

Microarray pig-zoonosis V4: Response analysis on mono-specific samples

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Development of a diagnostic microarray system for the detection of bacterial and viral zoonoses agents

Microarray Design

**Hybridization patterns
with mono-specific samples**

**Hybridization patterns
with environmental
samples**

Microarray Design

Sequences of Interest
in FASTA

Software

OligoPicker2.3.2

Unique
Sequences

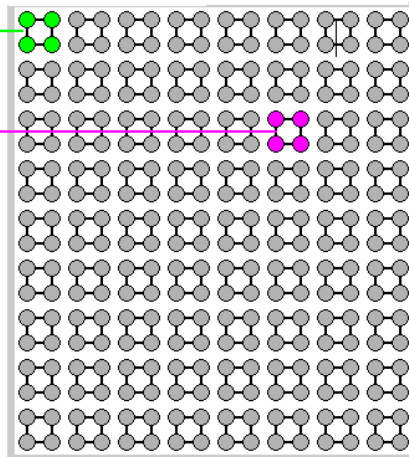
BLAST

ERIC, PATRIC,
NMPDR

Unique Sequences
are spotted 4 times

Positive
Control

Negative
Control



SUBARRAY



Collection of all sequences of interest
in FASTA format

Choice of the oligonucleotides:

- length (influence the number of mismatch for sequence)
- contained in CG
- homology between target and non target-genes
- number of genes by species
- selectivity power of genes

Positioning of the oligonucleotides in
the array (Spotting)

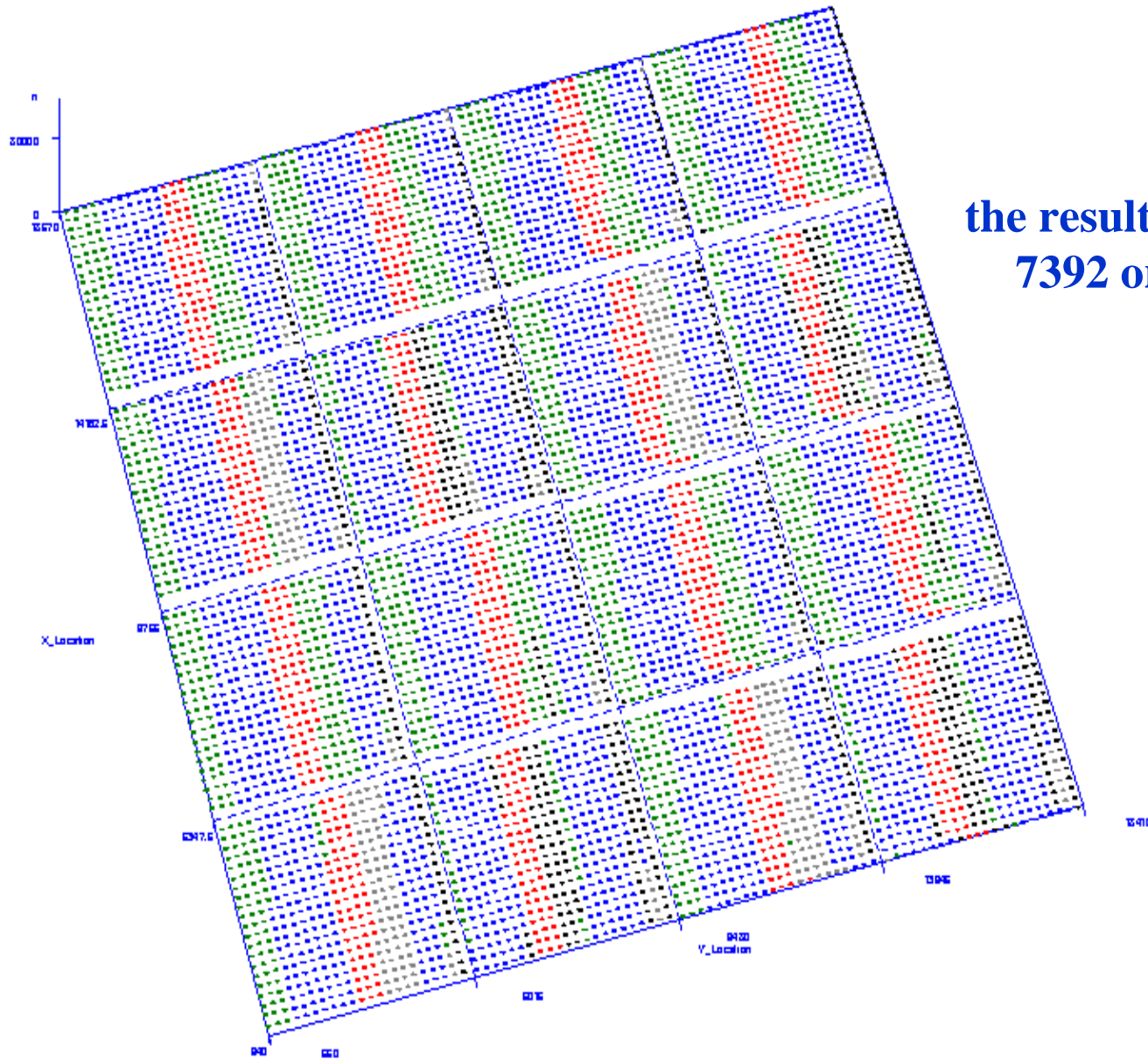
(from Tonelli et al. 2010)

Microarray Design

Genus	n° Spot	n° Genes	n° Species
Arcobacter	6	2	1
Campylobacter	1419	473	19
Clostridium	339	113	30
Enterococcus	225	75	26
Erysipelothrix	30	10	1
Escherichia	18	6	1
Hafnia	3	1	1
Helicobacter	48	16	10
Listeria	348	116	6
Salmonella	1860	620	5
Staphylococcus	1116	372	37
Streptococcus	660	220	42
Vibrio	15	5	1
Yersinia	948	316	14
Controls	357	119	4
Total	7392	2464	198

Microarray Design

L. innocua_ATCC33090_2



the result is a slide with
7392 orderly spots

As first validation the array has been hybridized to mono-specific samples

Zoonotic Agents

Staphylococcus species

Vibrio species

Enterococcus species

Campylobacter species

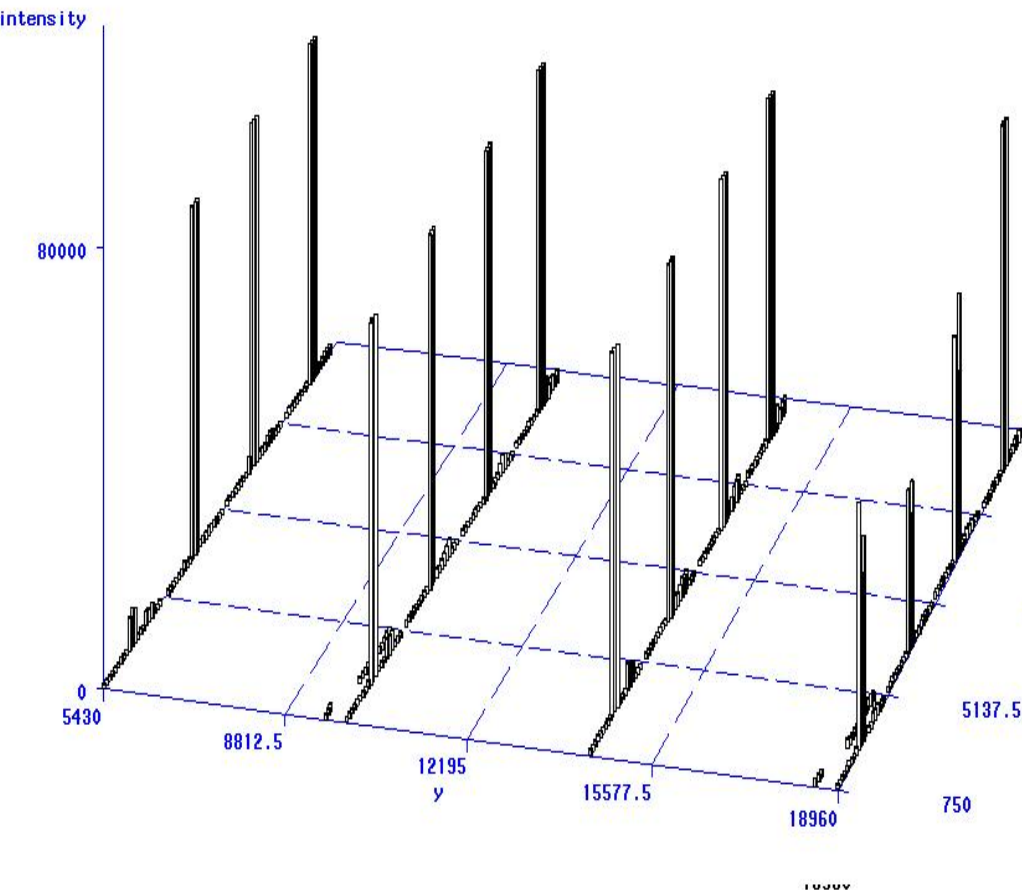
Escherichia species

Listeria species

Listeria cheese project

Microarray Design

L_innocua_ATCC33090 CONTROLLO

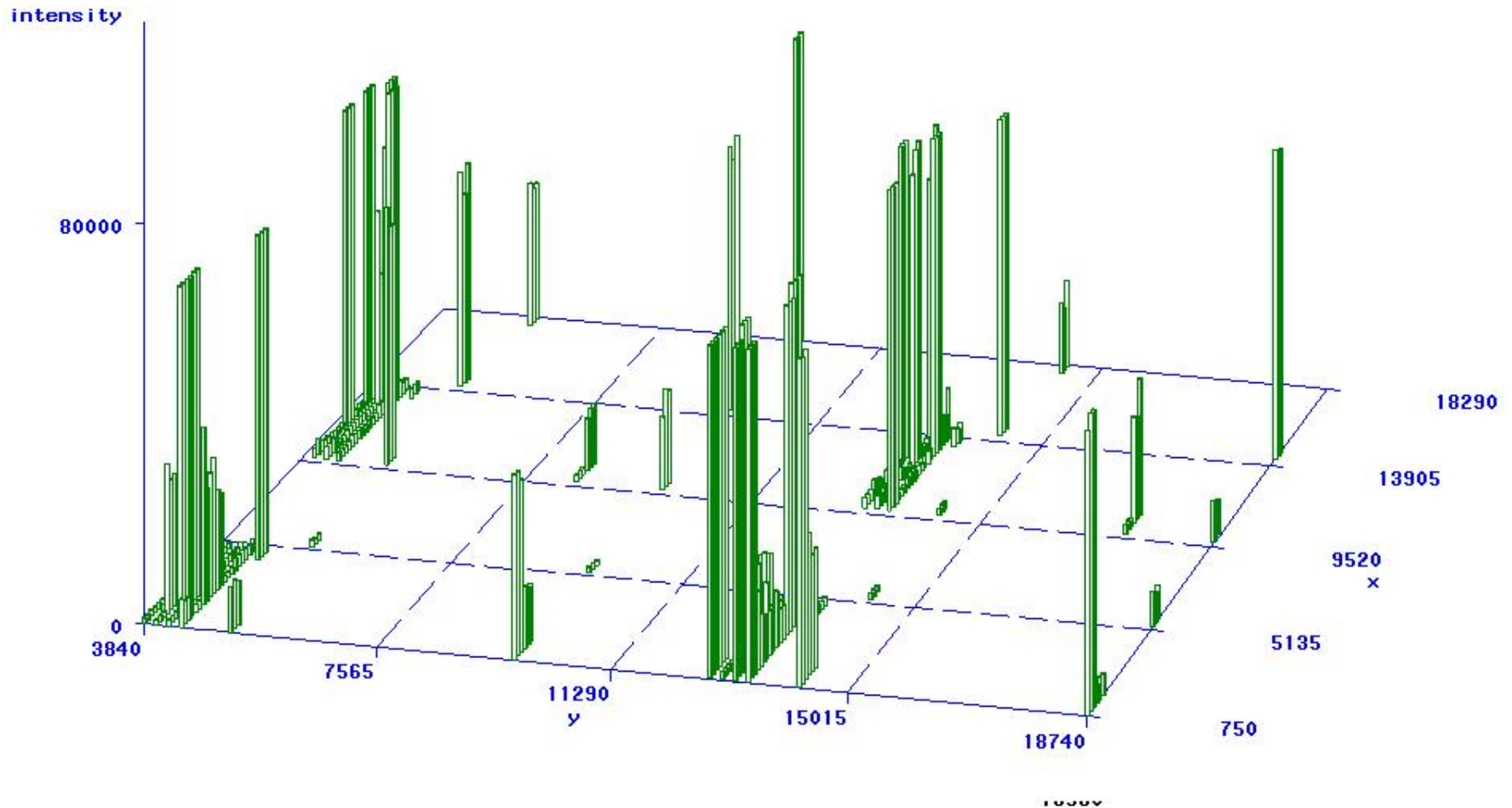


the array contains 357 spots of controls

Housekeeping type	Number
N/A	12
Negative control Arabid	51
Negative control gfp50	51
Positive control EUB33835	51
Empty	192

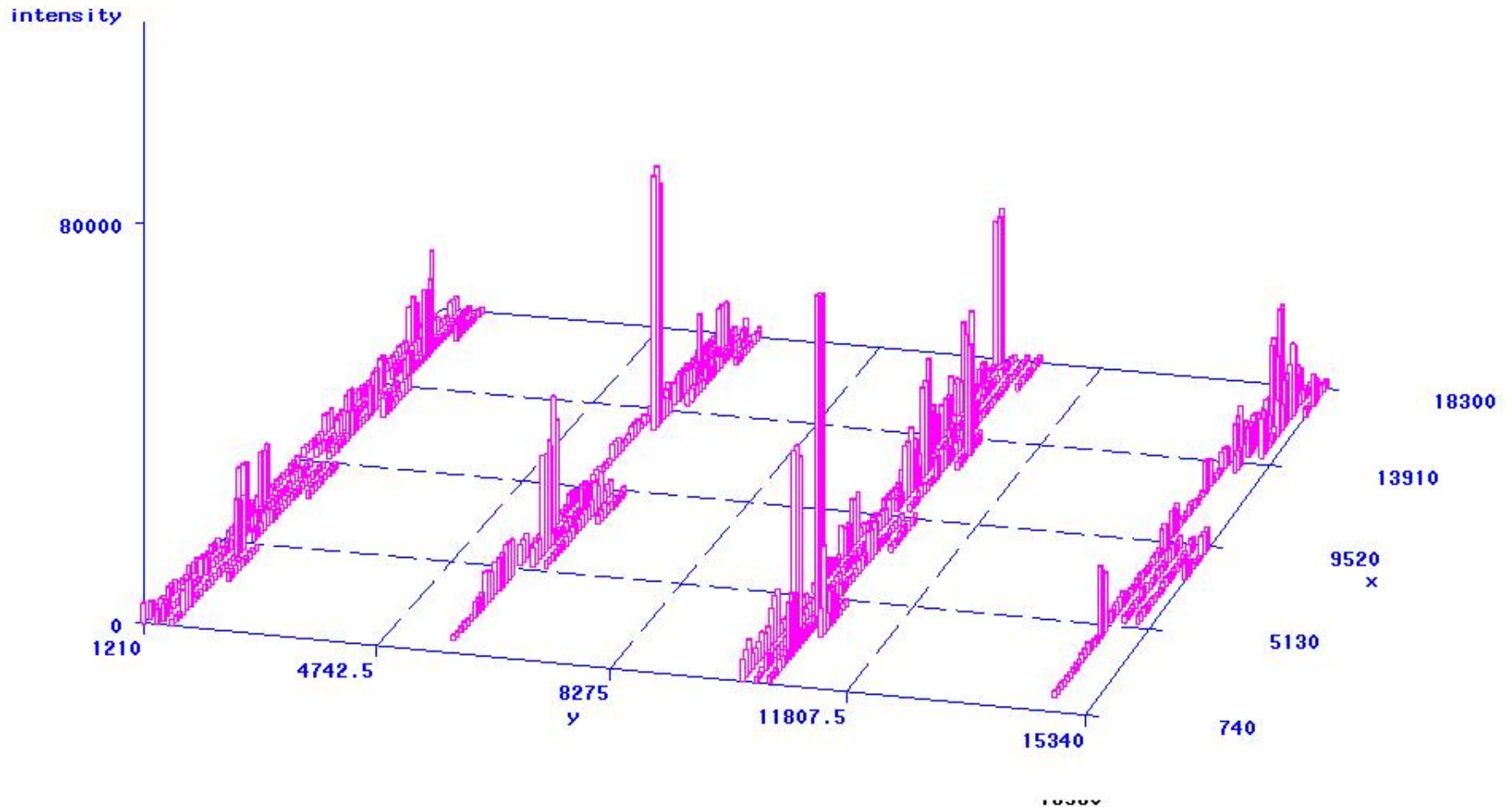
response of the **genes of *Listeria* sp.** when the array is hybridized with *Listeria innocua*

