Some Remarks About Laser-Induced Mass Spectrometry of Bacteria

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In order to investigate the availability of laser-induced mass spectrometry for the determination of bacteria, ten strains of the genera *Escherichia* and *Streptococcus* were chosen. Gram-negative as well as Gram-positive lysogenic and non-lysogenic, pathogenic and non-pathogenic strains were examined. Giant pulses of a Nd: YAG laser (frequency quadrupled, wave-length 265 nm) were used to vaporize and partly ionize the bacterial material. The time-of-flight mass spectra obtained could be discriminated by using the stepwise discriminant analysis provided by BMDP software package. Two different methods relating centrifugation and washing procedures were used. With the first method a correct classification between 50.0 and 90.9 per cent *(S. sanguis)* by means of mass spectra of positive ions and a correct classification between 27.1 and 80.4 per cent *(S. lactis-25)* by means of mass spectra of negative ions was observed. Using the second method a correct classification between 40.7 and 93.3 per cent *(S. lactis-25)* by means of mass spectra of positive ions and a correct classification between 56.7 and 95.0 per cent by means of mass spectra of negative ions was obtained. Here *S. sanguis* with 93.3 and *S. lactis-25* with 81.7 per cent were significantly differentiated. In this context it must be emphasized that a correct classification of 27 per cent must be considered statistically significant, if 10 different strains are used.

Introduction

In recent years several analytical techniques have been used to investigate bacteria and to find a method for a fast determination and classification of unknown microorganisms. Some of the techniques applied which sometimes gave encouraging results are *e.g.* pyrolysis gas-liquid chromatography [1−5], pyrolysis mass spectrometry [5−8] and direct probe mass spectrometry [9, 10].

Another method, the laser-induced mass spectrometry, having been applied to many biological, medical and technical problems [11, 12] was recently used to classify microorganisms [13−16] by the help of single cell analysis.

This paper reports about results obtained by using the Laser Microprobe Mass Analyser, LAMMA^R 500 (Leybold-Heraeus), analysing bacteria of the genera *Escherichia* and *Streptococcus* of Lancefield Group H and N in order to obtain some more information about the applicability of laser-induced mass spectrometry for bacterial determination.

Material and Methods

Laser-induced mass spectrometry

The LAMMA 500 instrument used for the experiments is a powerful tool in microanalysis [17]. In this instrument the material to be analysed is vaporized and partly ionized in the laser focus by a single ultra-short laser pulse (irradiance: $10^{10}−10^{11}$ W×cm^{-2}). The samples used must be stable in a high vacuum state and if layers or tissues are to be measured they must be perforable with a single laser pulse. A light microscope and an x-y-manipulator give the opportunity to choose the part of the sample to be analysed. The frequency quadrupled Q-switched-Nd: YAG laser emits light with a wave-length of 265 nm. Laser-induced ionformation produces ions of both positive and negative charge. As a result, depending on the polarities of electric potentials, a time of flight spectrum of either positive or negative ions is obtained by a single laser shot.

Bacteria, culture and preparation

The bacteria used for the experiments (see Table I) were cultivated in M17 liquid medium [18] at 37 °C till the cultures reached an optical density of at least 1.4 at 400−600 nm (Eppendorf Photometer). The

Table I. Names of the different bacterial strains, their abbreviations, the preparation methods used and where the strains were obtained from: A − Dr. Devoret, Gif-Sur-Yvette, France; B − Dr. Busse, Weihenstephan, FRG; C − F. L. Davies, Shinfield, U. K.

Name of the bacteria strain	Abbreviation	Prepared using method	Obtained from
Escherichia coli GY 5027	EC GY 5027	1 + 2	A
Escherichia coli GY 4015	EC GY 4015	1 + 2	A
Streptococcus sanguis	SS	1 + 2	B
Streptococcus cremoris 05M/22	SC 05M/22	1 + 2	B
Streptococcus lactis 4513	SL 4513	1 + 2	B
Streptococcus lactis 530/7	SL 530/7	1 + 2	B
Streptococcus lactis 530/12	SL 530/12	1 + 2	B
Streptococcus lactis 530/25	SL 530/25	1 + 2	B
Streptococcus lactis C2	SL C2	1 + 2	C
Streptococcus lactis C2-81	SL C2-81	1	(C)

bacterial cultures were centrifugated and then prepared in two different ways.

