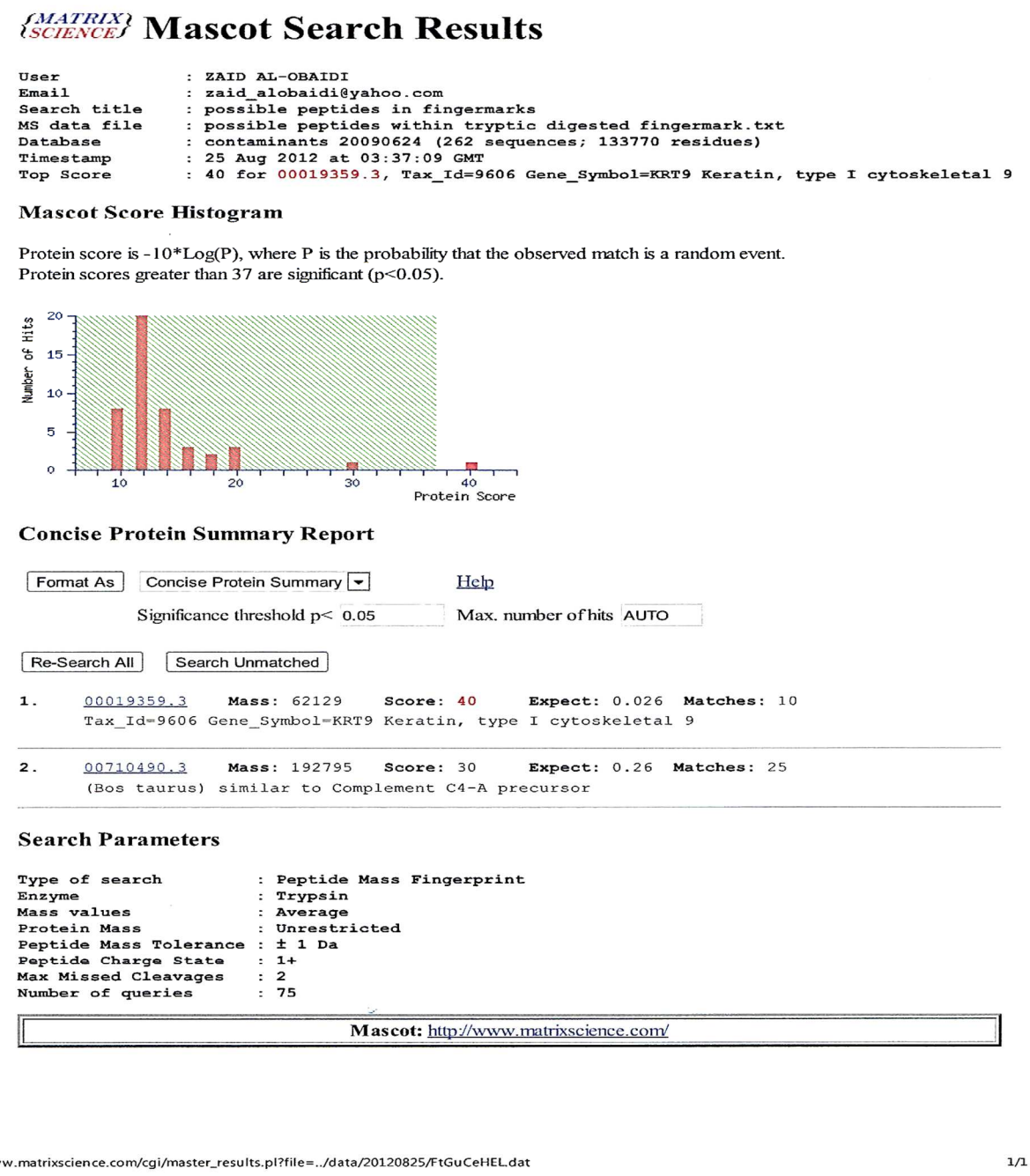
Search Parameters

Type of search : Peptide Mass Fingerprint
Enzyme : Trypsin
Mass values : Average
Protein Mass : Unrestricted
Peptide Mass Tolerance : ± 1 Da
Peptide Charge State : 1+
Max Missed Cleavages : 2
Number of queries : 82