L_innocua_ATCC33090 LISTERIA



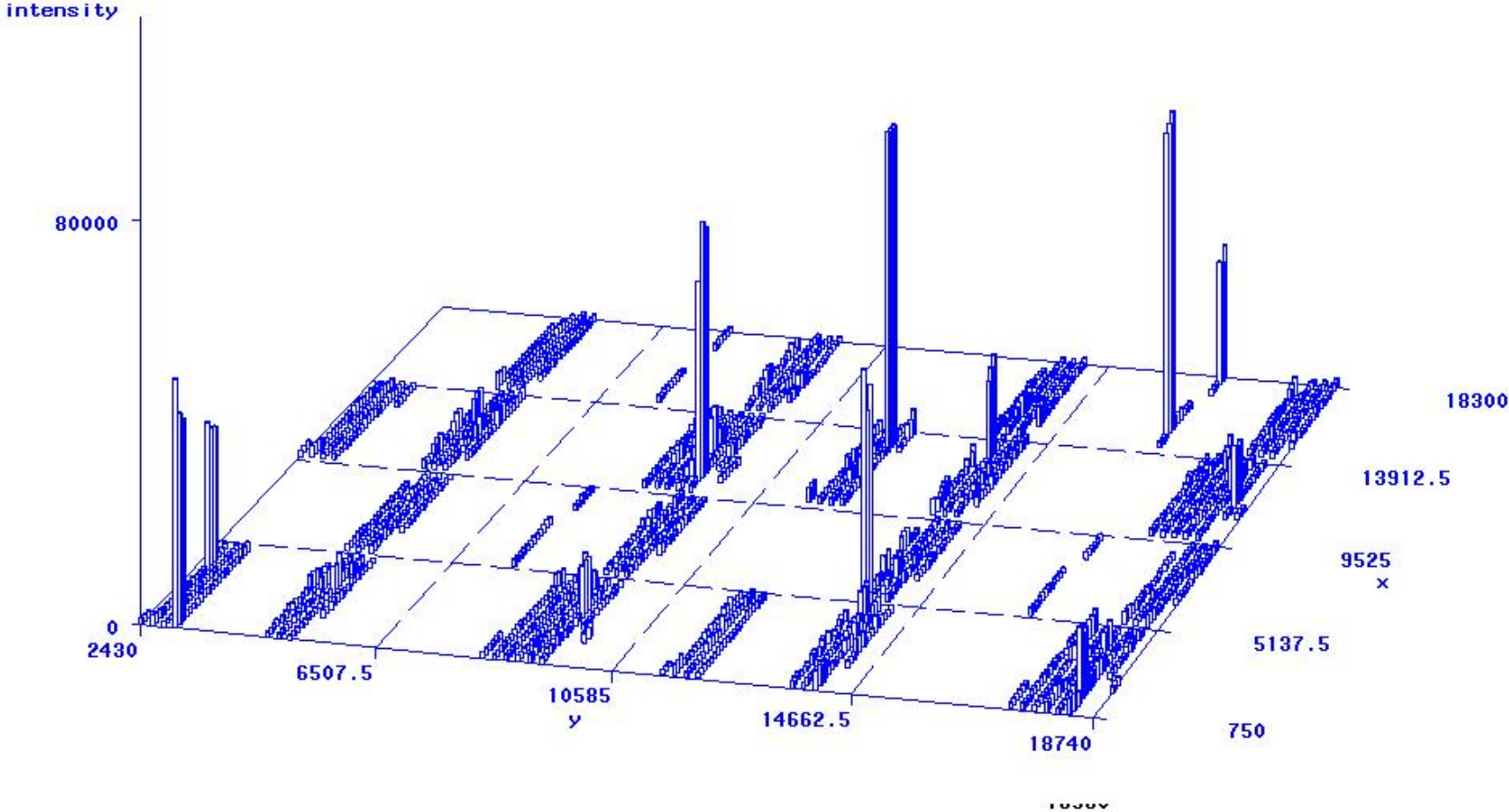
response of the genes of *Campylobacter* sp. when the array is hybridized with *Listeria innocua*

L_innocua_ATCC33090 LISTERIA



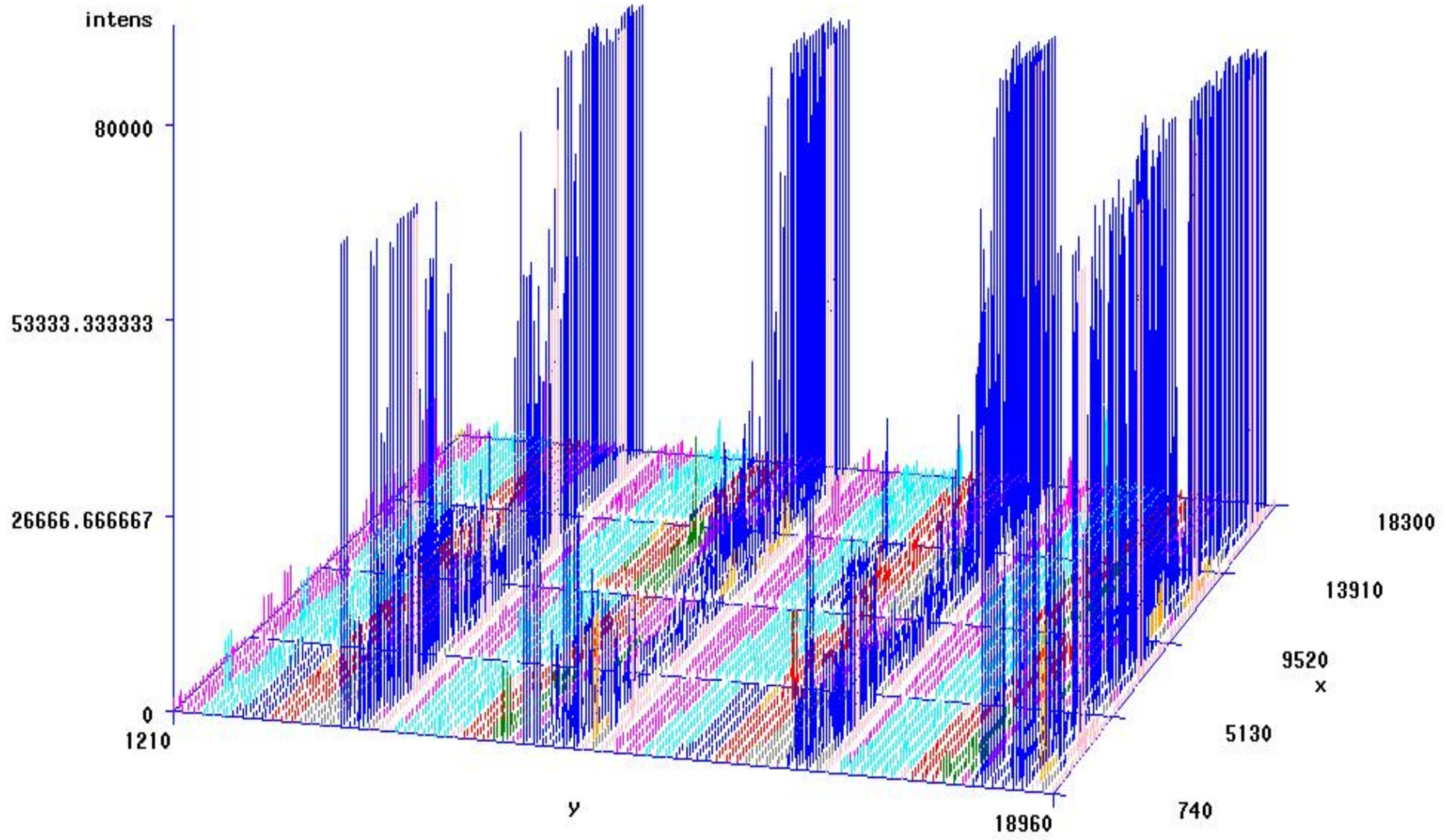
response of the genes of *Staphylococcus* sp. when the array is hybridized with *Listeria innocua*

L_innocua_ATCC33090 LISTERIA



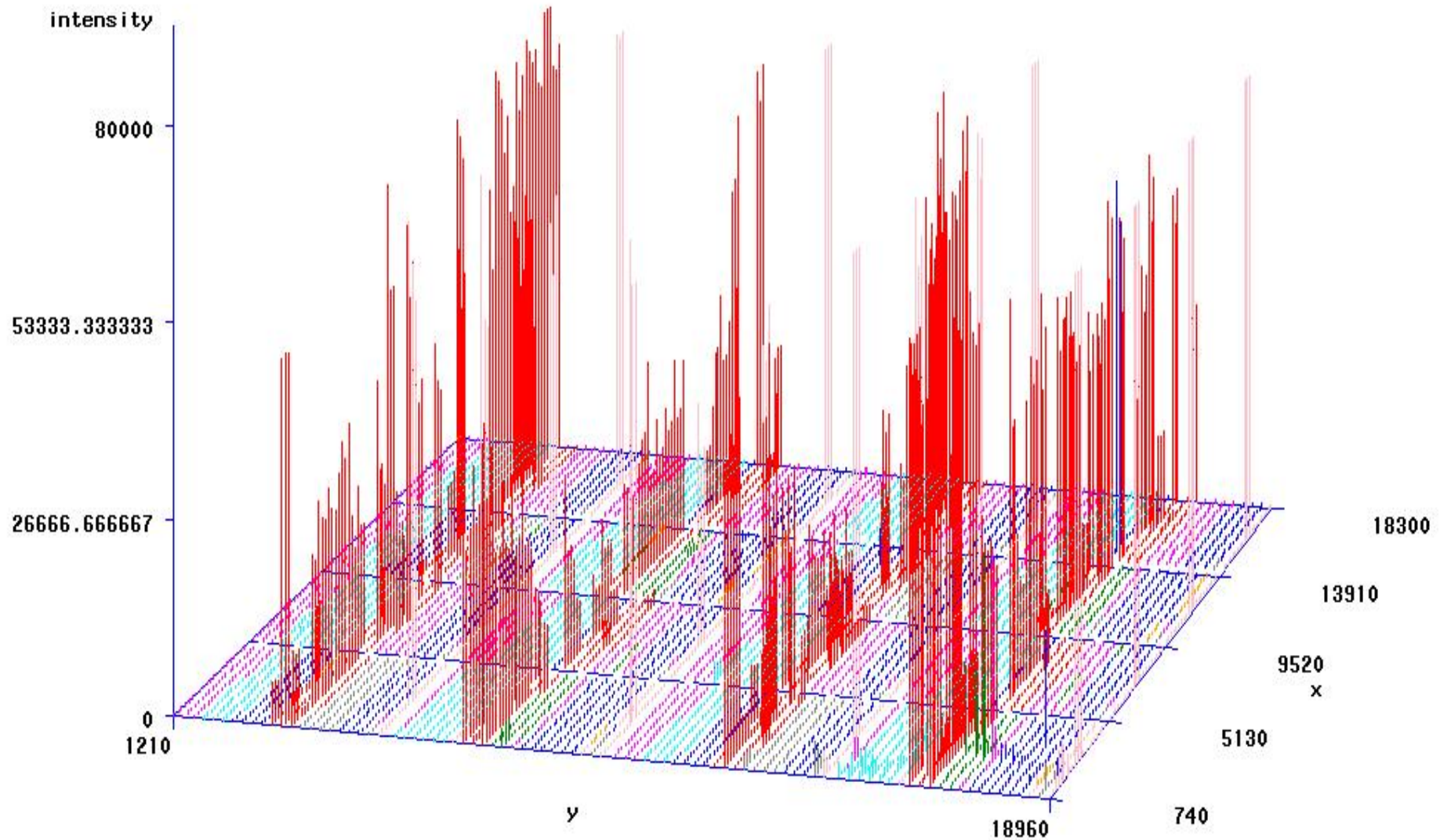
C_jejuni_ssp_jejuni_NCTC12560

example of response
(genes of Campylobacter sp. are reported in blue)



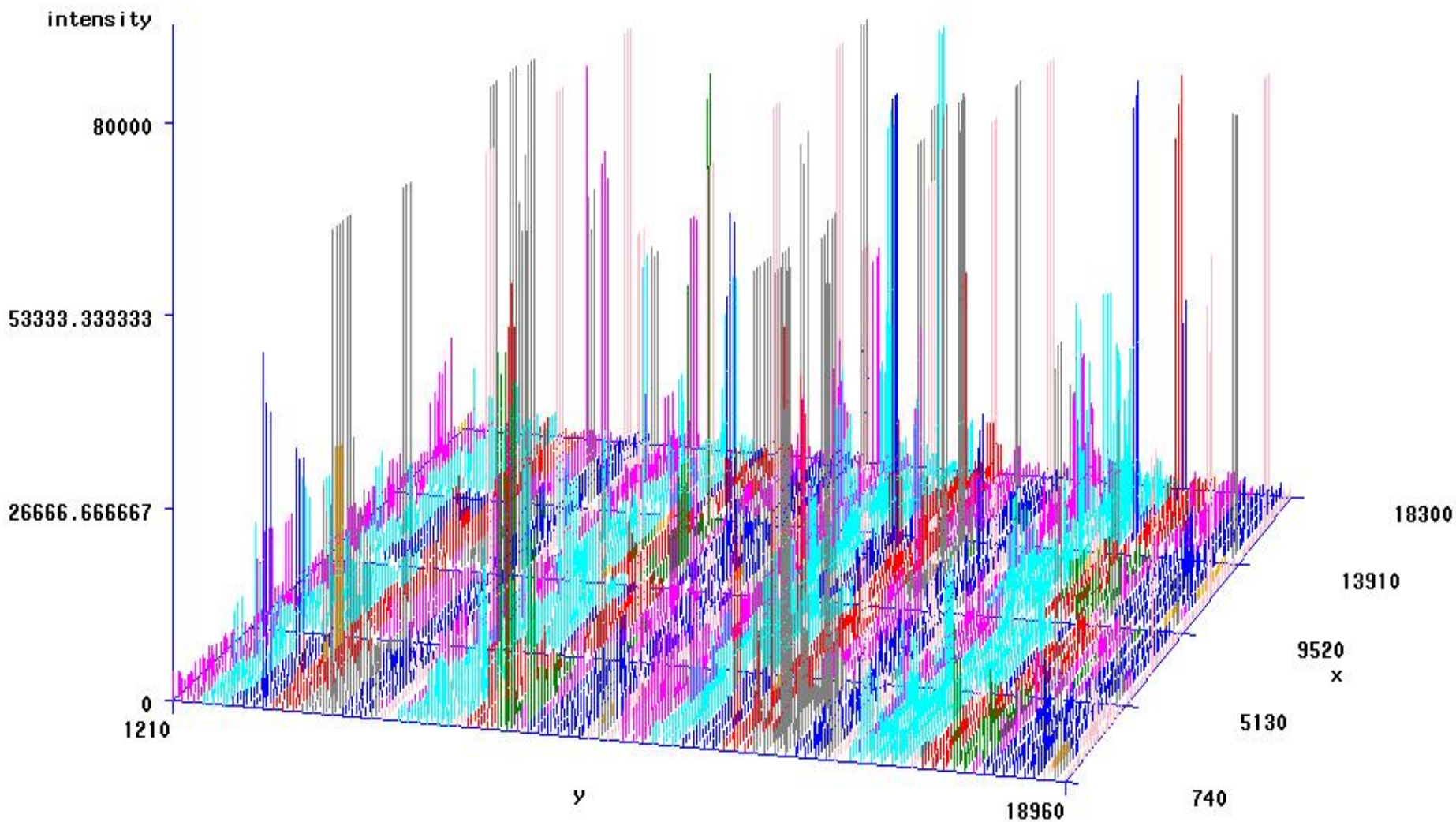
Staphylococcus_aureus_ATOC33862

example of response
(genes of Staphylococcus sp. are reported in red)



L_innocua_ATCC33090

example of response
(genes of *Listeria* sp. are reported in grey)



$$Y = \mu + F + e$$

μ = general mean

F = effect of classification factor

e = residual error

$$\text{Intensity} = \mu + \text{Genus} + \text{residual error}$$

La procedura GLM

Variabile dipendente: accrescimento

	Somma dei	Media	Valore	
Origine	DF	quadrati	quadratica	F
Modello	2	30700.00000	15350.00000	7.59
Errore	15	30350.00000	2023.33333	
Totale corretto	17	61050.00000		

Pr > F
0.0003

R-quadro 0.502867
 Var coeff 6.938017
 Radice MSE 44.98148
 Intensity Media 448.3333

