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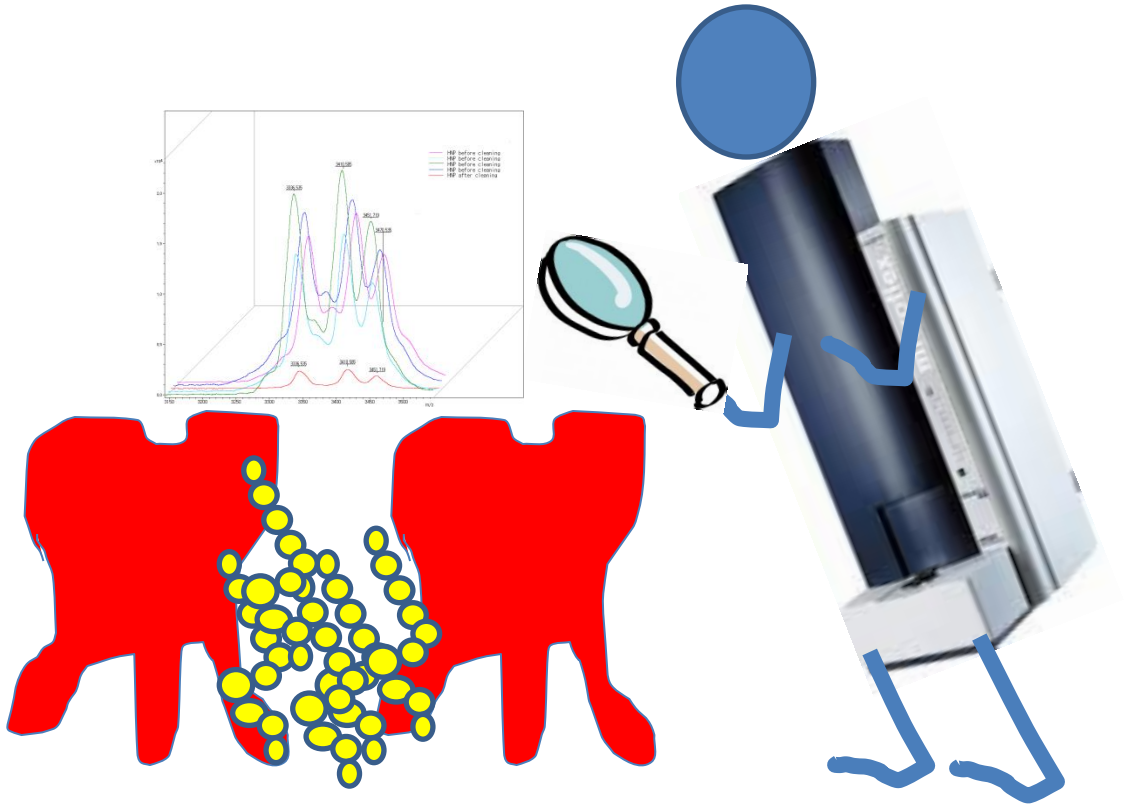

# MALDI-MS detection of bacteria in dental infection

# A brief case study demonstrating the applicability of MALDI Mass Spectrometry for detecting bacteria in dental samples

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**Abstract**

Samples collected from a gum infection site from a volunteer during surgery were directly analyzed using matrix assisted laser desorption mass spectrometry (MALDI-MS). This paper reports for the first time, the use of MALDI-MS for direct, rapid and sensitive analysis of bacteria from dental samples. The successful detection was owing to the extraction of the sample in microvolume of extractant (water) followed by preconcentration via vortexing coupled with centrifugation, A significant peptide cluster has been identified at the infection site, belonging to Human neutrophil peptides (HNPs). We were able to identify the presence of *Streptococcus mutans* in the dental samples, using MALDI-MS. This is a bacterium widely reported to be responsible for dental caries at dental infection sites. This approach for the first time reports and validates the unequivocal use of MALDI-MS in dental analysis yielding adequate information in a rapid way (only several minutes) leading to culture free detection of bacteria. This demonstrates that MALDI-MS can be applied as a rapid and sensitive tool for rapid clinical mass spectrometry (RC-MS) in future.