Mascot: http://www.matrixscience.com/

www.matrixscience.com/cgi/master_results.pl?file=../data/20120825/FtGuCeeEt.dat 1/1

Fig. 7 Mascot research predicts the presence of ' Dual specificity phosphatase (DUPD1 or DUSP27_HUMAN)' in fingermark that was digested by two constitutive enzymes (trypsin and carboxypeptidase Y respectively). This result was obtained after a submission of a pre-processed text file and can be seen at:
www.matrixscience.com/cgi/master_results.pl?file=../data/20120825/FtGuCeeEt.dat
Unsurprisingly, contaminant protein (as named by The Matrix Science) "Keratin" is detected in fingermark and also it has been predicted by Mascot as shown in figure 8 below.



MATRIX SCIENCE **Mascot Search Results**

User : ZAID AL-OBAIDI
Email : zaid_alobaidi@yahoo.com
Search title : possible peptides in fingermarks
MS data file : possible peptides within tryptic digested fingermark.txt
Database : contaminants 20090624 (262 sequences; 133770 residues)
Timestamp : 25 Aug 2012 at 03:37:09 GMT
Top Score : 40 for 00019359.3, Tax_Id=9606 Gene_Symbol=KRT9 Keratin, type I cytoskeletal 9

Mascot Score Histogram

Protein score is -10*Log(P), where P is the probability that the observed match is a random event.
Protein scores greater than 37 are significant (p<0.05).

Concise Protein Summary Report

Format As | Concise Protein Summary | Help

Significance threshold p< 0.05 Max. number of hits AUTO

Re-Search All | Search Unmatched

1. 00019359.3 Mass: 62129 Score: 40 Expect: 0.026 Matches: 10
Tax_Id=9606 Gene_Symbol=KRT9 Keratin, type I cytoskeletal 9

2. 00710490.3 Mass: 192795 Score: 30 Expect: 0.26 Matches: 25
(Bos taurus) similar to Complement C4-A precursor

Search Parameters

Type of search : Peptide Mass Fingerprint
Enzyme : Trypsin
Mass values : Average
Protein Mass : Unrestricted
Peptide Mass Tolerance : ± 1 Da
Peptide Charge State : 1+
Max Missed Cleavages : 2
Number of queries : 75

Mascot: http://www.matrixscience.com/

www.matrixscience.com/cgi/master_results.pl?file=../data/20120825/FtGuCeHEL.dat 1/1

Fig. 8 This figure shows the observed 'keratin' in fingermark that is predicted by the Mascot online freeware. The research considered keratin as a kind of contaminants. It was obtained after a submission of a pre-processed text file. The search confirms the existence of human dermcidin. Can be seen also at:
www.matrixscience.com/cgi/master_results.pl?file=../data/20120825/FtGuCeHEL.dat

- *Tryptic digest of fingermarks by using the spray coat technique:*

For the sprayed trypsin, 20, 2, 0.2 and 0.02 µg/mL concentrations were used with two different incubation times; 3 and 24 hours respectively. Unfortunately, there was a leakage within the auto-sprayer for the all trypsin concentrations except the 20 µg/mL. This in turn enforces the results to

be unreliable and to be excluded. The peaks of DCD-1L those got within 3 and 24 hours for the 20 µg/mL trypsin-fingermark digestion are shown in figures 9 and 10 appendix B.

- *Trypsin incubation time selection results:*

Multiple incubation periods have been tried starting with one hour incubation time then 2, 3, 24 hours incubations. The longer the incubation time the better the peptide digestion the larger the autolytic effects observed. So the long period will increase the trypsin autolytic activity and this increase the number of tryptic peaks and this badly affects the whole spectrum. After deep examination of the results, the three hours incubation time was the most fairly suitable with good digestion, reducing the time span for each digestion and more significantly reducing the autolysis beaks in the spectra. These effects can be seen in figures 3A, 6 and 8 in appendix B.