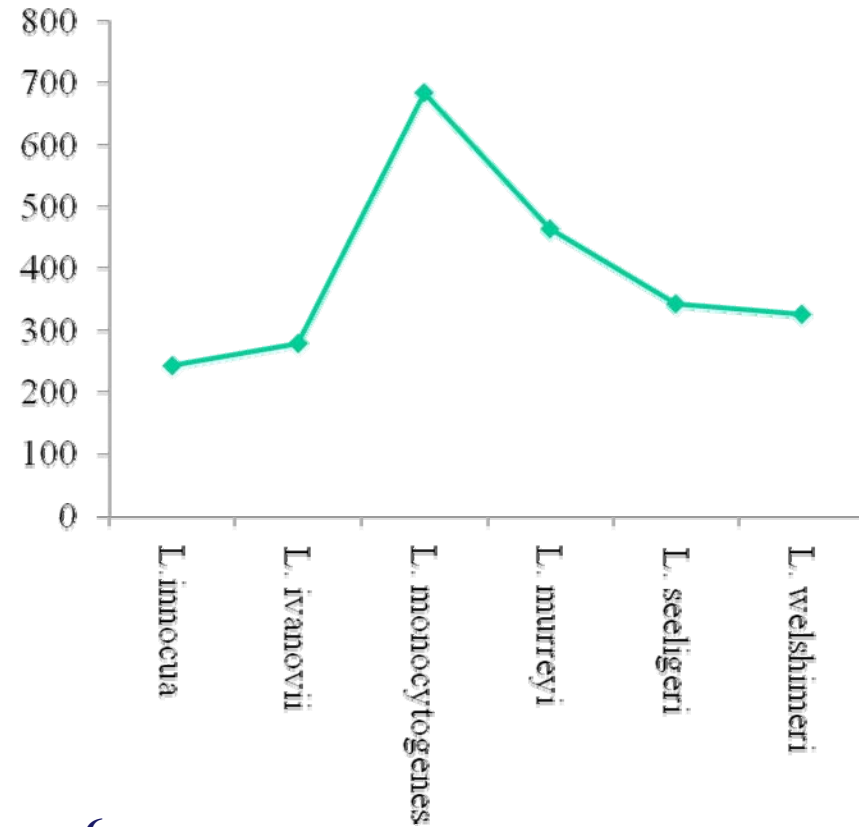
	DF	Tipo I SS	Media	Valore	
Origine			quadratica	F	Pr > F
Intensity	5	30700.00000	15350.00000	7.59	0.0003

	DF	Tipo III SS	Media	Valore	
Origine			quadratica	F	Pr > F
Intensity	5	30700.00000	15350.00000	7.59	0.0003

Hybridization patterns with mono-specific samples

MODEL

	ch1_Intensity	Numero
	LSMEAN	LSMEAN
LEVELS	242.642414	1
Listeria innocua	278.123474	2
Listeria ivanovii	683.617900	3
Listeria monocytogenes	463.034826	4
Listeria murrayi	342.738803	5
Listeria seeligeri	325.289662	6



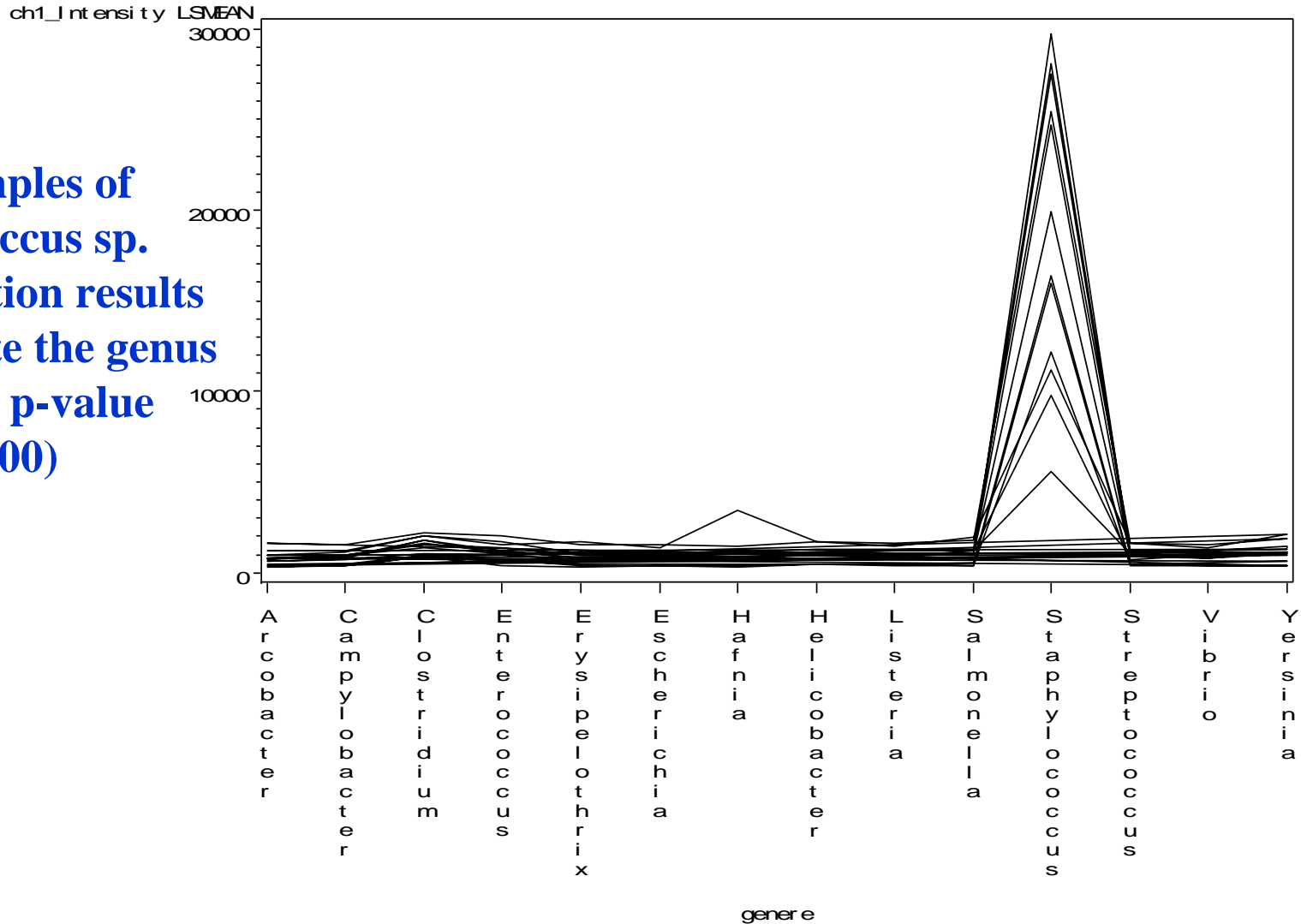
i/j	1	2	3	4	5	6
1		0.7364	<.0001	0.2752	0.6200	0.4612
2	0.7364		<.0001	0.3716	0.7548	0.6966
3	<.0001	<.0001		0.2530	0.0777	0.0002
4	0.2752	0.3716	0.2530		0.6549	0.5127
5	0.6200	0.7548	0.0777	0.6549		0.9339
6	0.4612	0.6966	0.0002	0.5127	0.9339	

Correction of the data

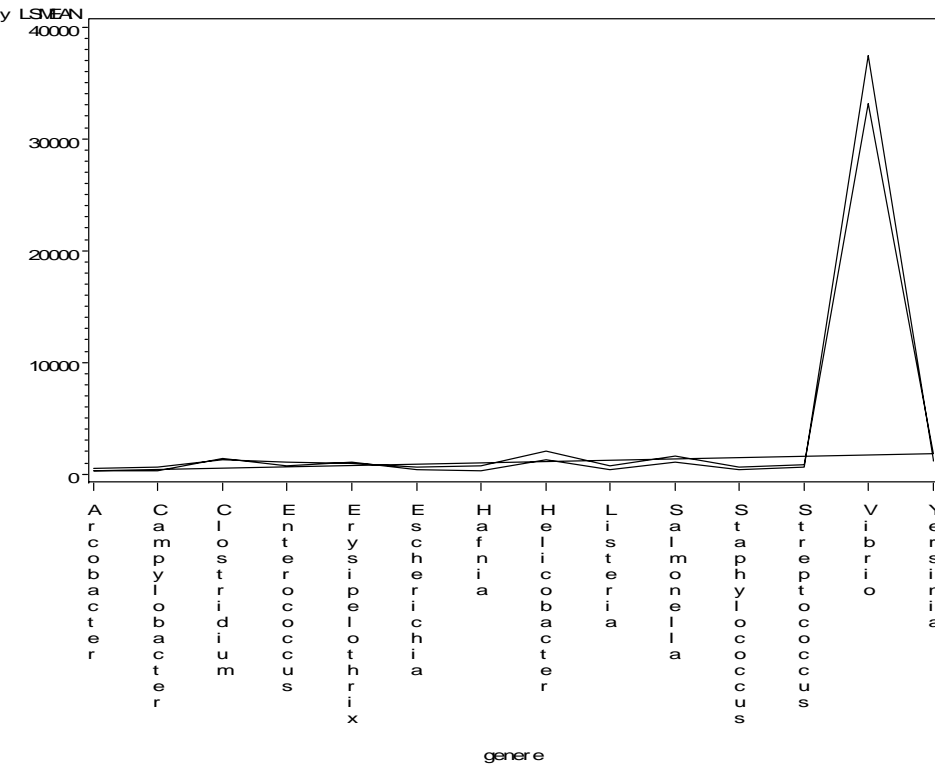
Plain	Correction	Non significant array	Non significant organism
1	row data	3	2
2	log2 (intensity)	5	4
3	intensity – background	5	3
4	Log2(intensity – background)	5	6
5	median of 3 spot	20	6
6	mean of 3 spot	5	3
7	intensity – back. and median	14	6
8	intensity – back. and mean	13	6
9	mean/dev.st of 3 spot	42	14
10	median/dev.st of 3 spot	42	14

consequently, following analyses have been conducted with row data

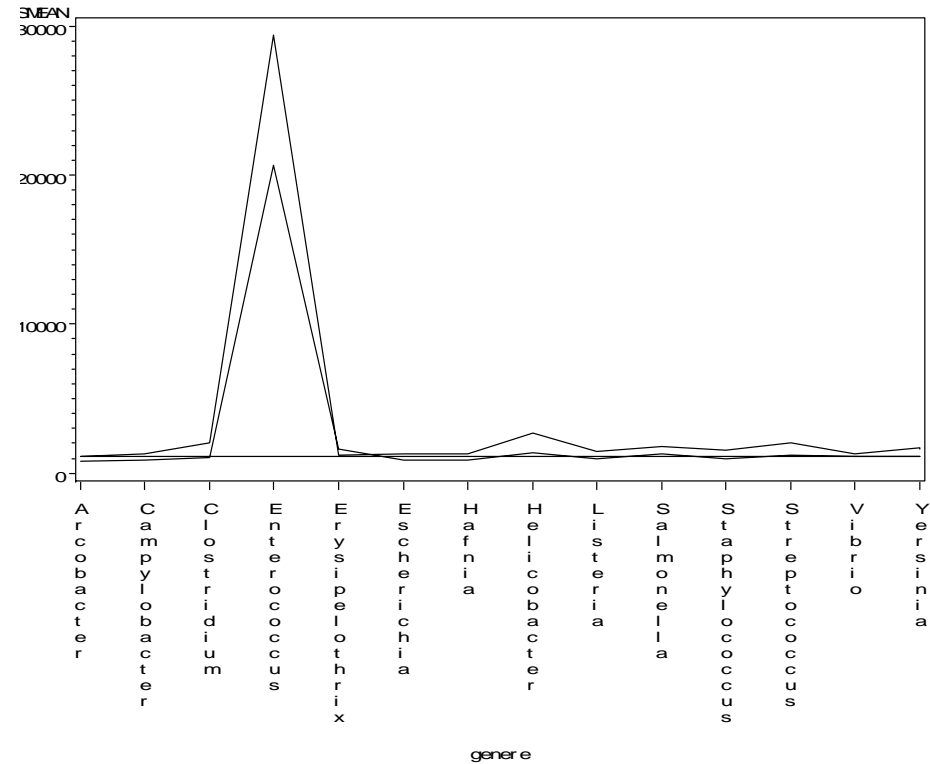
Staphylococcus



Vibrio

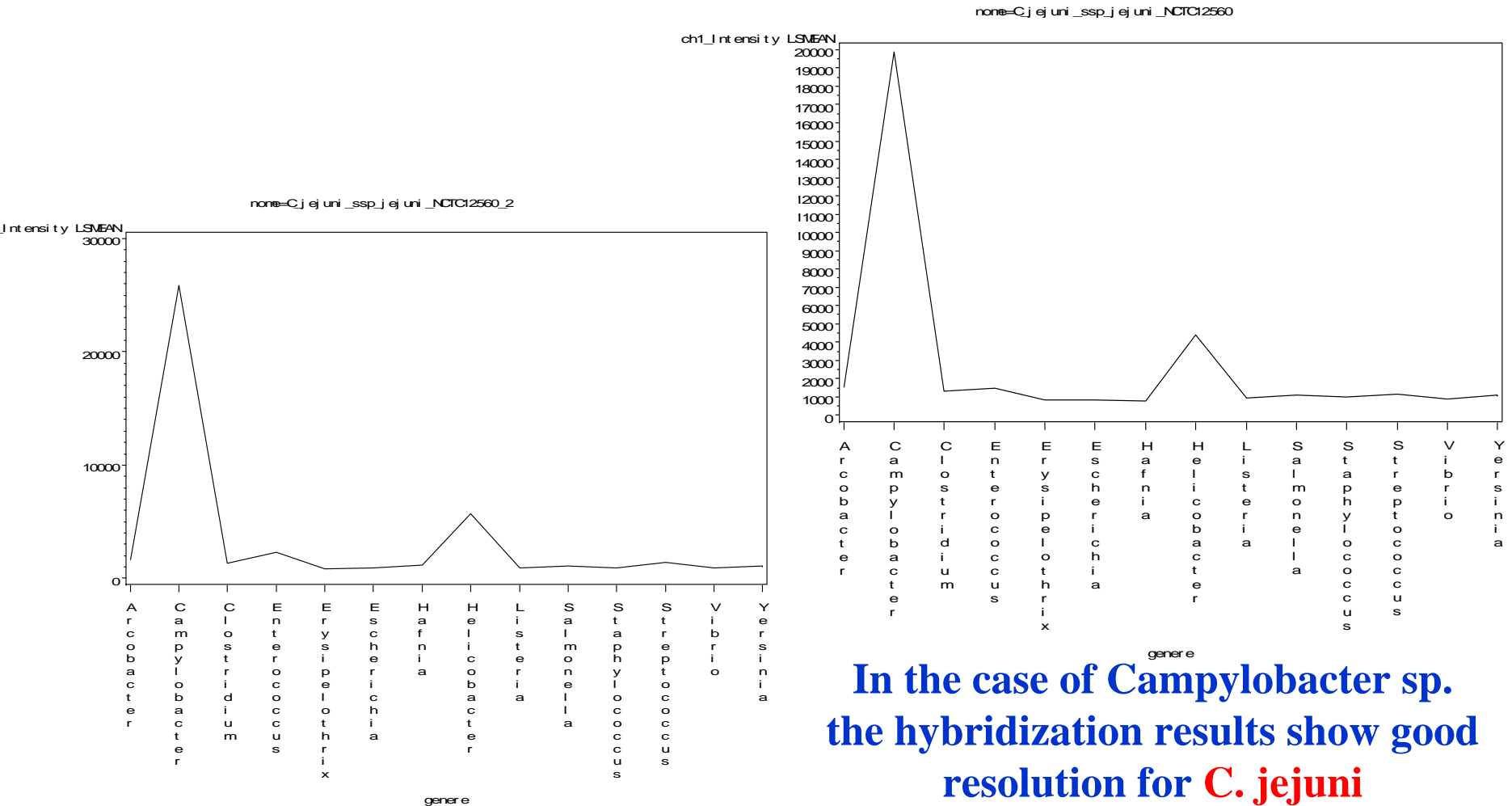


Enterococcus



The separation is also clean for *Vibrio* sp. and *Enterococcus* sp.