First method: A second and a third centrifugation was carried out as washing procedures in which saline was used for resuspending the bacterial material. The pellet of the last centrifugation was resuspended in saline too and the suspensions of the different bacterial strains were freeze-dried for about three days. The lyophilized material was stirred on a magnetic stirrer in a dry Erlenmeyer-flask. Thus the air in the flask was saturated with fine particles deriving from the material at the bottom. With an impactor used in aerosol-physics [19] the air-borne particulate material was sampled on a formvar-coated copper grid as used in electron microscopy. Thus it was possible to obtain particles nearly homogeneous in size by inspecting them in the light microscope.

Second method: The bacteria were washed twice by resuspending and centrifugating using aqua bidest. instead of saline. The pellet of the last centrifugation was resuspended in aqua bidest. A droplet of these suspensions brought on a formvar-coated grid produced a thin homogeneous layer of bacterial cells appropriate for perforation.

The formvar foil used to coat the grids must have the quality to be strong enough to hold the bacterial material and thin enough to produce only negligible background signals. For each bacterial strain and each preparation method about 60 spectra of positive as well as negative ions were taken. The spectra produced by the LAMMA 500 were recorded on floppy disks. These data were transferred to the ICL-computer of the University by first copying them to a magnetic tape as an interchange medium. The data were further processed by programs written by E. Schmid [20]. One of the programs changes the time-of-flight spectra into real mass spectra. At the end of the processing the data had the format necessary to perform a stepwise discriminant analysis calculated by the P7M-program of the BMDP-software package [21]. Of the various listings, the classification matrix represents the results best.

Results

At least two discriminant analyses have been calculated for the two preparation methods with the P7M-program, one for the positive and one for the negative spectra. Every set of about 60 spectra of positive as well as negative ions recorded for each bacterial strain formed one group for the multivariate analysis. Those groups of spectra were separated quite well for both preparation methods (see Tables II, III, IV and V). The second method yields a better discrimination for the different bacterial strains being more pronounced for the spectra of negative ions.

The mass ranges used for the discriminant analyses were 24−200 m/e excluding the mass lines of K^+ (39 and 41 m/e) for the positive ion spectra and 24−200 m/e for the negative ion spectra. The masses under 24 and above 200 m/e were not taken into consideration because they appeared very irregularly.

Table II. Classification matrix of mass-spectra of positive ions. Material prepared using the 1st method. Mass range 24−38, 40, 42−200.

Group	Percent correct	A	B	C	D	E	F	G	H	I	J
A EC GY 5027	50.9	29	—	—	4	16	—	—	8	—	—
B EC GY 4015	50.0	5	57	—	4	7	—	—	8	2	1
C SS	90.9	1	—	50	—	3	—	—	1	—	—
D SC 05M/22	61.1	—	—	—	33	7	—	—	11	3	—
E SL 4513	62.1	5	6	—	4	36	—	—	7	—	—
F SL 530/7	62.1	—	—	—	—	—	36	18	—	4	—
G SL 530/12	76.4	—	—	—	—	—	12	42	—	—	1
H SL 530/25	77.8	—	—	—	4	7	—	—	42	1	—
I SL C2	62.1	—	7	—	—	1	1	—	5	36	8
J SL C2-81	69.1	—	—	—	—	—	—	2	1	14	38
Total number	66.1	40	40	50	49	77	49	62	83	60	48

Table III. Classification matrix of mass-spectra of negative ions. Material prepared using the 1st method. Mass range 24−200.