4. Carboxypeptidase Y digestion

Carboxypeptidase Y was used to digest the tryptic-digested standard DCD-1L and its resulted peptides, also carboxypeptidase Y was used as *in situ* applied enzyme to digest the previous tryptic-digested peptides in fingermarks. This work was successfully performed to confirm the identity of previously detected tryptic-digested peptides.

4.1 Standard DCD-1L peptides species verification:

As mentioned previously trypsin splits peptide residues by breaking down the amide bond between two bonded amino acids at the C-terminal end, specifically of the amino acids lysine (K) and arginine (R), except those are next to proline (P) bearing in mind there is no single proline amino acid residue present in DCD. However, carboxypeptidase Y hydrolyzes the peptide chain of an amino acid residue at the carboxyl side. The idea of verification is simplified with the following example: as a result of tryptic digestion the following peptide chain is expected to be produced (GLDGAKKAVGGLGKLGKDAVEDLESVGKGA VDVKDVLDSVL) and it has m/z of 4160.7, so this peptide chain when subjected for further carboxypeptidase digestion, the expected digestion would be the cleavage of (L) leucine (bounded leucine has mass of 113.0 Da) at the C-terminal end. In terms of mathematics this means 4161.86 - 113 = 4048.86 *m/z* and what was found is 4048.42 *m/z*. Accordingly, the other tryptic digestion peaks can be calculated and verified by further digestion with carboxypeptidase as observed in figure 9 and listed in table 5**.**