Keywords; teeth; infection; mass spectrometry; analysis; bacteria; *Streptococcus mutans*

**Introduction**

Dental caries, also known as tooth decay or a cavity, is a disease where bacterial processes damage hard tooth structure (enamel, dentin, and cementum). These tissues progressively break down, producing dental caries (cavities, holes in the teeth). Two groups of bacteria are responsible for initiating caries: *Streptococcus mutans* and *Lactobacillus*. If left untreated, the disease can lead to pain, tooth loss, infection and in severe cases, also to death[1]. Today, caries remain one of the most common diseases throughout the world [2,3]. The mineral content of teeth is sensitive to increases in acidity from the production of lactic acid produced by lactic acid bacteria that are abundant in the mouth.

Gingivitis is a result of an over abundance of bacteria that are left to attack the soft tissue of the mouth. The bacteria can create large pockets of infection and further develop toxins, which easily speed up localized damage. There are several methods for reversing gum disease, but they will all depend on how severe the case is in order to determine which procedure would be the best. Common methods are pocket reduction and gum regeneration to get back to healthy gums, but first the entire infection must be eradicated before the mouth has a shot at maintaining a healthy environment again [4]. Matrix-assisted laser desorption/ionization mass spectrometry (MALDI MS)[5] has been proven to be quite versatile in many applications for the analysis of biological samples, especially to peptides and proteins. Typically, samples are mixed with an organic compound which can serve as a matrix to facilitate ionization of analytes in the samples. However, the use of MALDI-MS for dental studies is rather limited. But some reports using MALDI-MS for the analysis of bacteria from clinical samples and their further discrimination are available[6].

Traditionally, bacteria from clinical samples are detected by conventional plating methods which require extensive equipment, media and involve time consuming laborious procedures. The other alternative is the cost expensive and tedious gene amplification techniques. For the first time, we applied MALDI-MS for rapid and sensitive analysis of dental samples. The samples were collected from a gum infection site by a dentist during surgery to clear up the infection site suspected to be caused by bacteria. The volunteer removed one wisdom tooth 10 years back, since it was unable to grow out and was causing periodic pain. The gums in this area got infected and inflamed. Thus, the

surgery was performed to clear up the infected tissues. Samples obtained at the infection site and from the saliva before and after cleaning (the surgery process), were analyzed using MALDI-MS. Efforts were taken to detect the unknown bacterial peaks obtained from the mass analysis. The results prove that MALDI-TOF MS is a rapid and sensitive technique which can detect bacterial signals even from complex mixtures such as blood and saliva. The significant findings from the rapid analysis of these dental samples using MALDI-TOF MS are demonstrated.

**Experimental**

Samples (scraping) from the infection site below the wisdom tooth (located on the right side of the lower jaw) from a healthy (Taiwan) female volunteer (age 45) was collected by the dentist (Dr Chia Yu Hsieh, Dental Clinic of National Sun Yat-Sen University, Kaohsiung, Taiwan) and used for the MALDI-MS studies. The samples were collected before and after the infection tissues were removed. In addition, saliva samples from the same volunteer were also collected (2 days) prior to the dental visit which represents the initial stage of the infection. 10 mg of the sample (scrapings) was dispersed into 200μL sterile water in Eppendorf tubes. These samples were vortexed (VM 2000, Digi System Laboratory, Taipei, Taiwan) for 10 min; another set of samples were further centrifuged at 10,000 rpm for 5 min and spotted onto a target plate and overlaid with sinapinic acid (SA) and analyzed by MALDI-TOF MS. Each spot was overlaid with 2μL of SA (0.05M) in acetonitrile:water (3:1, v/v) containing 0.1% TFA and then air dried for a few minutes. A standard bacterium called *Steptococcus mutans* was purchased from BCRC culture collection, Taiwan. BCRC 10793 *Streptococcus mutans* (ATCC 25175; DSM 20523; IFO 13955; NCDO 2062; NCTC 0449). This bacterium which was also obtained from human dental caries, was used as a standard to identify the unknown bacterial peaks obtained from the infection site. All experiments were performed in triplicates (three spots from each sample were analyzed). All mass spectra were obtained in positive ion mode using MALDI-TOF MS (Microflex, Bruker Daltonics, Bremen, Germany). All experiments were performed in the linear mode with 63.2 μJ of laser energy.

**Results and Discussion**

Samples from the infection site were collected as scrapings and also from the saliva prior to the cleaning process and after the cleaning process. Figure 1 shows the comparative spectra of the

scrapings collected by the dentist from the infection site that were analyzed without prior sample preparation (Fig.1a) Fig. 1b shows the spectra obtained after simple and rapid sample pretreatment such as votexing (Fig.1b) and vortexing followed centrifugation (Fig.1c). It was observed that a combination of suspending the sample in micro volumes (200μL water) followed by vortexing coupled with centrifugation led to the successful detection of the bacterial signals in MALDI-MS. Shrivas and Wu[7], report the use of similar preconcentration and sonication methods leading to enhanced detection of organic compounds in mass spectrometric analysis.