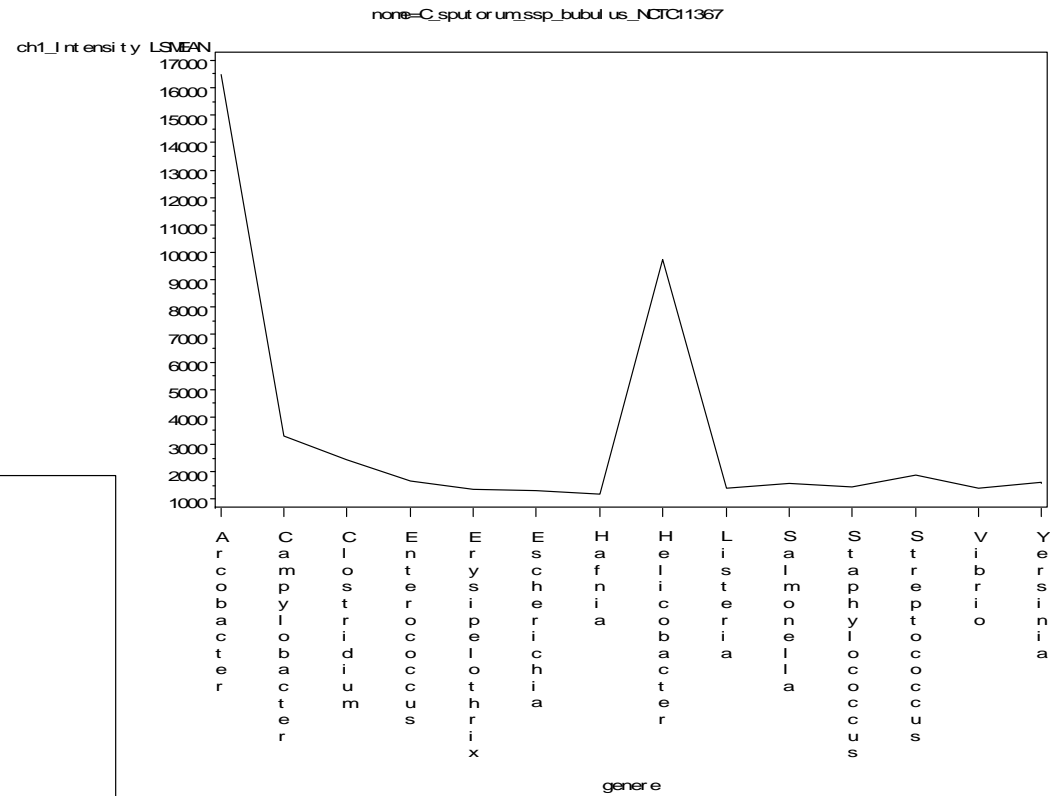
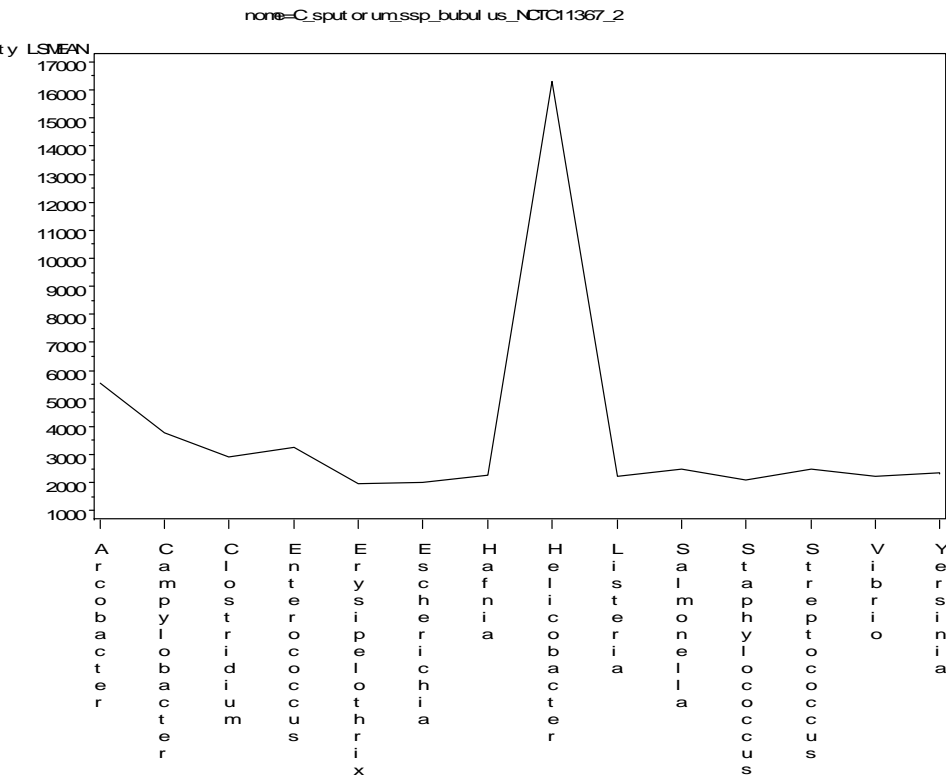
Camphylobacter



Hybridization patterns with mono-specific samples

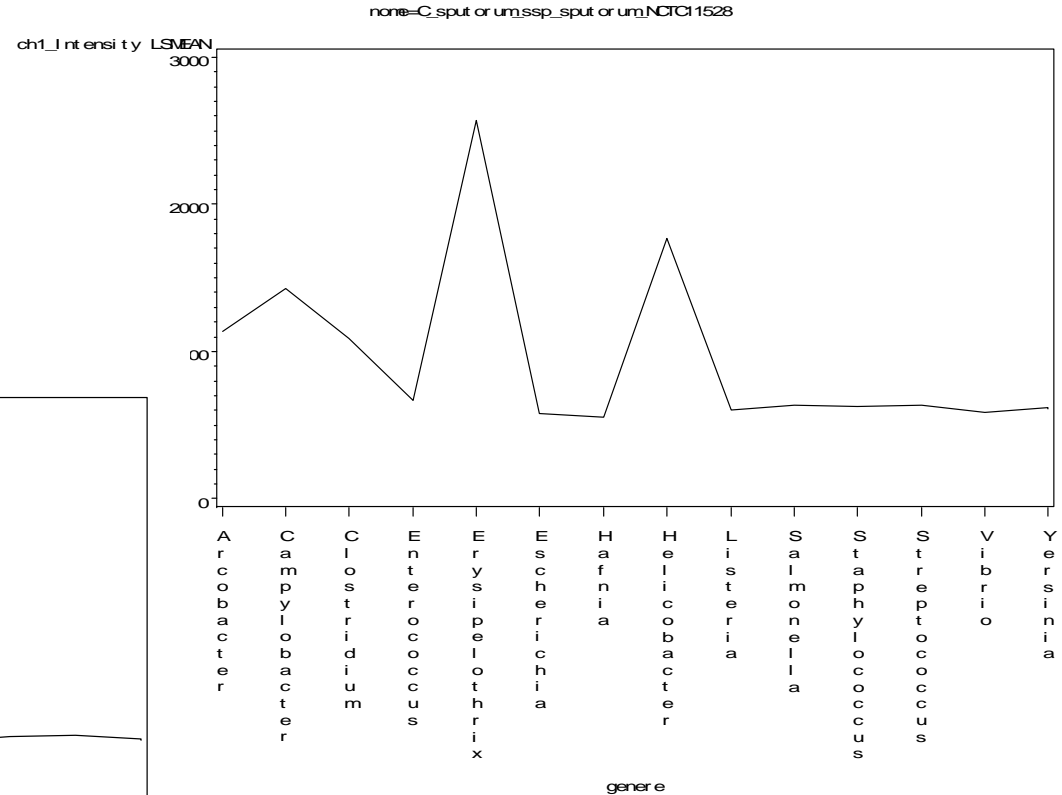
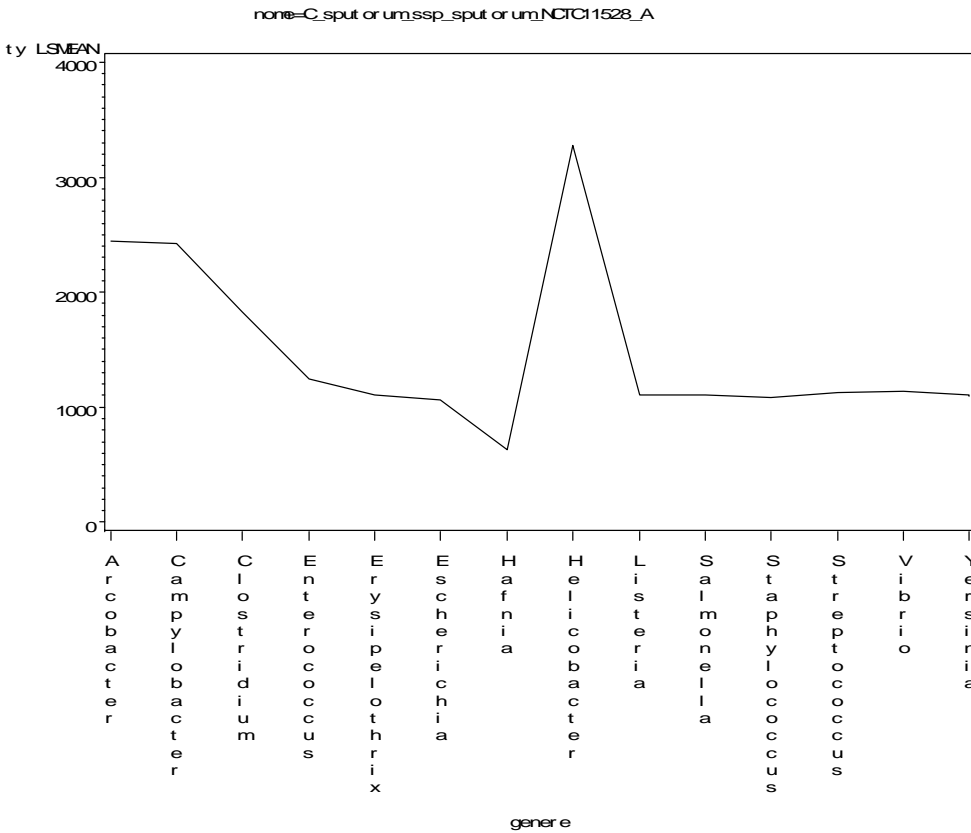
RESULTS

but contrasting patterns for
C. sputorum and *C. upsaliensis*



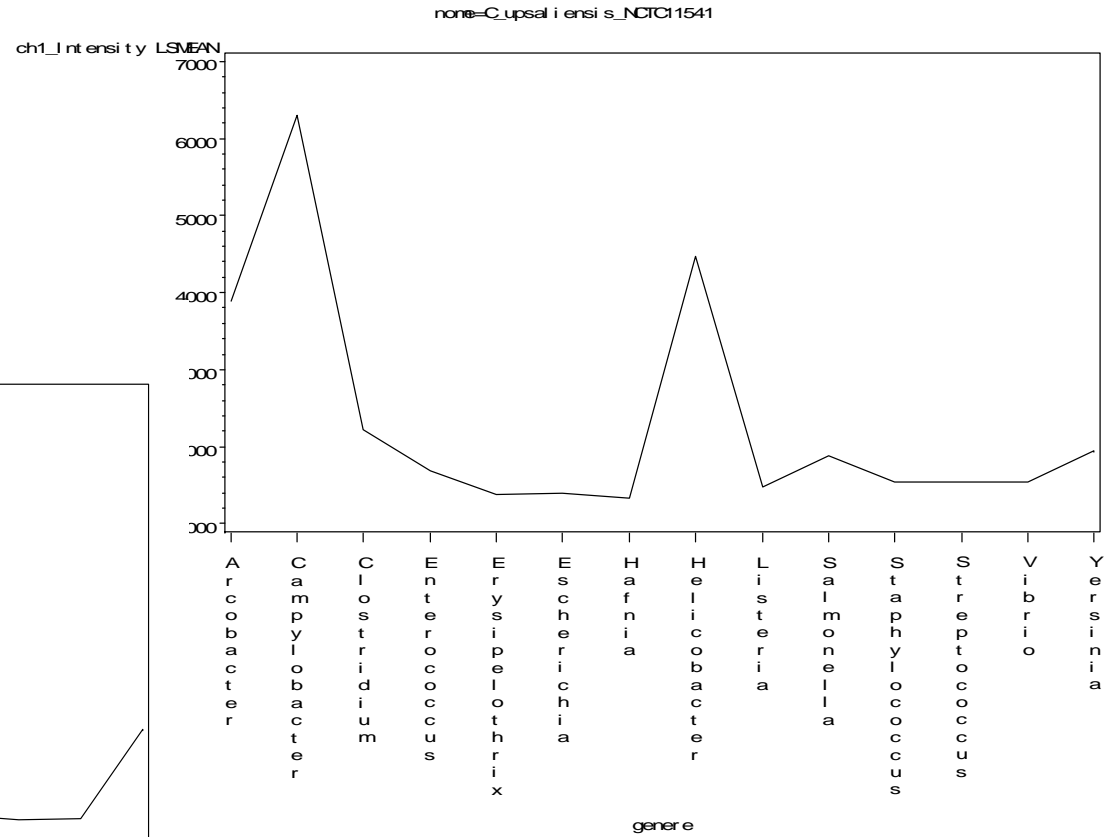
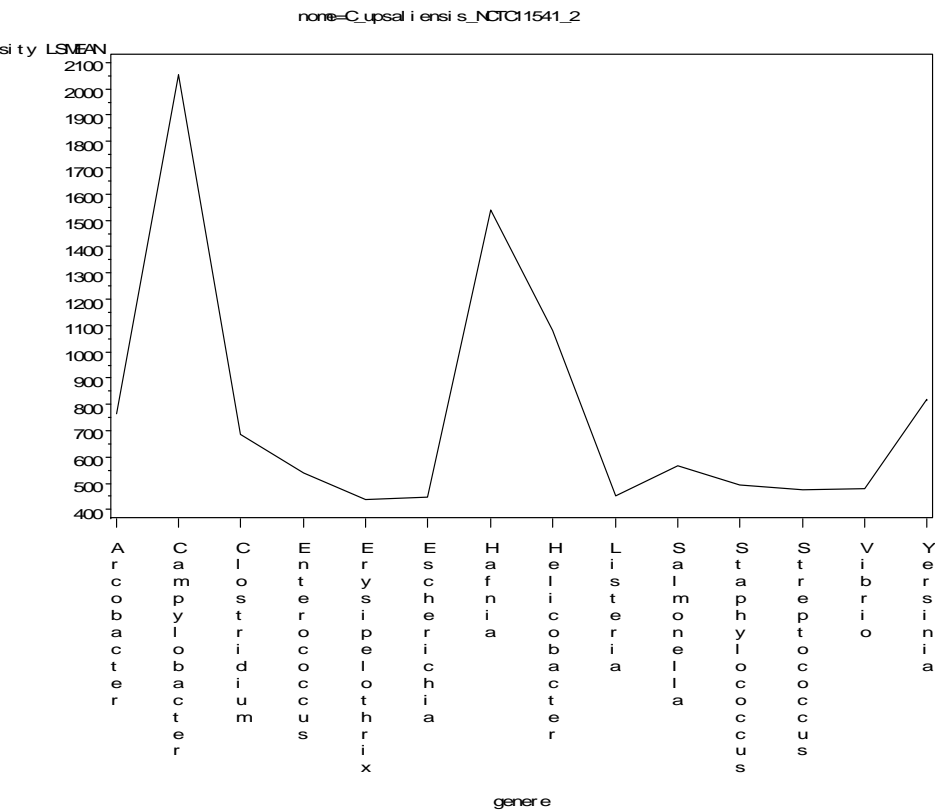
Hybridization patterns with mono-specific samples