Group	Percent correct	A	B	C	D	E	F	G	H	I	J
A EC GY 5027	51.9	28	6	—	1	3	—	2	6	5	3
B EC GY 4015	64.9	2	37	—	—	4	1	4	3	4	2
C SS	67.3	1	—	37	—	4	—	—	7	—	6
D SC 05M/22	27.1	—	5	—	16	9	—	4	9	10	6
E SL 4513	61.7	—	13	1	2	35	—	2	1	2	1
F SL 530/7	30.5	3	10	1	2	1	18	11	2	8	3
G SL 530/12	37.7	5	7	—	4	5	1	23	4	11	1
H SL 530/25	80.4	—	1	1	1	1	—	1	45	—	6
I SL C2	62.7	—	1	—	8	2	—	7	3	37	1
J SL C2-81	61.7	1	—	—	3	1	—	—	11	2	29
Total number	54.1	40	80	40	37	65	20	54	91	79	58

Table IV. Classification matrix of mass-spectra of positive ions. Material prepared using the 2nd method. Mass range 24−38, 40, 42−200.

Group	Percent correct	A	B	C	D	E	F	G	H	I
A EC GY 5027	58.3	35	11	—	—	—	3	—	11	—
B EC GY 4015	65.0	1	39	—	—	1	2	—	17	—
C SS	85.0	2	—	51	—	1	—	—	6	—
D SC 05M/22	83.3	—	4	—	50	—	—	1	5	—
E SL 4513	81.0	—	—	—	—	47	1	—	10	—
F SL 530/7	40.7	—	—	—	6	7	24	11	9	1
G SL 530/12	73.3	—	1	—	1	—	1	44	10	4
H SL 530/25	93.3	2	1	—	—	—	—	1	56	—
I SL C2	73.3	—	—	—	—	—	—	7	9	44
Total number	72.6	40	56	51	57	56	31	64	133	49

Table V. Classification matrix of mass-spectra of negative ions. Material prepared using the 2nd method. Mass range 24−200.

	Group	Percent correct	Number of cases classified into group								
			A	B	C	D	E	F	G	H	I
A	EC GY 5027	95.0	57	2	−	−	−	−	−	1	−
B	EC GY 4015	88.3	5	53	−	1	−	−	−	1	−
C	SS	93.3	−	1	56	−	1	−	−	2	−
D	SC 05M/22	78.0	−	1	−	46	−	2	6	4	−
E	SL 4513	88.3	−	−	1	−	53	2	−	4	−
F	SL 530/7	81.0	−	−	−	1	−	47	4	6	−
G	SL 530/12	56.7	1	−	−	2	−	13	34	10	−
H	SL 530/25	81.7	1	1	−	−	1	4	4	49	−
I	SL C2	91.5	−	1	−	1	−	2	−	1	54
	Total number	83.8	63	59	57	51	55	70	48	78	54

Table VI. Classification matrix of mass-spectra of positive ions. Same spectra as used in Table IV only calculated with another mass range. Material prepared using the 2nd method. Mass range 1−220 without 39 and 41.

	Group	Percent correct	Number of cases classified into group								
			A	B	C	D	E	F	G	H	I
A	EC GY 5027	63.3	38	8	−	−	−	−	−	14	−
B	EC GY 4015	66.7	1	40	−	−	1	−	−	18	−
C	SS	85.0	2	−	51	−	1	−	−	6	−
D	SC 05M/22	86.7	−	1	−	52	−	−	4	3	−
E	SL 4513	81.0	−	−	−	−	47	2	−	9	−
F	SL 530/7	39.0	−	−	−	8	7	23	10	9	−
G	SL 530/12	75.0	−	−	−	1	−	2	45	9	3
H	SL 530/25	96.7	−	1	−	−	1	−	−	58	−
I	SL C2	80.0	−	−	−	−	−	−	2	10	48
	Total number	74.9	41	50	51	61	57	27	61	136	53

The masses 23 (Na^+), 39 and 41 (K^+) $m/4$ of the positive ion spectra were not used because their signals often exceeded the dynamic range of the transient recorder.