We also attempted sample preparation by centrifugation combined with vortexing, which resulted in greatly improving the spectrum (Fig. 1c). The spectrum shows the appearance of many peaks within this mass region, which are suspected to be from bacteria. In addition, these spectra were consistent and highly reproducible. In Fig. 1b, the samples spotted after vortexing, did not lead to many peaks. The samples spotted without any sample pretreatment (direct analysis) also do not show many peaks (Fig.1a). Thus, we found that preconcentration of the analyte by votexing followed by centrifugation prior to MALDI-MS analysis is required to increase the detection sensitivity of these dental samples, in order to facilitate successful MALDI-MS analysis. This method would prove useful especially in the case of real samples (clinical samples) where the bacteria are in significantly lesser concentrations. Also, the intial step where the samples were suspended in micro volumes of water, is crucial, since suspending the samples in higher volumes will lead to the dilution of the bacterial counts and hence lead to decreased or no detection of bacterial signals in MALDI-MS.

The obtained unknown peaks were matched with the mass profile of bacteria present in the mouth (oral microbial flora) available in literature. It was interesting to observe that the observed peaks from the scrapings at the infection site matched with *Streptococcus sps*[7]. Fig. 2 compares the MALDI-MS spectra collected from the samples obtained at the infection site, using votexing combined with centrifugation method prior to MALDI-MS detection. As seen in Fig. 2a, the spectra collected during dental surgery (before cleaning up) show many peaks suspected to match with the *Streptococcus mutans* standard (Fig. 2b). The unknown peaks were matched with the peaks obtained from standard *Streptococcus mutans* BCRC 10793 whose source was from a dental infection site too. As indicated in the figure the unknown peaks obtained at the infection site matched those of *Streptococci mutans*.

Streptococci are important components of the human oral bacterial flora. They comprise the human pathogenic mutans Streptococci, which are crucial for the development of dental caries. These organisms normally colonize the occlusal fissures and contact points between the teeth, and this correlates with the incidence of decay on these surfaces[8].

Another vital clue yielded by the MALDI-MS analysis in this study is the presence of a predominant cluster ions (three peaks) shown at m/z 3300-3500 from the dental samples (Fig. 3a-c). The peaks obtained from samples taken from the infection site (Fig. 3b & Fig. 3c) showed 3.6 to 10 fold intensity (10000 and 3300) than that of the spectrum in Fig. 3a (900). These peaks were identified to belong to a group of peptides called Human neutrophil peptides (HNPs) (or α – defensins) [9]. They were composed of three peptides which form close clusters named HNP1, HNP2 and HNP3, respectively. HNPs are abundant peptides in saliva[10]. These peptide molecules have cationic antimicrobial peptides that are the products of activated and deteriorating neutrophils[11]. HNPs play very important roles in the innate immune response; they are among the most important components for intrinsic immunity against viruses, fungi and bacteria[12-14]. The intense peaks of HNPs shown in Fig. 3b indicate that the defense of the human body towards the infection stage is at its highest. Further, as observed in Fig. 3c, the spectra which was obtained after cleaning procedure (immediately after cleaning), showed a distinct decrease in the HNPs peak intensity. It was interesting to observe that the HNPs peaks do not disappear immediately after cleaning, but continue to remain (at decreased levels) at the infection site. This could be a preventive measure taken by the system, to guard the infection site, and also during replacement of the cleaned tissues. Figure 4 shows the stacked view, of replicates of spectra collected before cleaning showing the high reproducibility of the HNPs cluster in all the spectra. The marked decrease in the HNPs peaks in the spectra from samples analyzed after the cleaning procedure is evidenced from fig. 4. The presence of these antimicrobial peptides in the infected samples and later their decrease in after clearing of the infection site, confirm that the infection is microbial in origin. Thus, HNP's at infection sites can be considered as the important biomarker peptides to confirm or rule out the role of bacteria in various infections.

The current study thus uses a simple microvolume extraction method combined with vortex cum centrifugation techniques for the successful detection of bacteria at a dental infection site. The

technique could also be extended for the detection of crucial antimicrobial HNPs markers at the dental site.