RESULTS



Hybridization patterns with mono-specific samples

RESULTS



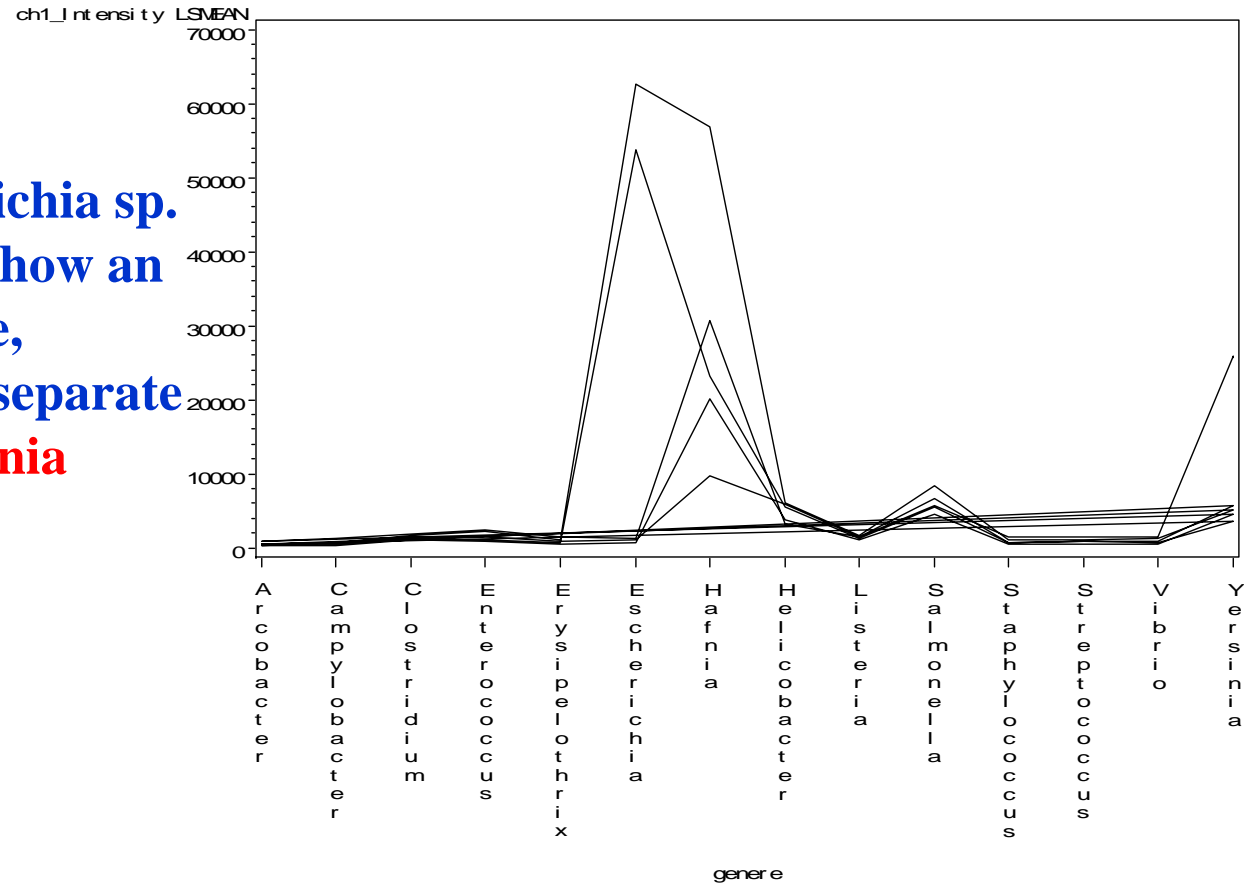
Helicobacter
Campylobacter
Arcobacter
Hafnia
Erysipelothrix

are the genus that mostly react
 when the arrays are hybridized
 with Campylobacter sp. samples

Organismo ibridizzato	Genere	p-value
C_sputorum_ssp_sputorum_NCTC11528_A	Erysipelothrix	0,055754
C_sputorum_ssp_bubulus_NCTC11367	Escherichia	0,093715
C_sputorum_ssp_sputorum_NCTC11528_A	Helicobacter	0,123145
C_sputorum_ssp_sputorum_NCTC11528_A	Escherichia	0,123212
C_sputorum_ssp_bubulus_NCTC11367_2	Escherichia	0,123295
C_sputorum_ssp_bubulus_NCTC11367	Vibrio	0,137295
C_sputorum_ssp_bubulus_NCTC11367_2	Enterococcus	0,146986
C_upsaliensis_NCTC11541	Hafnia	0,169748
C_sputorum_ssp_sputorum_NCTC11528_A	Vibrio	0,184806
C_sputorum_ssp_sputorum_NCTC11528	Escherichia	0,189894
C_sputorum_ssp_bubulus_NCTC11367_2	Vibrio	0,217498
C_upsaliensis_NCTC11541_2	Campylobacter	0,228154
C_sputorum_ssp_sputorum_NCTC11528	Vibrio	0,235477
C_upsaliensis_NCTC11541	Campylobacter	0,345405
C_sputorum_ssp_bubulus_NCTC11367_2	Campylobacter	0,368935
C_sputorum_ssp_sputorum_NCTC11528	Helicobacter	0,402555
C_sputorum_ssp_sputorum_NCTC11528_A	Hafnia	0,405986
C_sputorum_ssp_bubulus_NCTC11367	Hafnia	0,457731
C_sputorum_ssp_sputorum_NCTC11528	Hafnia	0,579974
C_sputorum_ssp_bubulus_NCTC11367_2	Hafnia	0,585699
C_upsaliensis_NCTC11541_2	Hafnia	0,733746
C_sputorum_ssp_sputorum_NCTC11528	Campylobacter	0,794605
C_sputorum_ssp_sputorum_NCTC11528_A	Campylobacter	0,993182

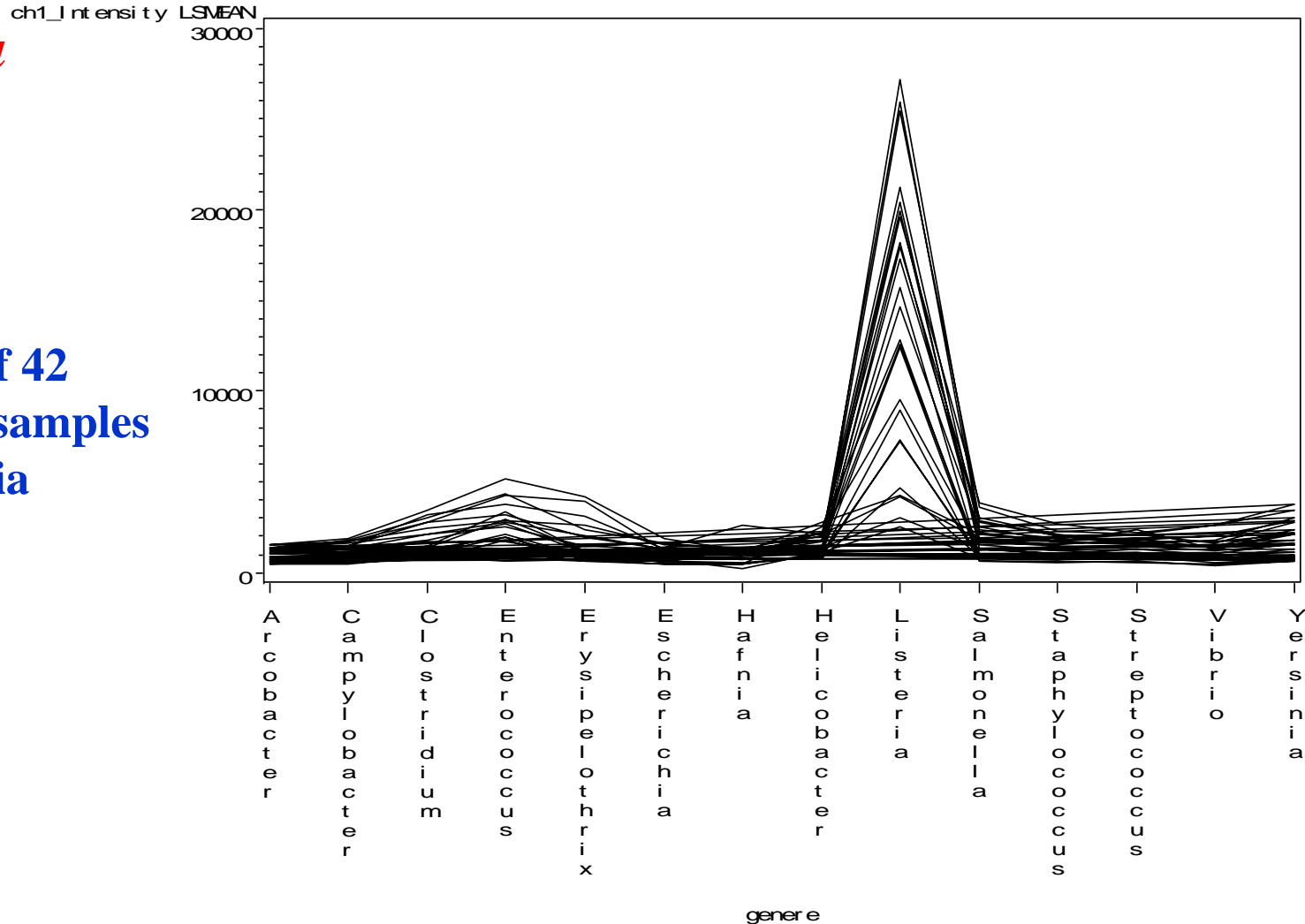
Escherichia

Also In the case of *Escherichia* sp. the hybridization results show an ambiguous response, it is not possible to clearly separate *Escherichia* from *Hafnia*



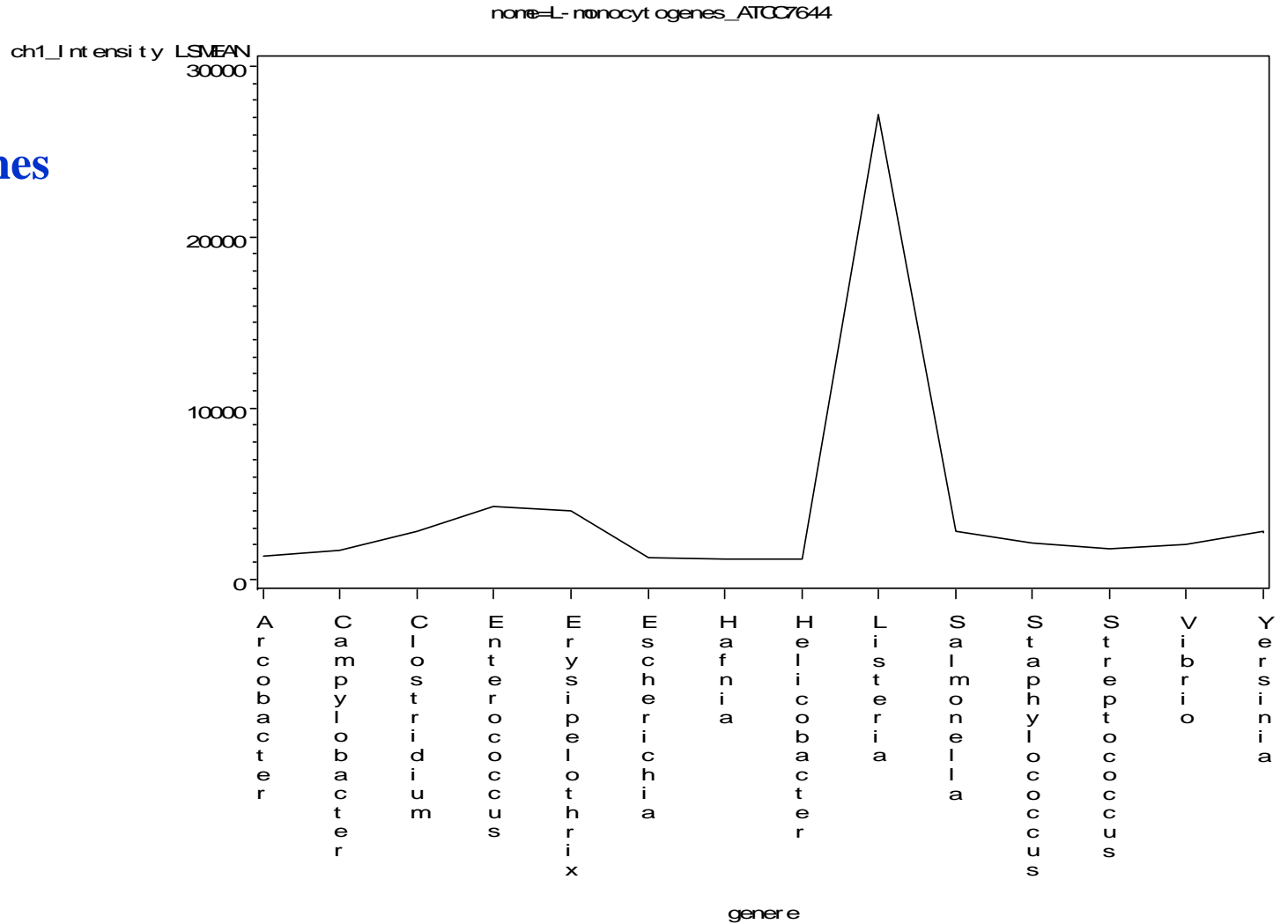
Listeria

response of 42
mono-specific samples
of *Listeria*



In almost all the cases it is possible to distinguish the hybridized genus with a highly p-value ($p < 0.000$)