Another discriminant analysis was calculated using the mass range 1−230 m/e without 39 and 41 m/e (see Table VI) for the spectra of positive ions achieved from the material prepared by using the second method.

This was done because the signal of the mass 23 did not exceed the dynamic range of the transient recorder or in the case of *S. sanguis* (see Figs. 1 and 2) totally disappeared because no saline was used in this preparation. In order to make sure that no other information was lost in the previous calculations the other masses (except the lines of K^+) were taken into account as well. However, the discriminant analysis showed that these masses did not provide any additional information for discriminating these bacteria. The lack of Na^+ for *S. sanguis* was obscured by the saline used in the first preparation method. Considering the mass line 23 m/e the classification matrix (Table VI) showed a slight improvement of present classifications.

Comparing Figs. 1 and 2 as well as Figs. 3 and 4 it becomes quite clear that the homogeneity was much better for positive and negative ion spectra when the bacteria were prepared by the second method. This is probably an effect of inadequate volumes which were vaporized from samples prepared using the first method because of the slight differences in particle size.
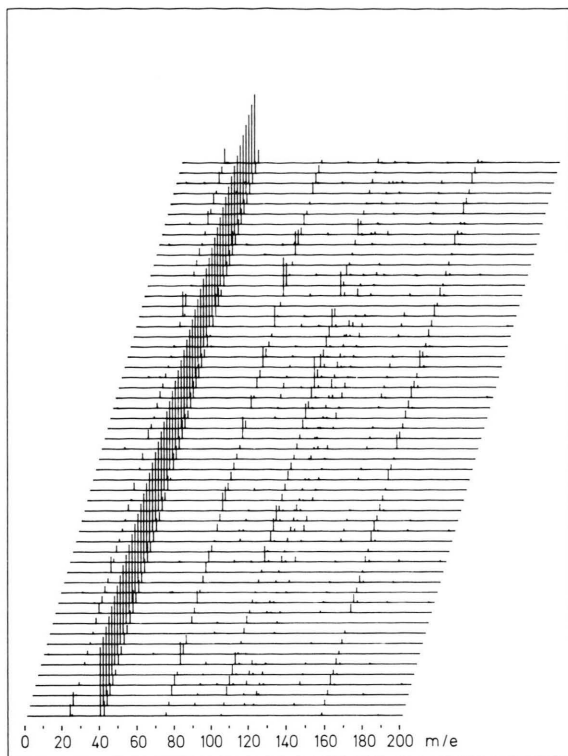
Fig. 1. 55 mass-spectra (positive ions) originating from *Streptococcus sanguis;* 1st preparation method.
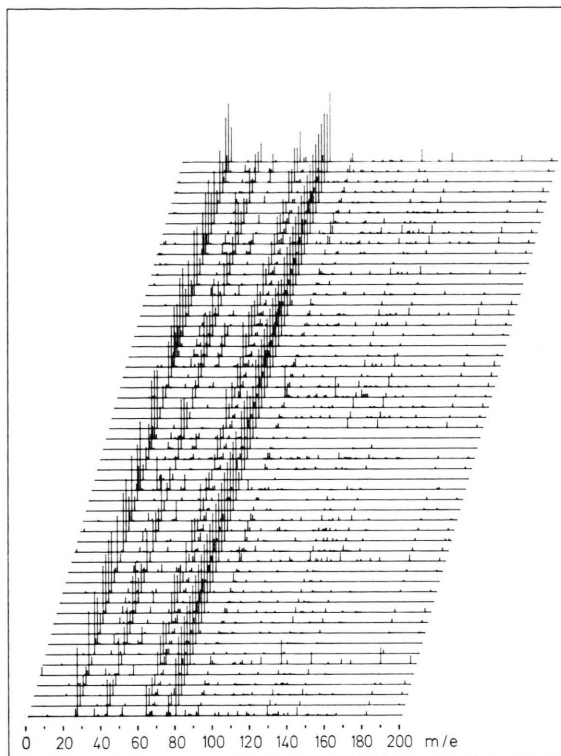