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**Conflict of Interest**

None of the authors of this manuscript have any direct financial relation with the commercial identities mentioned in the paper that might lead to a conflict of interest.

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**Figure Captions**

Fig1. MALDI-MS spectra of samples collected from gum infection site during the surgery (before cleaning up) (a) without any pretreatment (b) votexed for 10 min (c) vortexed for 10 min and then centrifuged at 10,000 rpm for 5 min. The predominant peaks at m/z 3300-3500 belong to HNPs and peaks at m/z 15138 and 15882 belong to human blood.

Fig. 2 MALDI-TOF MS spectra comparing the bacterial peaks obtained from (a) the gum infection site during surgery (prior to cleaning) showing bacterial peaks belonging to Streptococcus mutans [∗] identified by comparing against the mass profile of (b) standard BCRC 10793 *Streptococcus mutans* isolated from human dental caries; the peaks matched with the infection sample are marked with the symbol [∗]. Spectra were collected in linear mode with a laser energy of 63.2 μJ.

Fig. 3 MALDI-MS spectra showing the presence of the human neutrophil peptides (HNPs) indicated by the peak cluster at m/z 3300-3500 of varying intensities in samples taken from (a) saliva samples (2 days) before surgery (b) gum infection site during surgery (before cleaning) (c) gum infection site after cleaning. Peaks of HNPs [◯] and the human blood peaks [◆] are represented by symbols in the spectra.

Fig. 4 MALDI-MS stacked spectra showing the reproducibility of the HNPs peak cluster (during cleaning) in multiple spectra and the significant decrease in the HNPs peak intensity after the cleaning procedure.