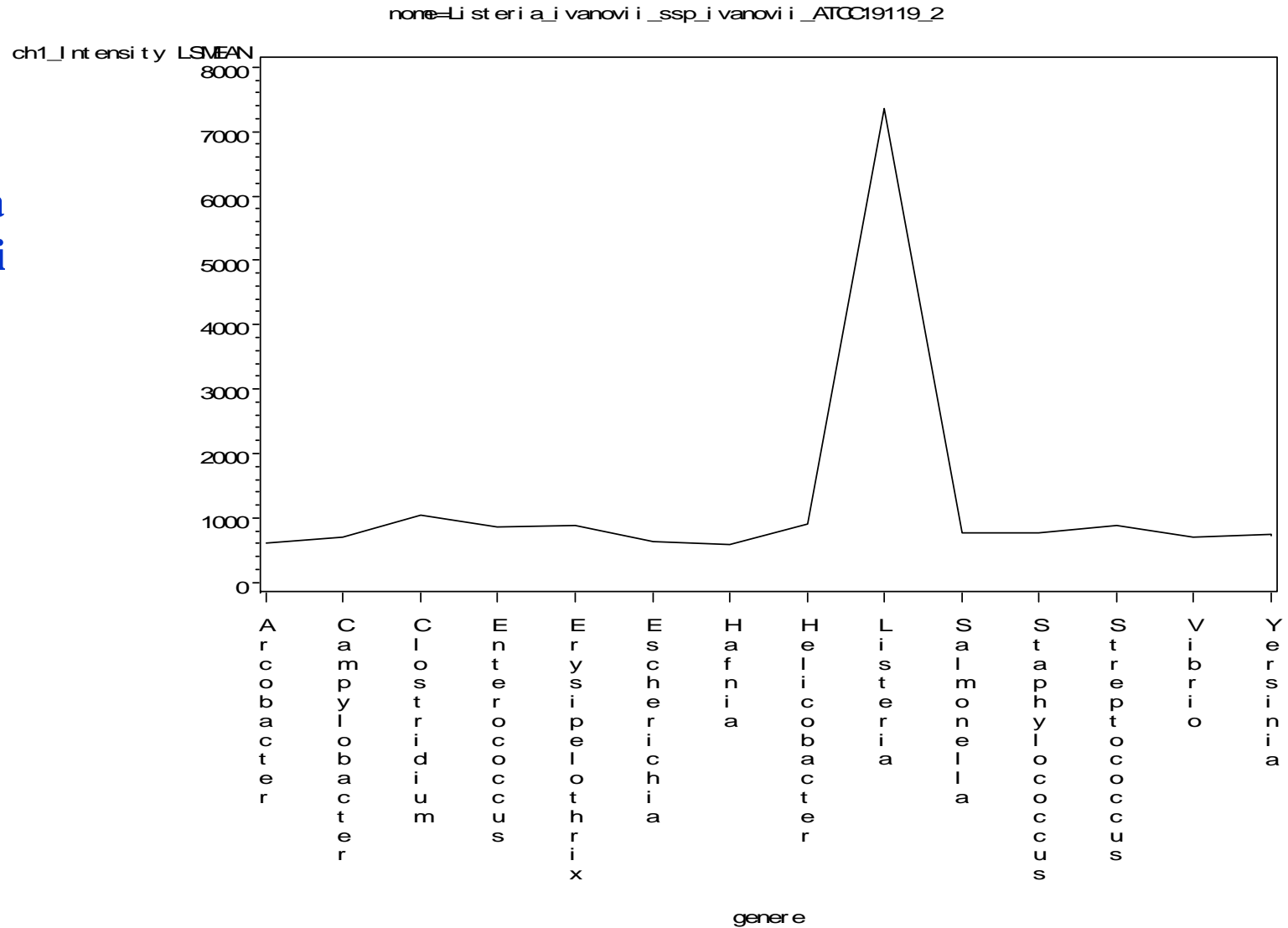
Listeria monocytogenes



Hybridization patterns with mono-specific samples

RESULTS

**Listeria
ivanovii**



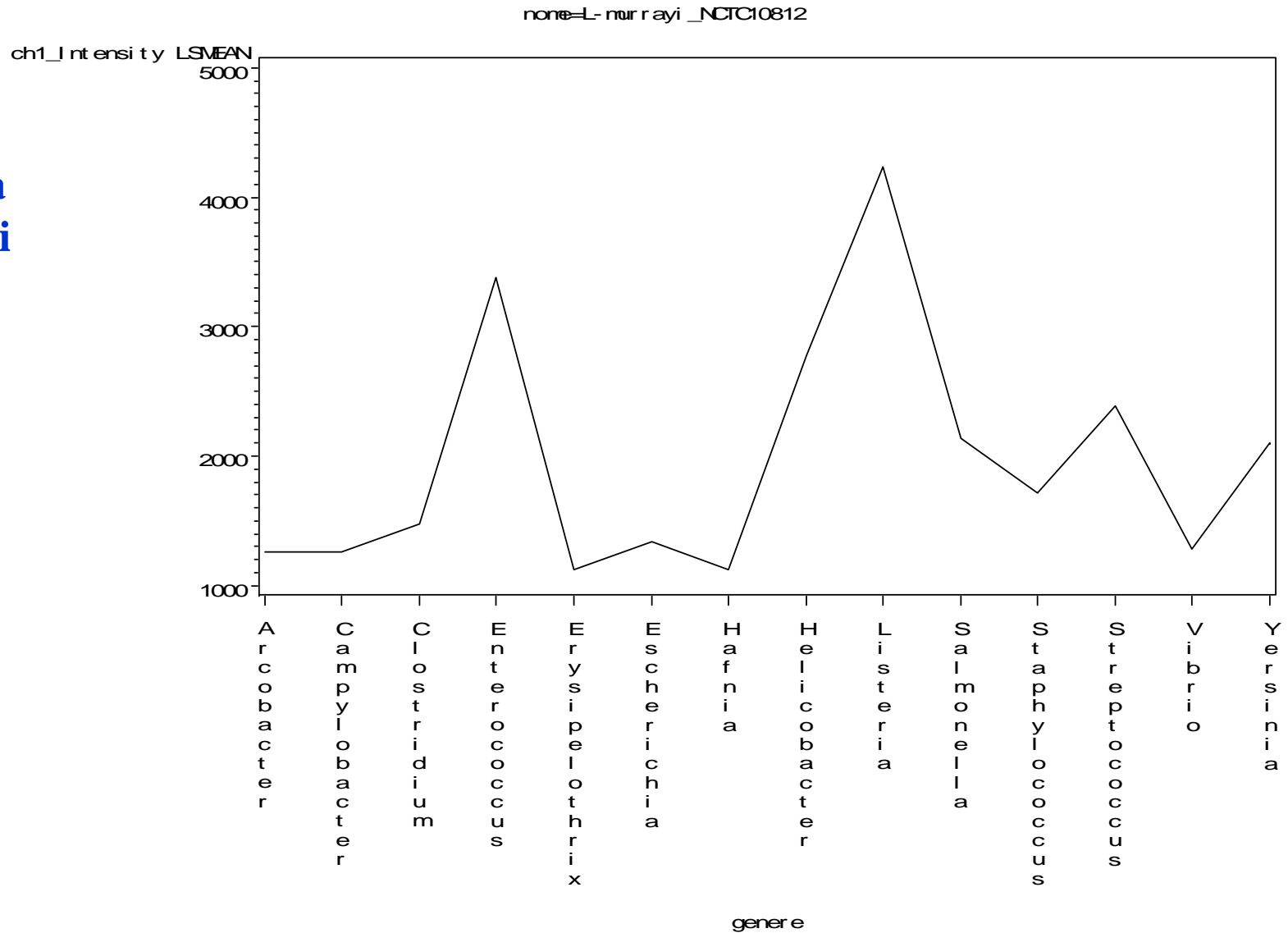
Hybridization results obtained with *L. murrayi* are less clear: **Enterococcus** and **Hafnia** don't statistically result different from *Listeria*

Organismo ibridizzato	Genere	p-value
L-murrayi_NCTC10812_2	Enterococcus	0,077
L-murrayi_NCTC10812	Enterococcus	0,110
L-murrayi_NCTC10812_2	Hafnia	0,121
L_grayi_ATCC19120_2	Hafnia	0,199
L-murrayi_NCTC10812	Hafnia	0,234
L_grayi_ATCC19120	Hafnia	0,400

Hybridization patterns with mono-specific samples

RESULTS

**Listeria
murrayi**



Hybridization patterns with mono-specific samples

with the aim to verify the discriminating power of the array with regard to level - species the same model has been used for **Listeria sp.**

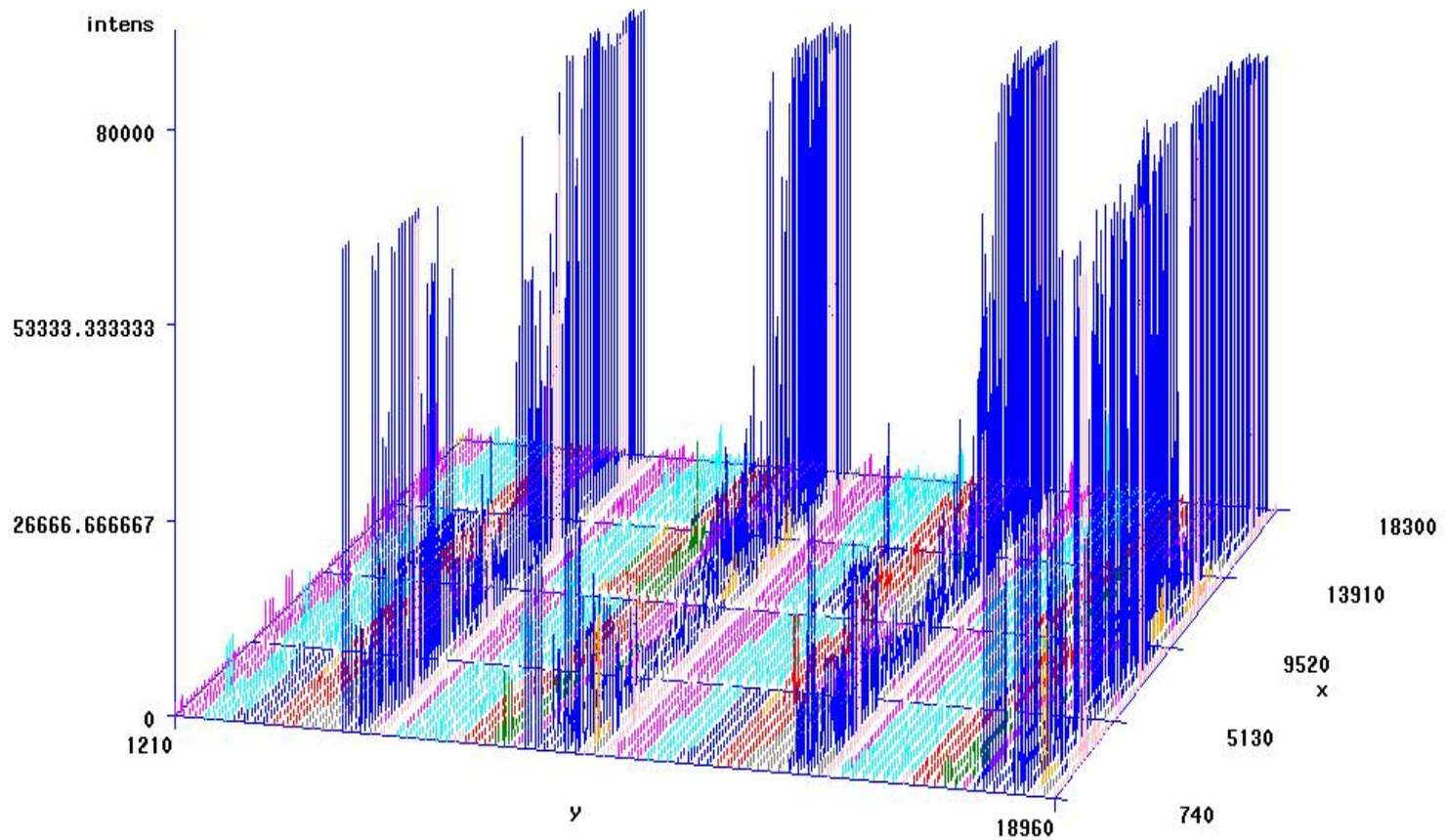
In this case: **Intensity = general mean + species + residual error**

In this case, for every species, the average of the intensity of the genes of *Listeria sp.* has been calculated on all the arrays except those of the species in object

Such value has been taken out from the intensity of the species analysed with the purpose of "to polishing up" the signal from the response of the genes of a species if that one is lacking

Hybridization patterns with mono-specific samples

C_jejuni_ssp_jejuni_NCTC12560

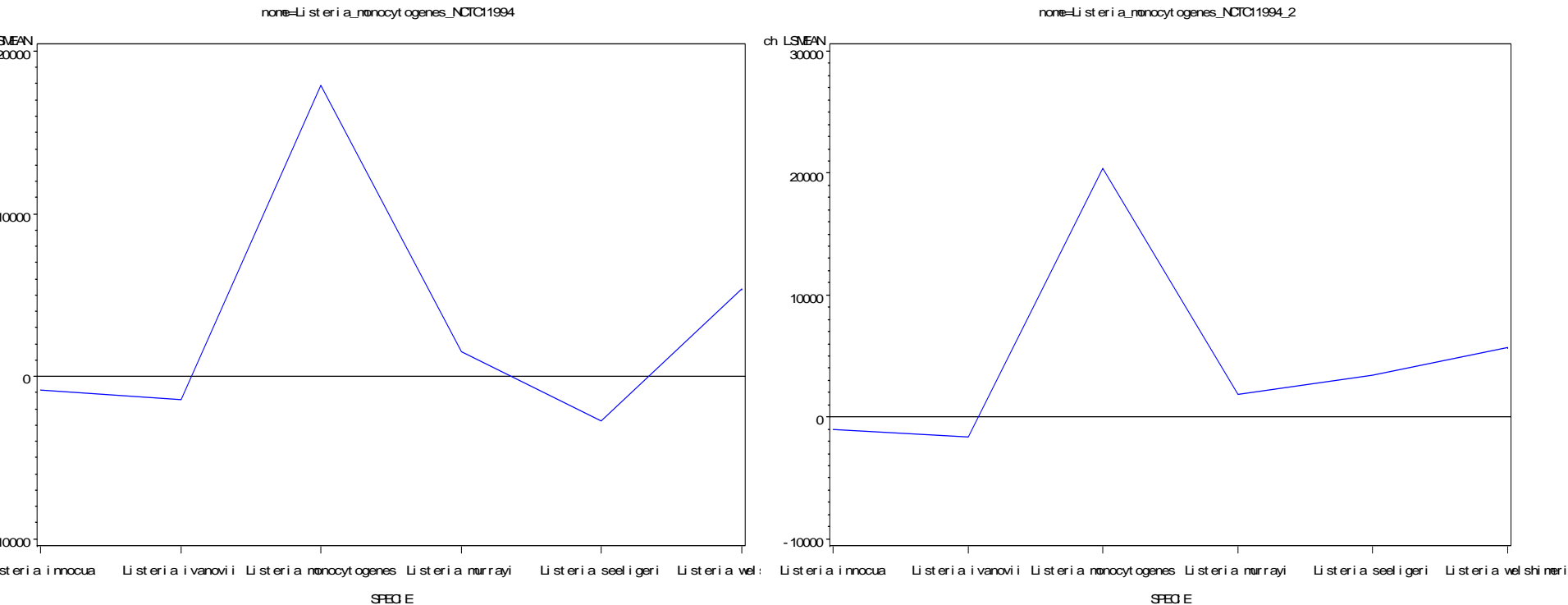


Listeria monocytogenes

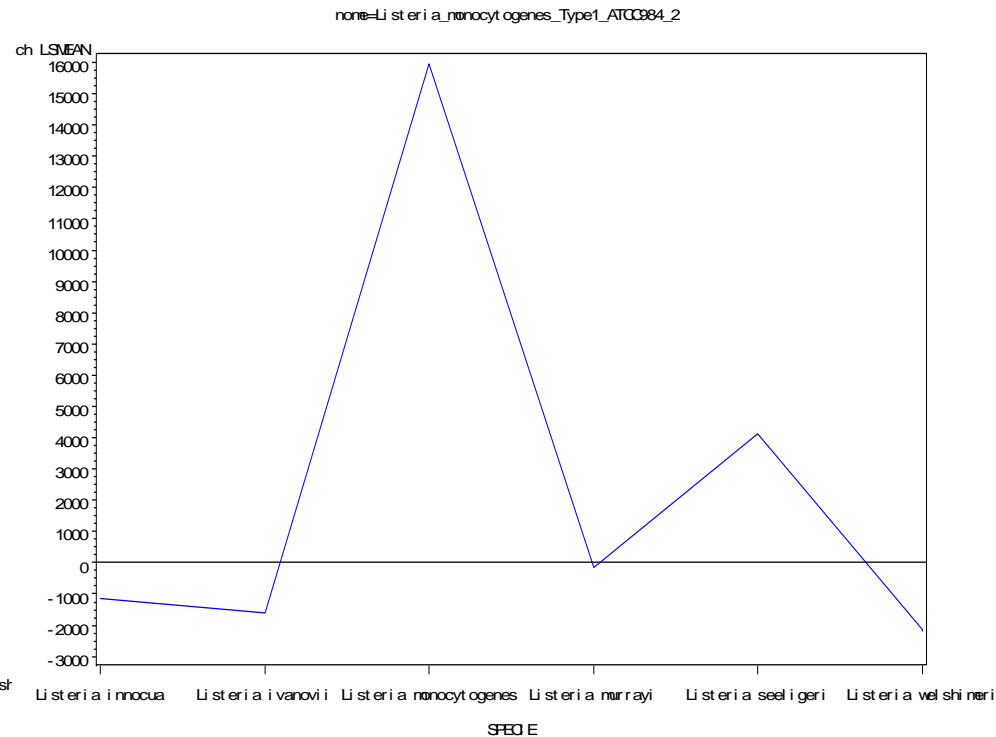
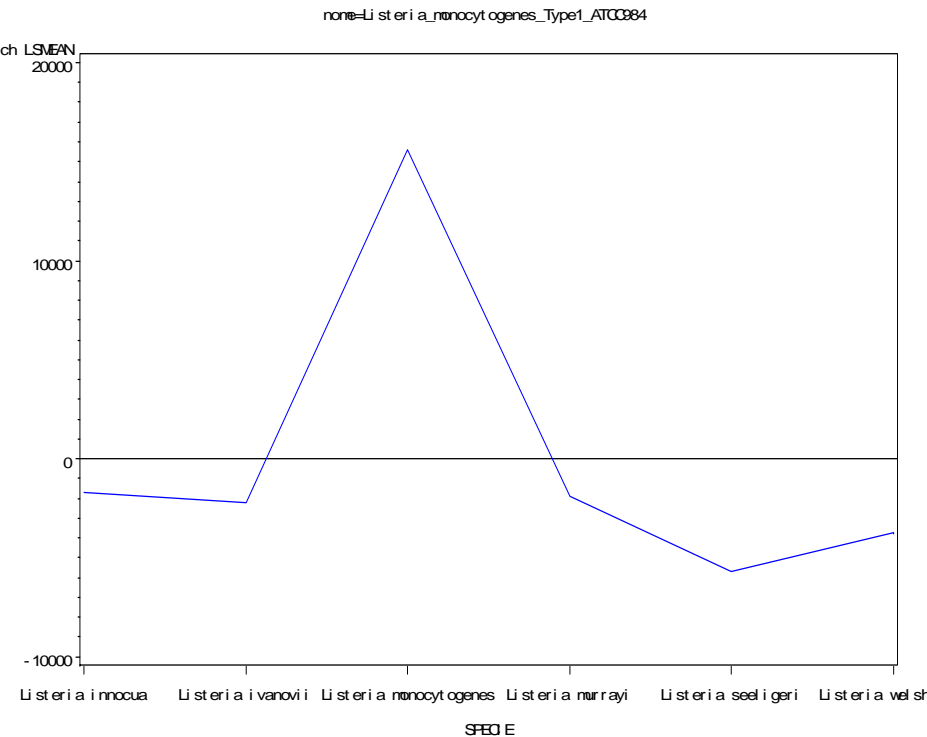
Array	Duplicato
Listeria_monocytogenes_ATCC10888	Listeria_monocytogenes_ATCC10888_2
Listeria_monocytogenes_NCTC11994	Listeria_monocytogenes_NCTC11994_2
Listeria_monocytogenes_Type1_ATCC984	Listeria_monocytogenes_Type1_ATCC984_2
Listeria_monocytogenes_USDA_FS15_H2426	Listeria_monocytogenes_USDA_FS15_H2426_2
Listeria_monocytogenes_serotype4b_ATCC13932	Listeria_monocytogenes_serotype4b_ATCC13932_2
Listeria_monocytogenes_serovar1_2a_NCTC10887	Listeria_monocytogenes_serovar1_2a_NCTC10887_2
Listeria_monocytogenes_serovar1a_NCTC4886	Listeria_monocytogenes_serovar1a_NCTC4886_2
Listeria_monocytogenes_serovar4c_NCTC4883	Listeria_monocytogenes_serovar4c_NCTC4883_2
Listeria_monocytogenes_serovar7_NCTC10890	Listeria_monocytogenes_serovar7_NCTC10890_2

for nine samples of *L. monocytogenes* the species is perfectly individualized with good repeatability between arrays and replicas