Fig. 3. 55 mass-spectra (negative ions) originating from *Streptococcus sanguis;* 1st preparation method.
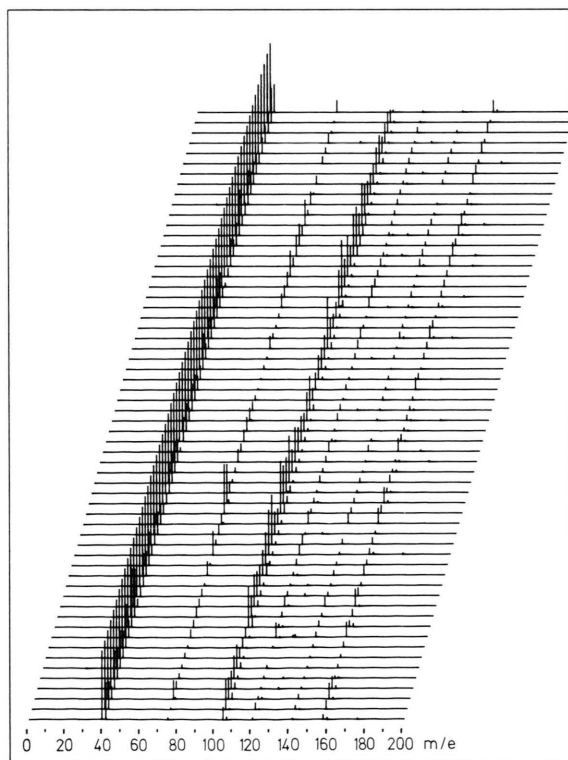

Fig. 2. 60 mass-spectra (positive ions) originating from *Streptococcus sanguis;* 2nd preparation method.
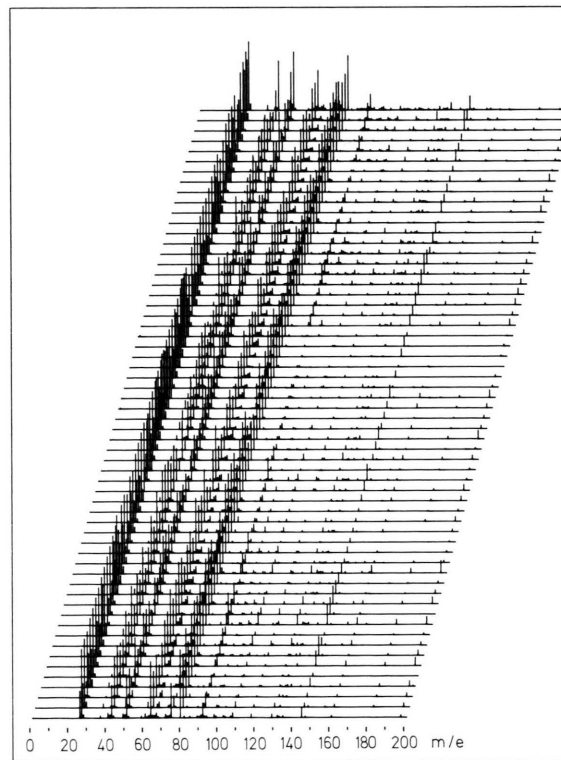

Fig. 4. 60 mass-spectra (negative ions) originating from *Streptococcus sanguis;* 2nd preparation method.

Conclusion

The results show that encouraging results are obtainable with the LAMMA[R] 500 instrument by doing, in contrast to single cell analysis, bacterial determination with freeze-dried and lyophilized bacterial material. Suitable multivariate statistical methods available in well-known software packages such as BMDP can be used to discriminate the different bacterial strains. Because of the unknown behaviour of the highly complex bacterial material in the laser-focus nothing can be said about the origins and the structure of the characteristic molecular ion species caused by laserpyrolysis and laserdesorption processes. In other words this way of determining bacteria may become an interesting technique for bacteriologists but there are still many questions left to answer before making up one's mind about it.

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