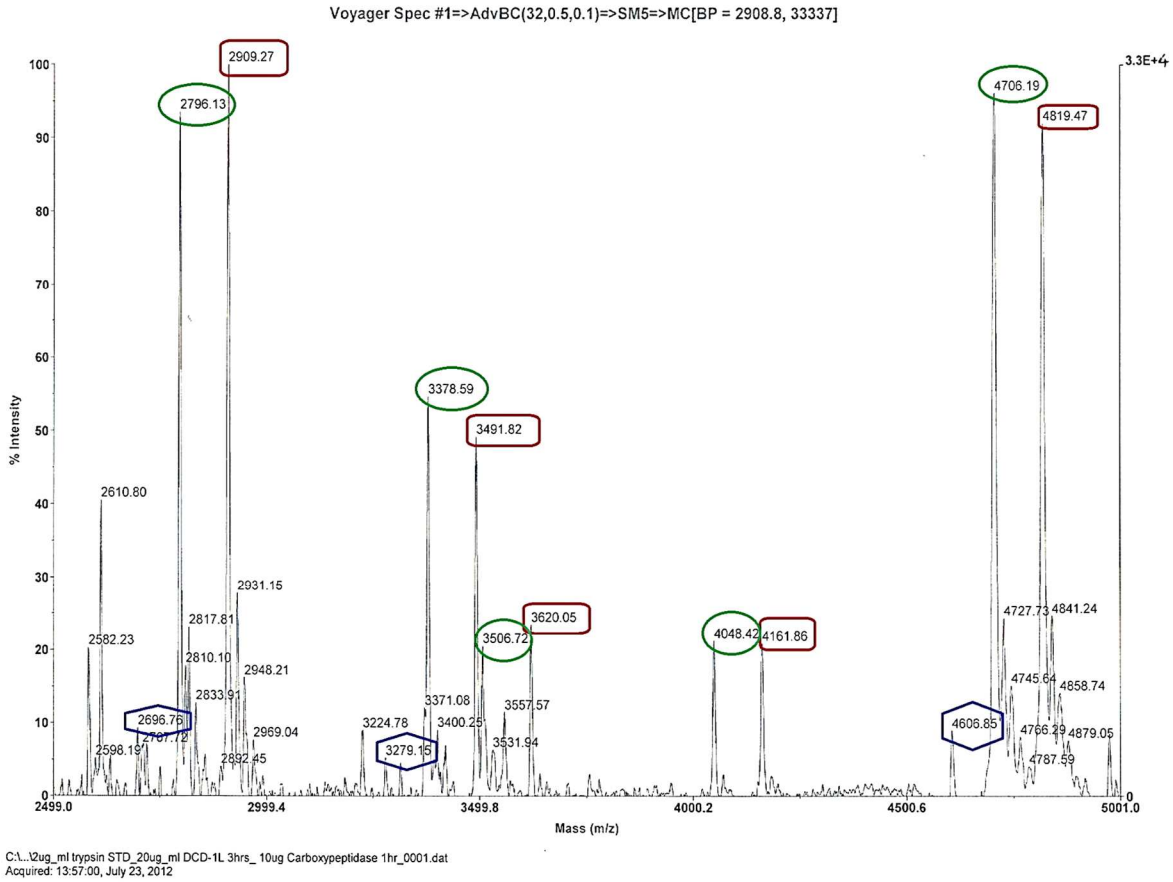


Fig. 9 MALDI-TOF mass spectrum of the 20 µg/mL DCD-1L peptide digested by 2 µg/mL trypsin for 2hours and then by 10 µg/mL of carboxypeptidase Y for 1 hour (the total digestion time for trypsin was 3hours).

= DCD-1L and dermcidin-Tryptic digested peptides.

= The carboxypeptidase digested peptides. (Also can be seen in table 4)

= The secondary digested carboxypeptidase-digested peptides. (Listed in table 4)

Table 5. Resulting peptide map following double (serial) enzymatic digestion of standard DCD-1L with 2 µg/mL trypsin and 10 µg/mL carboxypeptidase.

*represents DCD-1L without any tryptic digestion.

** Means further second digestion by carboxypeptidase.

No.	Amino acid sequence achieved with tryptic digestion	*m/z*	Symbol / Mass (Da) of the cleaved amino acid (C-terminal) after the digestion with carboxypeptidase	Resultant *m/z*
1	SSLLEKGLDGAKKAVGGLGK LGKDAVEDLESVGKGAVHD VKDVLDSVL*	4819.47	L/ 113.0	4706.19
2	SSLLEKGLDGAKKAVGGLGK LGKDAVEDLESVGKGAVHD VKDVLDSV**	4706.19	V/ 99.0	4606.85
3	GLDGAKKAVGGLGKLGKDA	4161.86	L/ 113.0	4048.42

	VEDLESVGKGAVHDVKDVL DSVL			
4	KAVGGLGKLGKDAVEDLES VGKGAVHDVKDVLDSVL	3620.05	L/ 113.0	3506.72
5	AVGGLGKLGKDAVEDLESV GKGAVHDVKDVLDSVL	3491.82	L/ 113.0	3378.59
6	AVGGLGKLGKDAVEDLESV GKGAVHDVKDVLDSV**	3378.59	V/ 99.0	3279.15
7	LGKDAVEDLESVGKGAVHD VKDVLDSVL	2909.27	L/ 113.0	2796.13
8	LGKDAVEDLESVGKGAVHD VKDVLDSV**	2796.13	V/ 99.0	2696.76

4.2 *In situ* DCD-1L peptides species verification:

Many fingermarks digestions with either 10 µg/mL, 1 µg/mL, 0.1 µg/mL and 0.01 µg/mL concentration of carboxypeptidase Y enzyme were done, among which, the 10 µg/mL was the best digestive concentration (can be seen in figure 14 appendix B and the other concentration and the fingermark without any digestion are show in figures 15-18 appendix B). The digestions with carboxypeptidase Y also were performed for 20, 30 and 60 minutes digestion times (the time optimisation comparative/sequenced results are not shown); form these the 60 minutes was the best. Even with the best conditions used it still too hard to assign the peaks of interest, this probably due to the complexity and/or the masking effect of the multi-peptides double-enzymatic digestion environments. Nevertheless three peaks were obtained and are listed in table 6 and are shown in figure 10 below.