Listeria monocytogenes

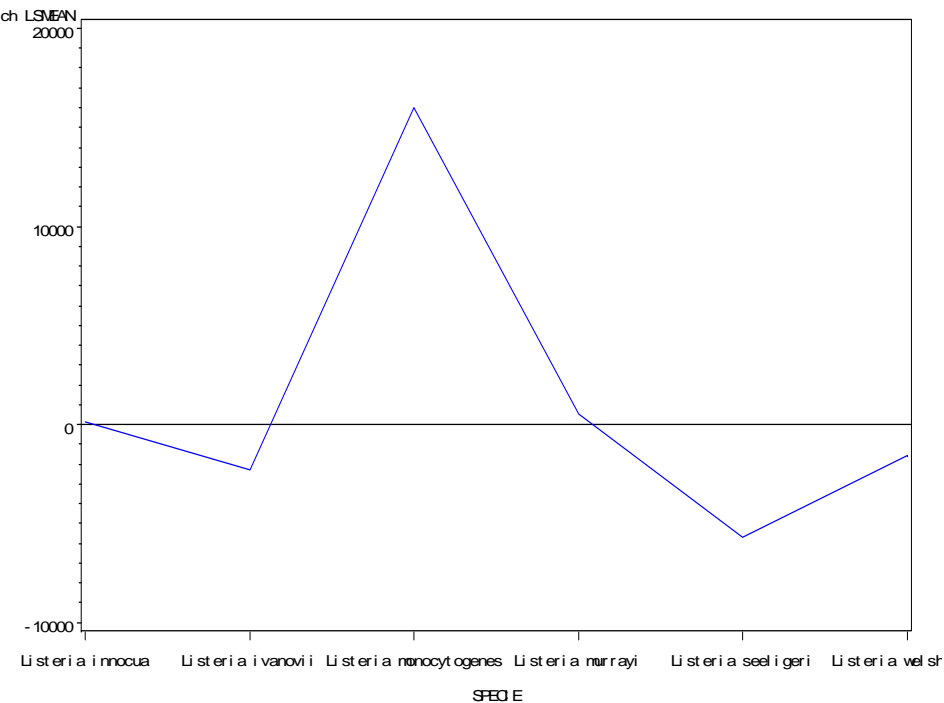


Listeria monocytogenes

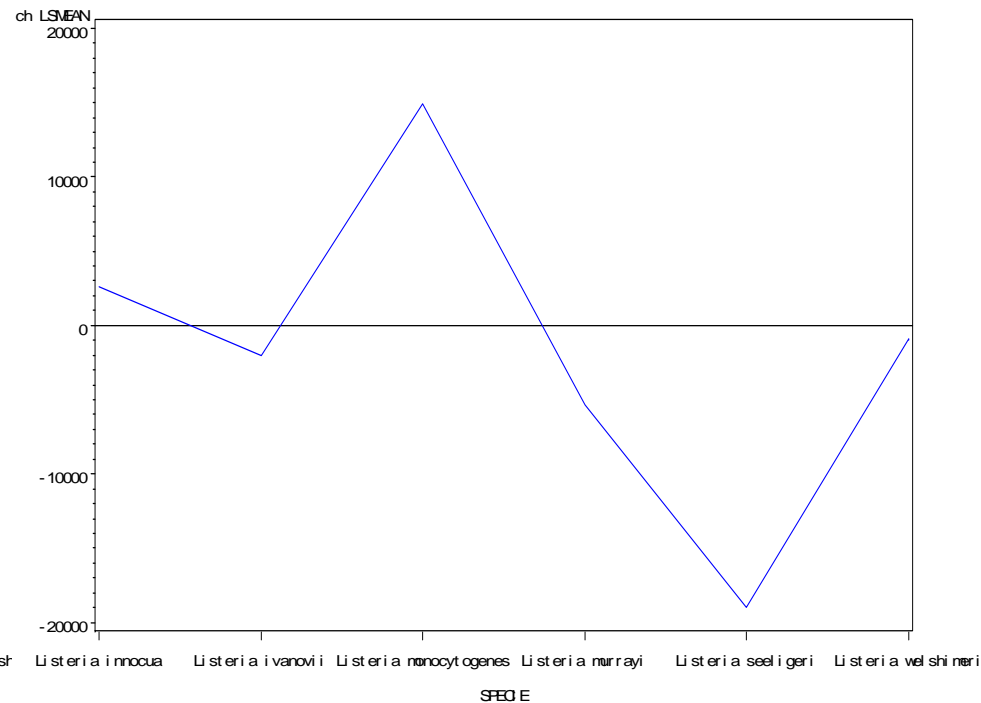


Listeria monocytogenes

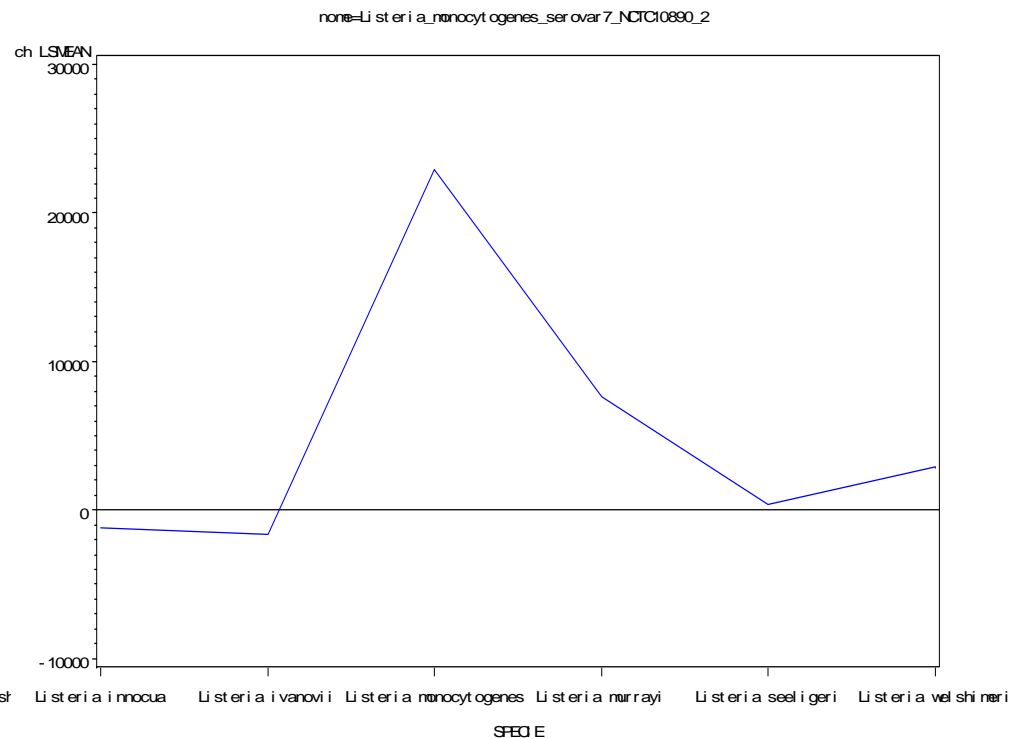
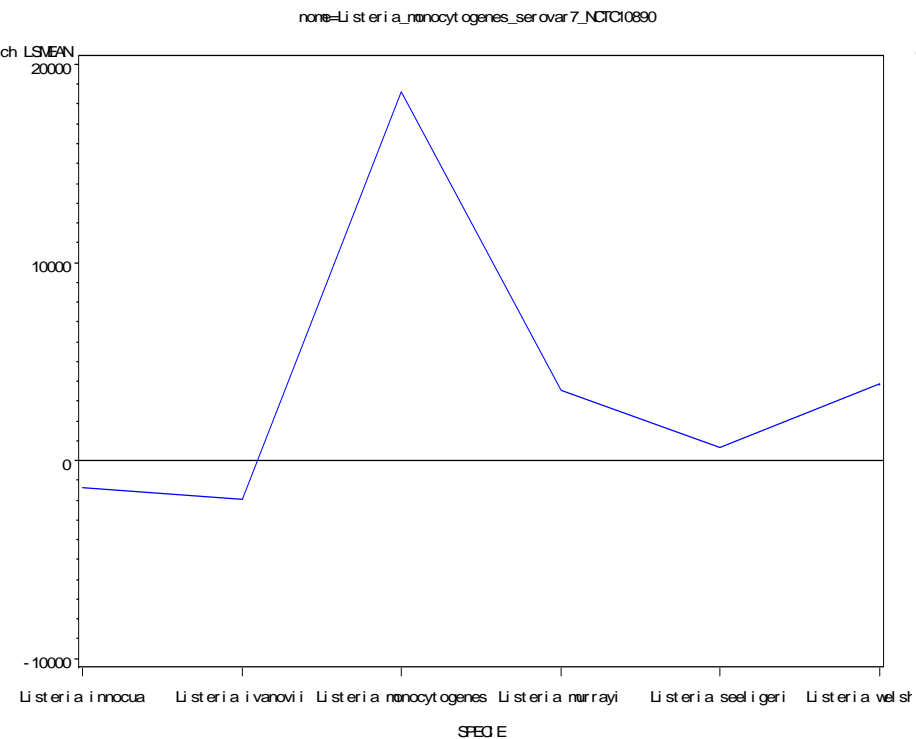
none=*Listeria monocytogenes*_serovar1_2a_NCTC10687



none=*Listeria monocytogenes*_serovar1_2a_NCTC10687_2



Listeria monocytogenes



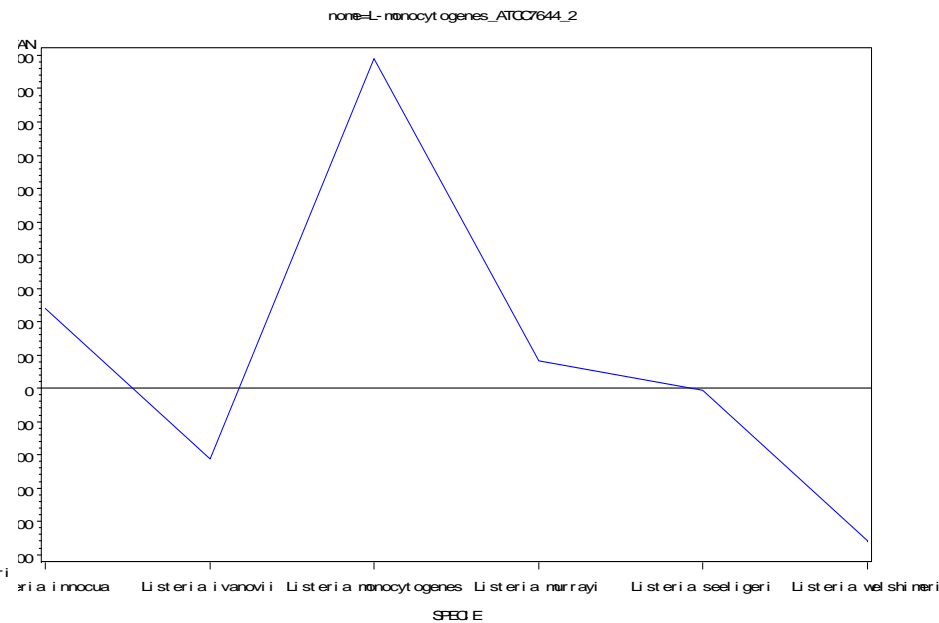
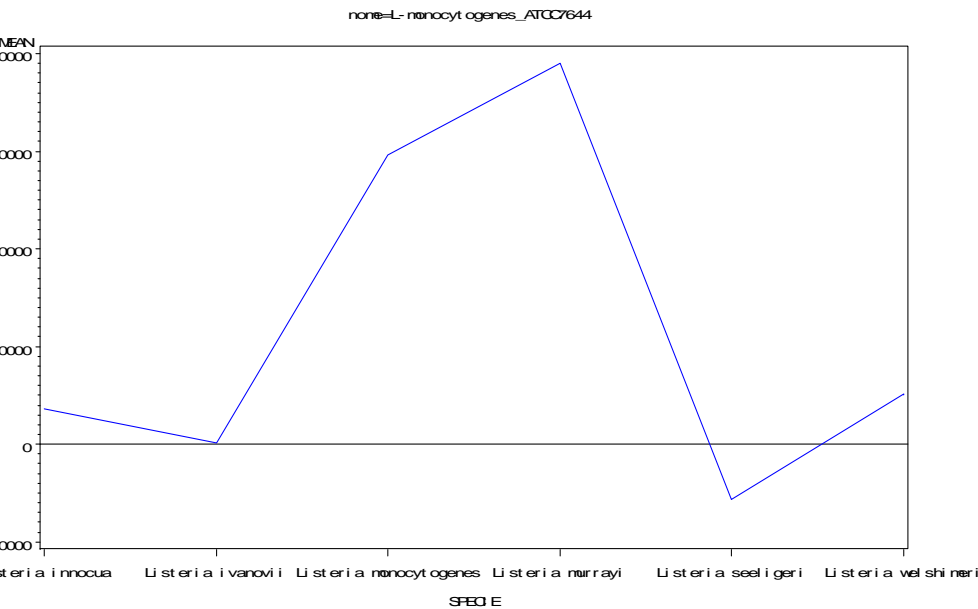
Listeria monocytogenes

for the following samples a low repeatability was found between the array and his duplicate,

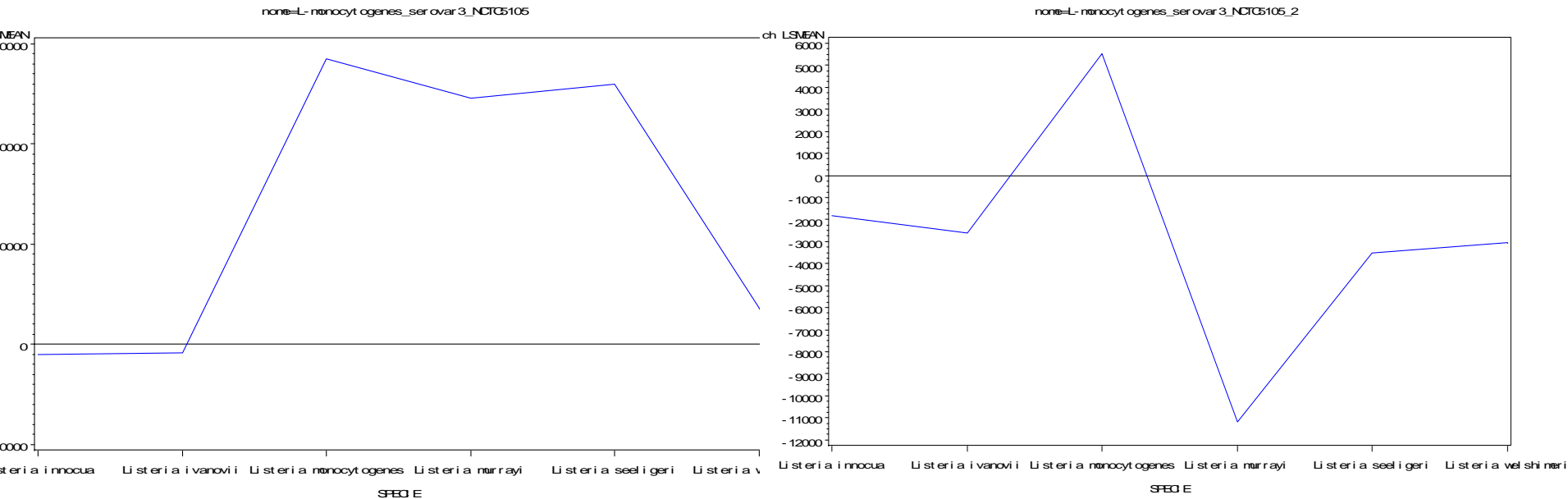
Array	Duplicato
L-monocytogenes_ATCC7644	L-monocytogenes_ATCC7644_2
L-monocytogenes_serovar3_NCTC5105	L-monocytogenes_serovar3_NCTC5105_2
L-monocytogenes_sierotype4_ATCC19115	L-monocytogenes_sierotype4_ATCC19115_2
L-monocytogenes_typeII_ATCC9525	L-monocytogenes_typeII_ATCC9525_2

with a precise response in a case (*L. monocytogenes* is individualized in univocal way) and an ambiguous one in the other