# Fig.1

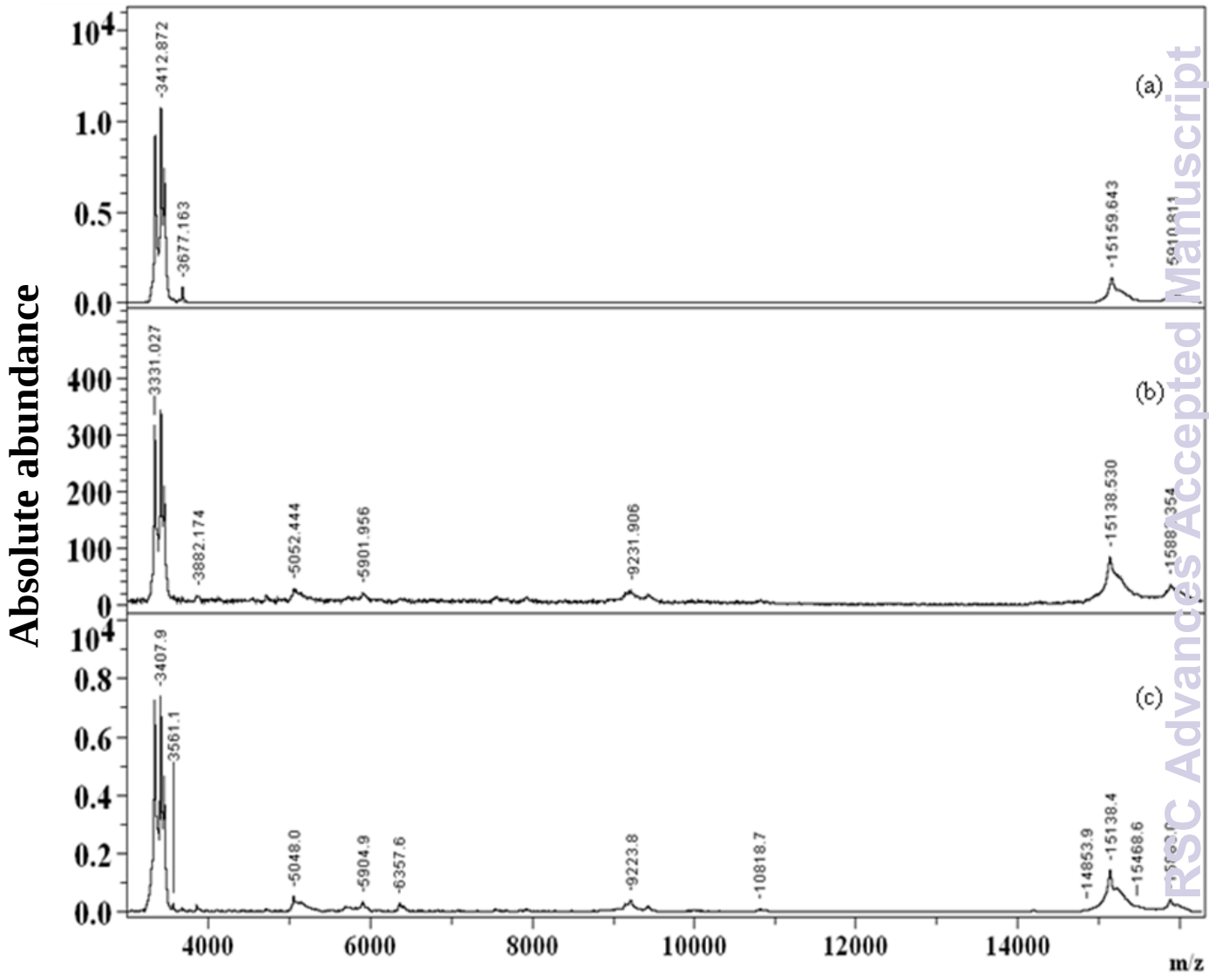
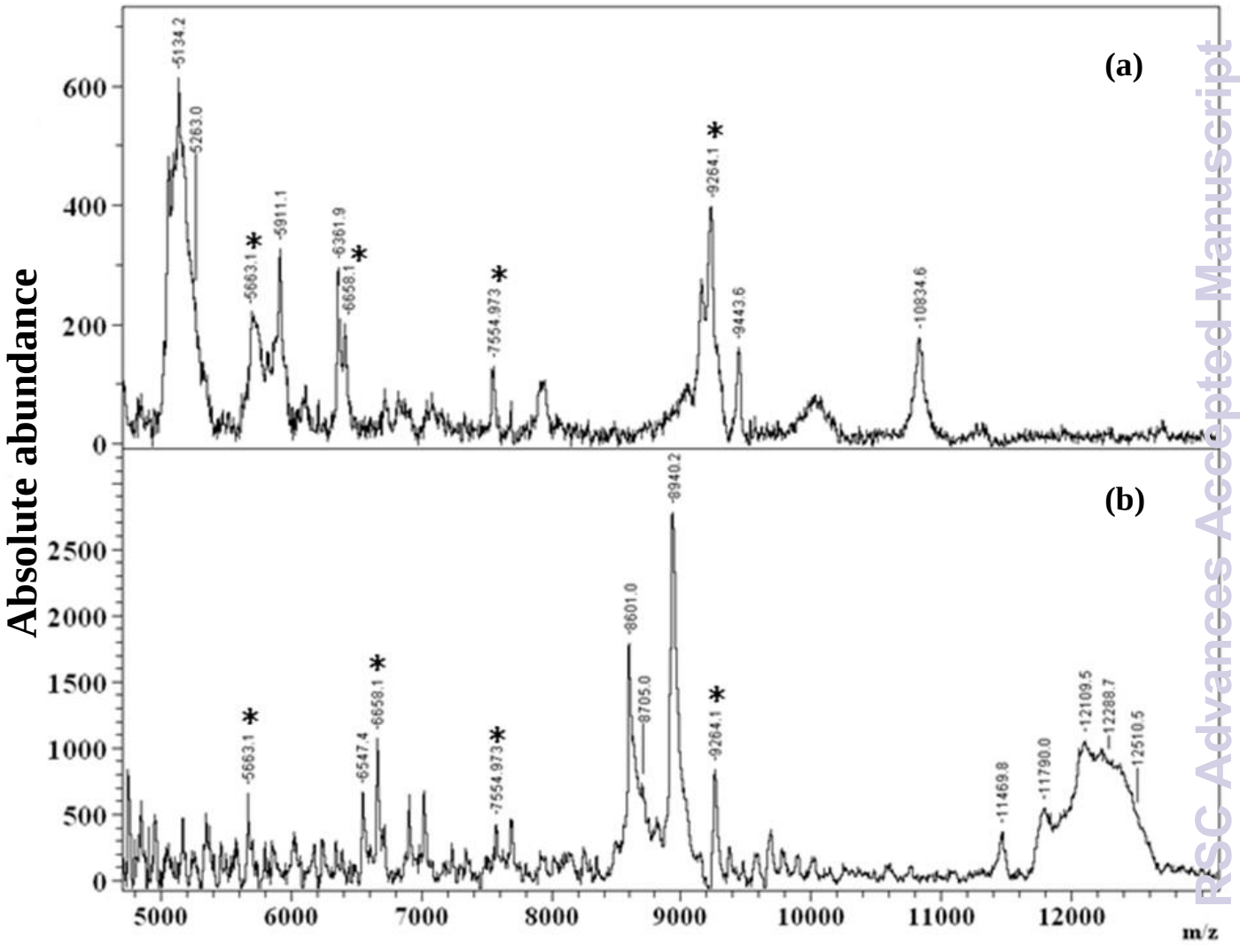