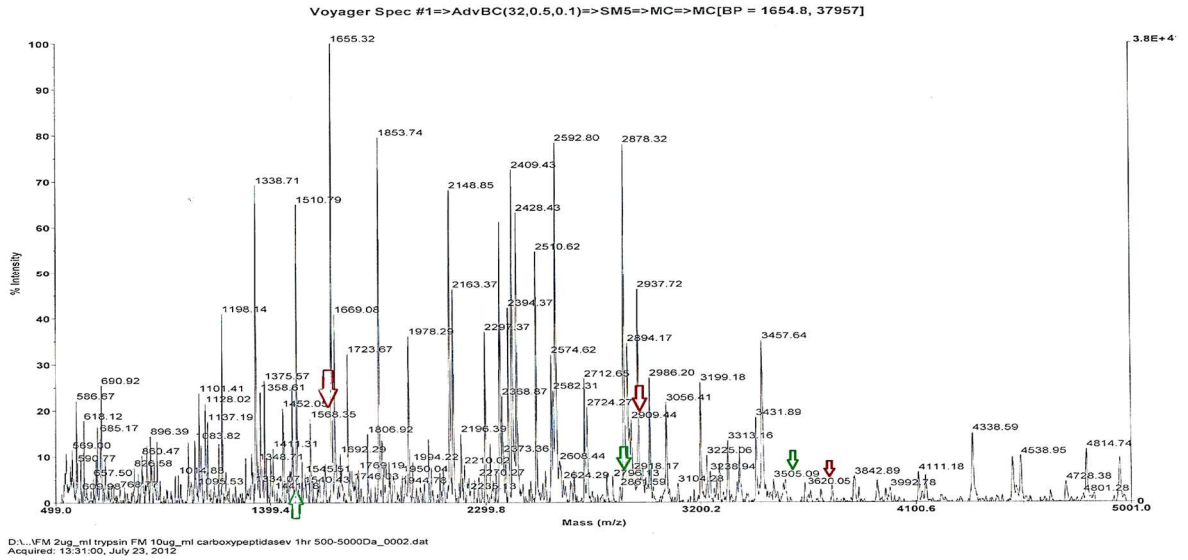


Fig. 10 MALDI-TOF mass spectrum of a groomed fingermark that was digested with 2 µg/mL trypsin for 2 hrs and further digestion with 10 µg/mL carboxypeptidase enzyme for 1 hr was done. The total digestion time for trypsin was 3 hrs.

⇩ = dermcidin-tryptic digested peaks.

⇩ = carboxypeptidase digestion of the tryptic digested peaks.

Table 6 Tryptic digestion followed by carboxypeptidase digestion of a groomed fingermark. The expected amino acid sequences of the digested peaks are listed. The observed and the theoretical peptides masses are shown with the percentages of accuracies before and after carboxypeptidase digestion.

No.	Amino acid sequence achieved with tryptic digestion	*m/z theoretical/ observed*	% Accuracy	Symbol / Mass (Da) of the cleaved amino acid (C-terminal) after the digestion with carboxypeptidase	Resultant *m/z theoretical/ observed*	% Accuracy
1	KAVGGLGKLGKDAVEDLESVGKGAVHDVKDVLDSVL	3620.10 / 3620.05	99.99862	L/ 113.0	3507.10/ 3505.09	99.9427
2	LGKDAVEDLESVGKGAVHDVKDVLDSVL	2909.24/ 2909.44	99.99313	L/ 113.0	2796.24/ 2796.13	99.99607
3	GLDGAKKAVGGLGKLGK	1569.85/ 1568.35	99.905	K/ 128.0	1441.85/ 1441.18	99.9536

4.3 Verification of DUPD1 (or DUSP 27) peaks:

The carboxypeptidase enzyme has the same role of cutting the C-terminal end of the peptides. The tryptic-digested DUPD1 then submitted to carboxypeptidase digestion for confirmation purposes the results observed are shown in figure 11 below. The peaks observed in figure 11 B were processed using mMass software and the peaks of interest only are shown by using manual peak labelling. Even so the intensities of these peaks are not as high as the peaks for dermcidin or trypsin autolysis or keratin, but the peak masses are very interesting. Especially when carboxypeptidase digestion was performed and the expected peaks were there. These peaks are listed with more details in table 7. In which the explanation of the observed peaks in contrast to the theoretically calculated peaks and how accurate the results is done.