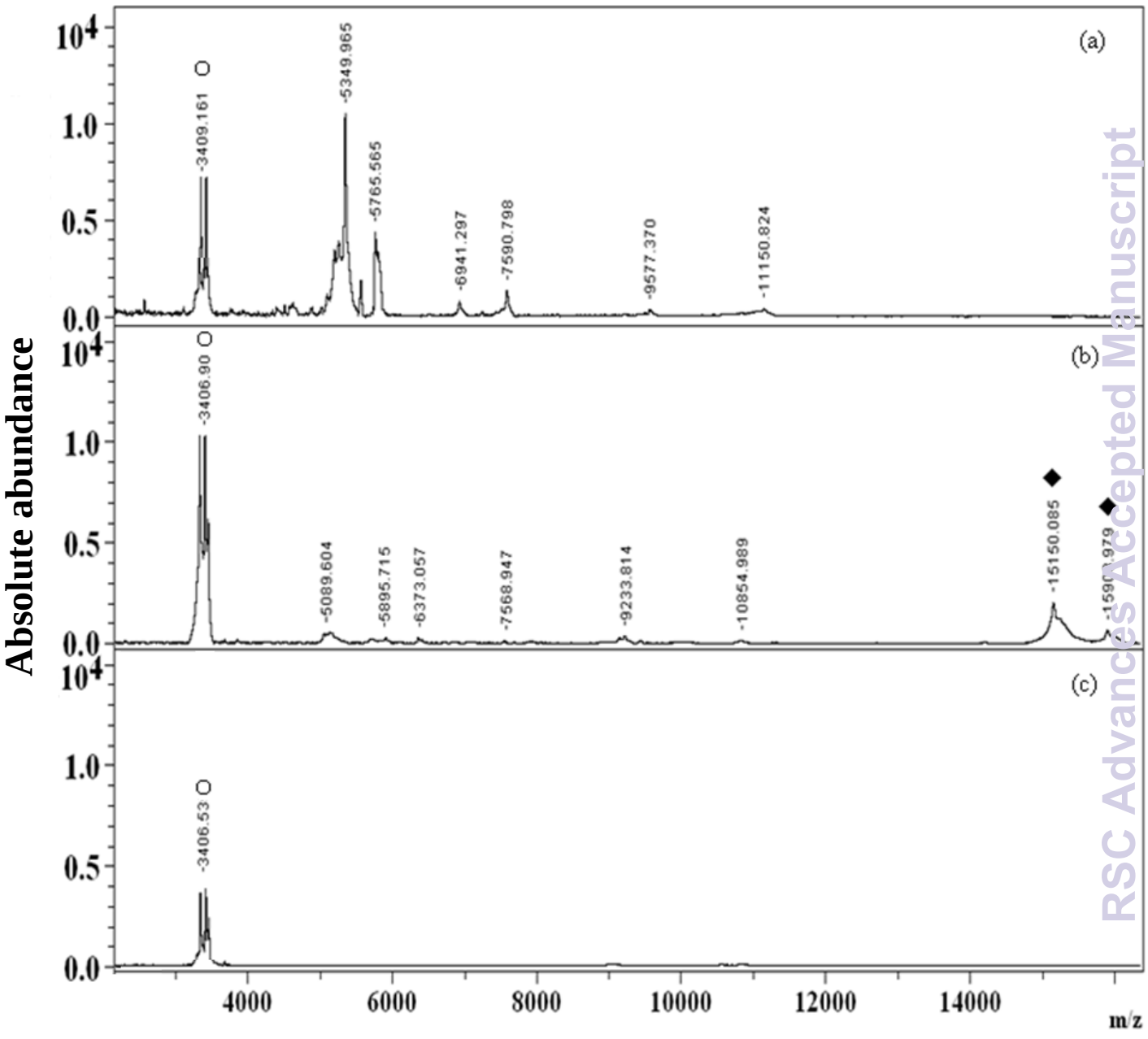


**Fig.2**



# Fig.3



# Fig.4