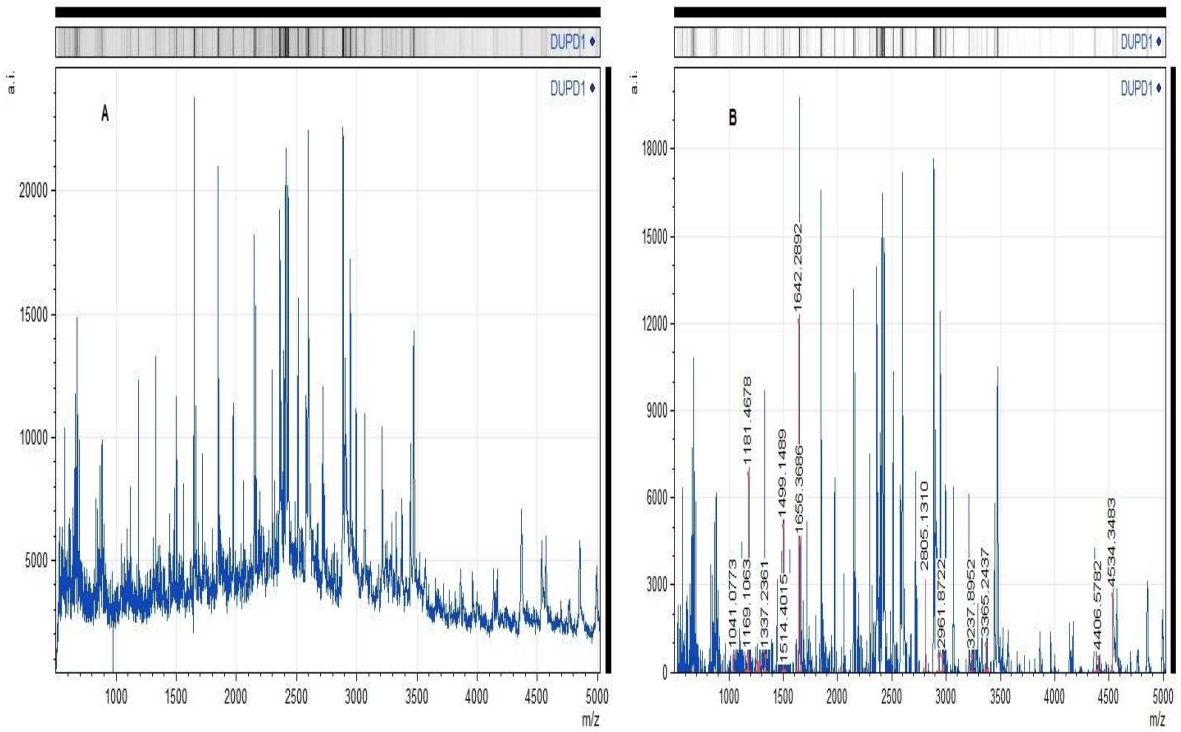


Fig. 11 Comparison of the mass spectral display of data from MALDI mass spectrum explains the expected DUSP27 peaks those gained from tryptic digestion and further digested with carboxypeptidase. (B) Has been processed (baseline correction, noise removal, smoothing and manual peaks picking): The figure was taken from mMass software and shows the only the peaks of interest which are shown in table 7. An on-tissue digest obtained from a MALDI MS image (A) without any processing: The MALDI mass spectrum was converted to text file and displayed again using the freeware package mMass (http://www.mmass.org/).

Table 7. In this table the predicted tryptic-digestion of the DUSP27 protein with the amino acid sequences are listed. The observed and the theoretical peptides masses are shown with the accuracies percentages of the yield before and after carboxypeptidase digestion.

No.	Amino acid sequence achieved with tryptic digestion	*m/z theoretical/ observed*	% Accuracy	Symbol / Mass (Da) of the cleaved amino acid (C-terminal) after the digestion with carboxypeptidase	Resultant *m/z theoretical/ observed*	% Accuracy
1	DMDIQYHGVEADDLPTFDLSVFFYPAAAFIDRALSDDHSK	4534.95/ 4534.34	99.98655	K/ 128.0	4406.95/ 4406.57	99.99138
2	LSPKMEEEGEEEDYCTPGAF ELERLFWK	3364.72/ 3365.24	99.98455	K/ 128.0	3236.72/ 3237.89	99.96385
3	GSPQYTHVNEVWPKLYIGDE ATALDR	2961.25/ 2961.8	99.97906	R/ 156.0	2805.25/ 2805.13	99.99572

		7				
4	LYIGDEATALDRYR	1656.83/ 1656.36	99.97163	R/ 156.0	1500.83/ 1499.14	99.8874
5	GSPQYTHVNEVWPK	1642.81/ 1642.28	99.96774	K/ 128.0	1514.81/ 1514.40	99.97293
6	LYIGDEATALDR	1337.47/ 1337.23	99.98206	R/ 156.0	1181.47/ 1181.46	99.99915
7	TSLKNAYSSAK	1170.30/ 1169.10	99.89746	K/ 128.0	1042.30/ 1041.07	99.88199

Discussion

Depending on the method of preparation, the fingermarks are classified into three major types; the ungroomed, the groomed and the eccrine fingermarks. The first one is the *ungroomed* fingermarks which was preferred due to short time consuming as no fingermark-washing protocols were required. However, the ungroomed fingermark production has a poor reproducibility. This in turn enforces the replicates to be done just to make sure that the data is reliable, unfortunately this would cost more time. The second type of fingermarks is the *groomed* fingermarks which was better than the ungroomed as the peaks were more intense. On the other hand, groomed fingermark has extra peaks that were detected. This could be due to the grooming procedure includes rubbing the fingers with the skin which due to friction few of the skin build up peptides and proteins are transferred to the fingermark which considered as a contaminant and could cause false results. An example of such proteins is keratin. The third and last fingermarks type used in this work is the *eccrine* fingermarks in which the results were good, but it also consumed a lot of time to prepare. So the same time for ungroomed fingermark preparation plus 10 minutes for sweating induction, added to approximately 15 minutes for fingermark-washing process, so the total time was 25-30 minutes which results in a very long preparation time. Also there were variables in the used washing method leading to differences in results obtained and affect the observed spectra. These variables are: the concentration of ammonium acetate, the time, and the specific way of washing. Fortunately these variables are affixed once a time to reach the final optimised method. Simply, the reason behind washing can be explained as follows: Eccrine fingermark are produced by eccrine sweat from eccrine glands. Eccrine sweat contains cationic salts, mainly sodium and potassium and to less extent ammonium ions[13]. These cations can hinder

the peak detection by MALDI MS. This justifies the use of cationic scavenger washing solution like ammonium acetate and pyridinium acetate. The C-terminal of the peptides usually occupied by such cations. So if the peptide is assigned as Ptn^- and the cation is assigned as M^+, the theoretical chemical equation would be:

$$Ptn^- M^+ + NH_4^+ CH_3COO^- \longrightarrow CH_3COO^- M^+ + Ptn^- NH_4^+$$

So the salt $CH_3COO^- M^+$ would be removed easily as it is water soluble but this introduces another problem which is the attachment of the ammonium groups to the protein. This cationic replacement causes the ammoniated species to be detectable in the MALDI mass spectrum which adds extra difficulties for peaks interpretations. Unfortunately, this strategy resulted insufficient to detect peptides and proteins from eccrine fingermarks. This might be due to that the concentration/washing time need to be explored further. Overall, a noisy baseline has been observed even with the best eccrine fingermarks spectrum.

Many studies were done to improve the tryptic digestion by using the effect of increase solubility by the detergents[14,15,16,17,18,19]. In this study, 0.5% w/v of the detergent β-octyl-D-glucopyranoside was employed because it has been reported to positively influence the digestion and it will be far away from the critical micelle concentration (i.e. 0.6716% w/v). The mentioned amount was added to tryptic digestion buffer as it has been proven to increase the MALDI-MS peptides signals with speculations of increasing the proteins and peptides solubility and hence increase the enzyme efficiency to digest these substrates.

For tryptic best work concentration, it was very obvious that the 20 µg/mL was the best for digestion but it was not the best for the overall work; this could be due to the *trypsin autolysis* effect (bearing in mind trypsin is still protein in nature and has the ability to digest itself), so the higher the concentration of trypsin, the higher the autolytic activity. The real unwanted effect with trypsin autolysis is that the peaks of the peptides of the cleaved enzyme would appear as intense as the peaks of the target peptides or even more intense which simply can mask the peaks of interest.

In addition to trypsin, carboxypeptidase Y also has been used to confirm the peptides mapping or identity. Carboxypeptidase Y act as a protease enzyme that cleaves peptide bonds from the C-terminal ends, one amino acid at a time. This adds an extra confirmation of the observed peptides data are related to the expected theoretical data as what has been obtained with this study[20]. Fair employment of enzymatic digestions were achieved. Also some of the digestions were applied on fingermarks with carboxypeptidase only (i.e. without any tryptic digestion). This was done particularly for comparison reasons (to allocate the carboxypeptidase digestions if any) and also to select the right concentration of this enzyme to be used fairly to digest the peptides within the fingermarks which eventually was 10µg/mL for 1 hour incubation period.

The digestion with two constitutive enzymes within a fingermark is very complicated. It is assumed the presence of many peptides within fingermarks. Very few were detected and many suspected with no sufficient information about them yet. These assumptions can be confirmed by the presence of many unknown peptides peaks after enzymatic digestion applied to the fingermark. With all the mentioned difficulties the finding of three expected tryptic digestion peaks with their corresponding carboxypeptidase digestion peaks can be considered as a gain within these circumstances.

Bioinformatics, for example online software and freeware, were used and have been employed to aid peptide identification and successfully reduce the time required to analyse a huge number of data. For instance, this work generates more than 1600 spectra all of them need to be pre-processed. The pre-processing processes are required prior any further data analysis to be done. These spectra pre-processing stages encompass the correction of the baseline, signal to noise improvement, peaks intensities normalization, spectral alignment, smoothing, manual peak picking and the most important the calibration. This study utilised a combined usage of MALDI-MS data explorer software (from applied bioscience: limited access, available with MALDI instrument only and not available online), mMass freeware, ExPASy Bioinformatics Resource Portal (free access at: http://expasy.org/) and the online data processing programme Protein Knowledgebase (UniProtKB) (freely accessed at: http://www.uniprot.org/) and lastly the free online software Mascot (also accessible at: http://www.Matrixscience.com/). In this work a combined employment of all mentioned bioinformatics tools has been successfully achieved.

Conclusions

The species of dermcidin and dermcidin tryptic digested peptides have been successfully identified directly in fingermark. Also, in addition to the obvious presence of keratin, an unexpected protein was also identified namely human dual specific protein phosphatase, DUSP27. However more focus studies are suggested to be done on this particular protein and maybe the usage of other techniques would be valuable. The involvement of other laboratory techniques for pre-isolation purposes like HPLC, LC, GC and others would be helpful in resolving the complexity exists in the fingermarks system and could enhance/ facilitate the use of MALDI-MS to detect more possible proteins/peptides in fingermarks.

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