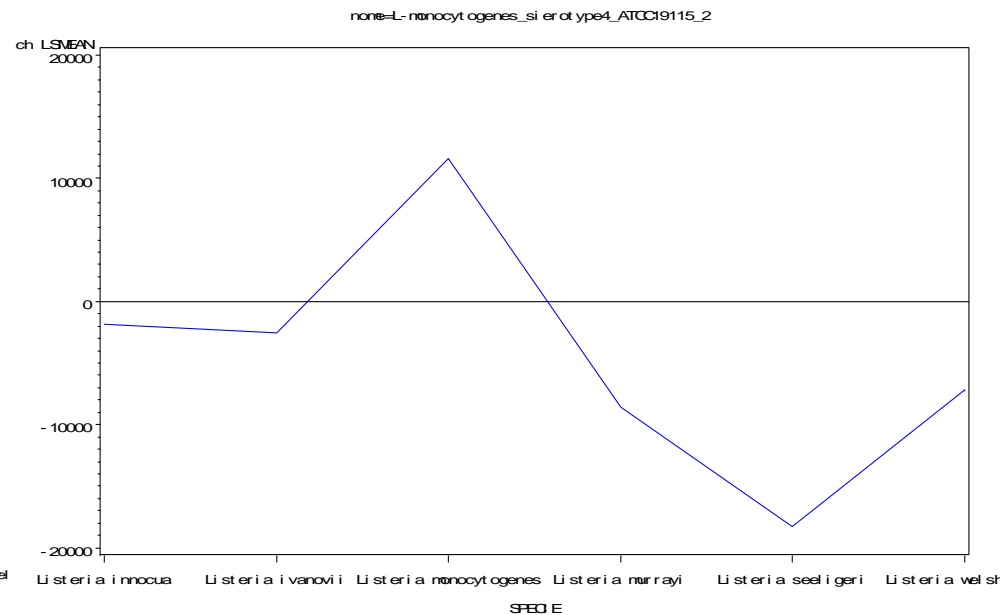
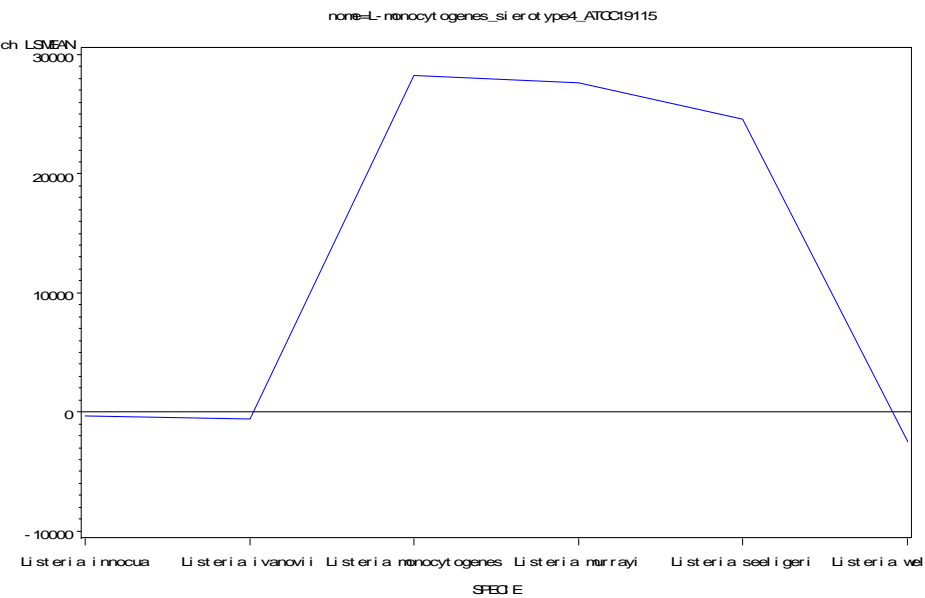
Listeria monocytogenes



Listeria monocytogenes

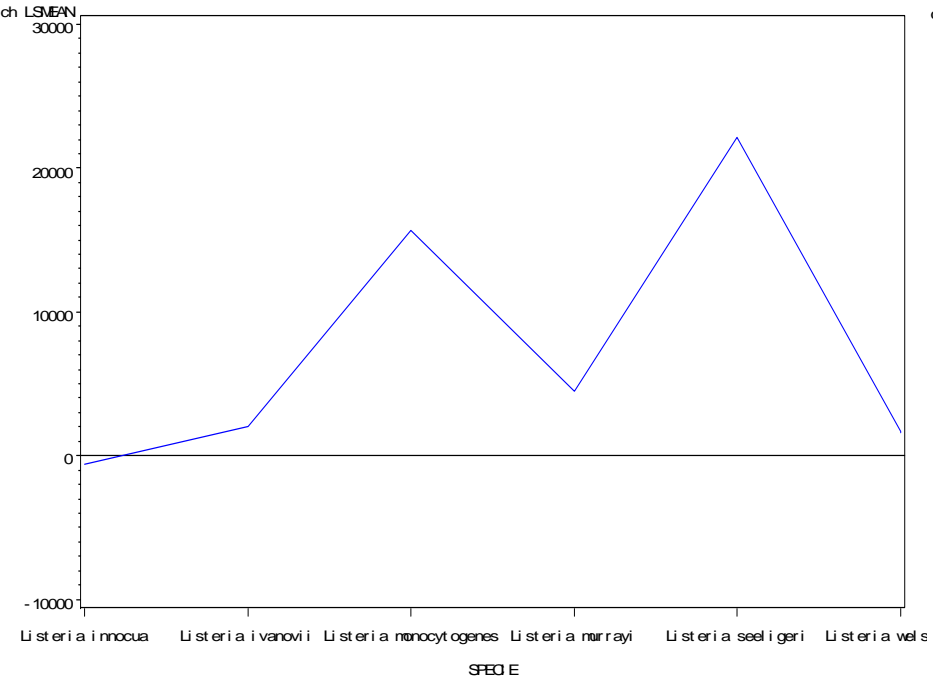


Listeria monocytogenes

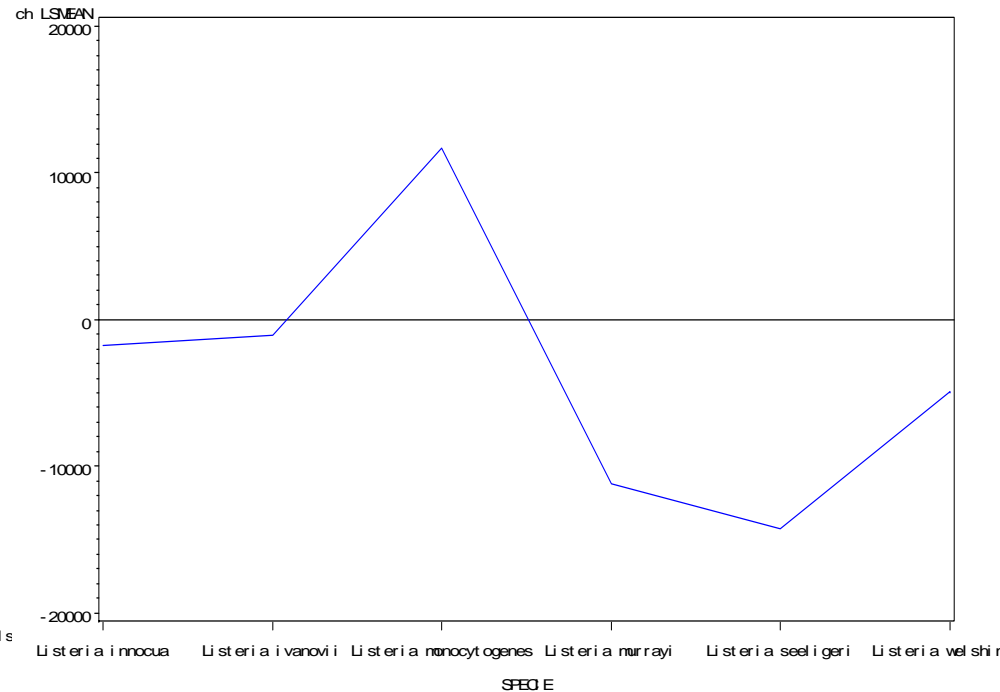


Listeria monocytogenes

none-L-monocytogenes_typel1_ATCC3525

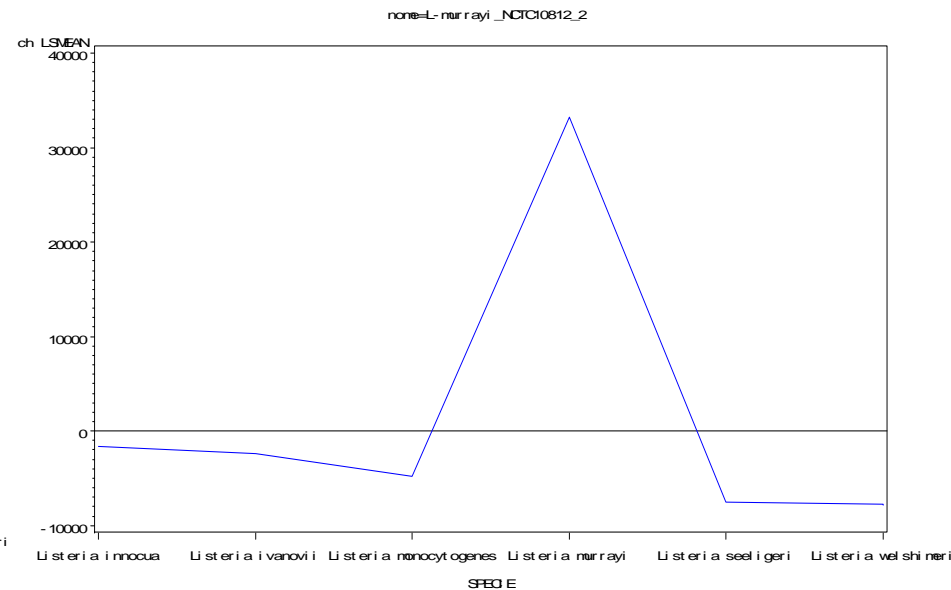
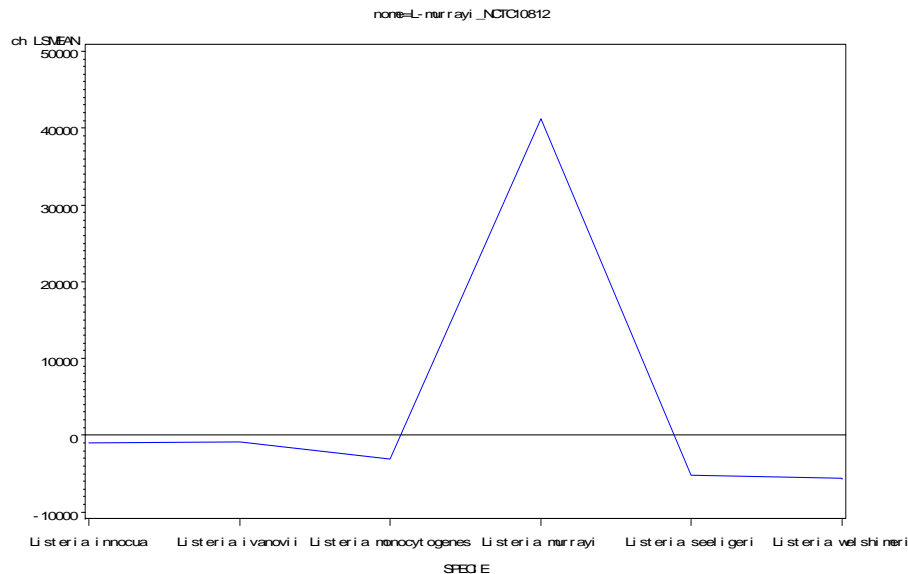


none-L-monocytogenes_typel1_ATCC3525_2



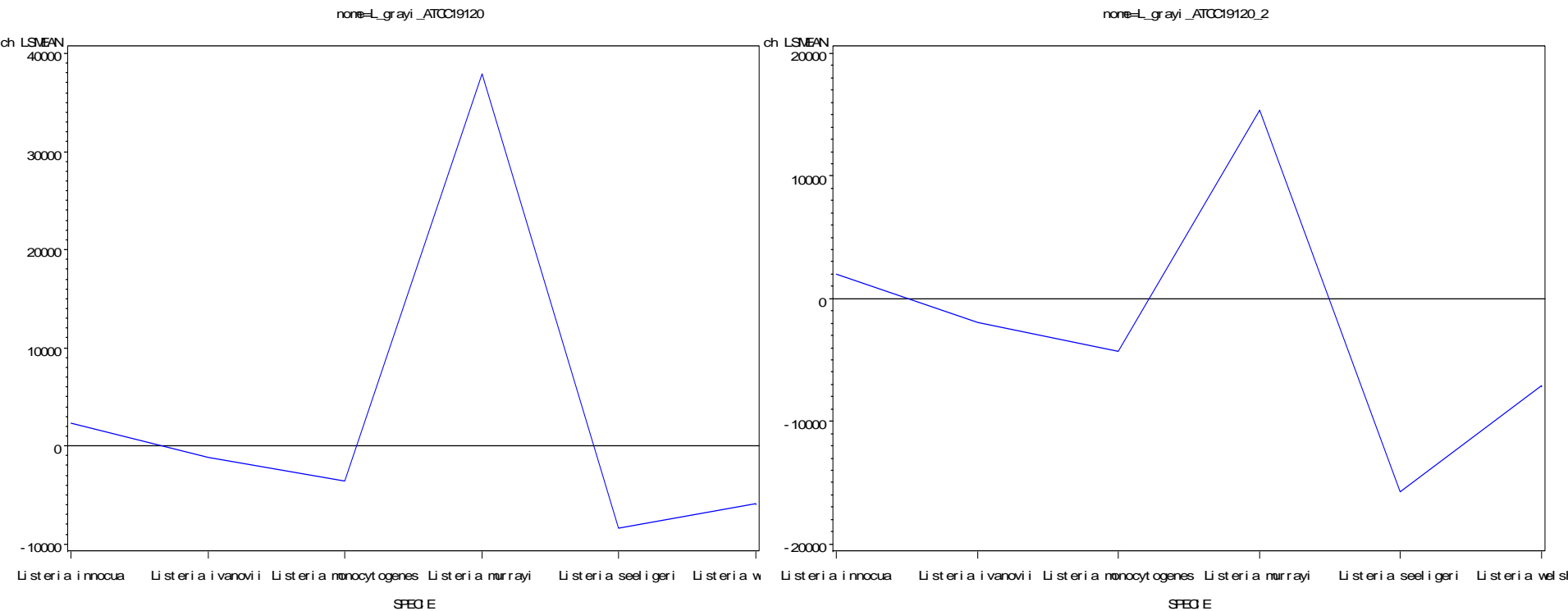
Listeria murrayi

Array	Duplicato
L-murrayi_NCTC10812	L-murrayi_NCTC10812_2
L_grayi_ATCC19120	L_grayi_ATCC19120_2
L_grayi_ssp_grayi_NCTC10815	L_grayi_ssp_grayi_NCTC10815_2



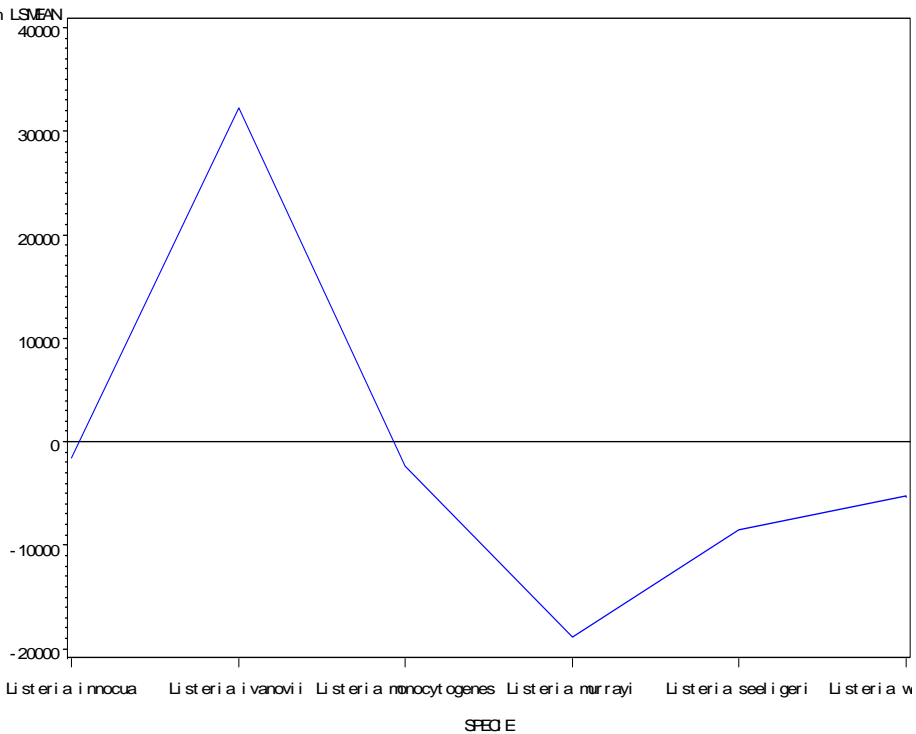
in the case of *L. murrayi* the species is perfectly individualized

Listeria murrayi

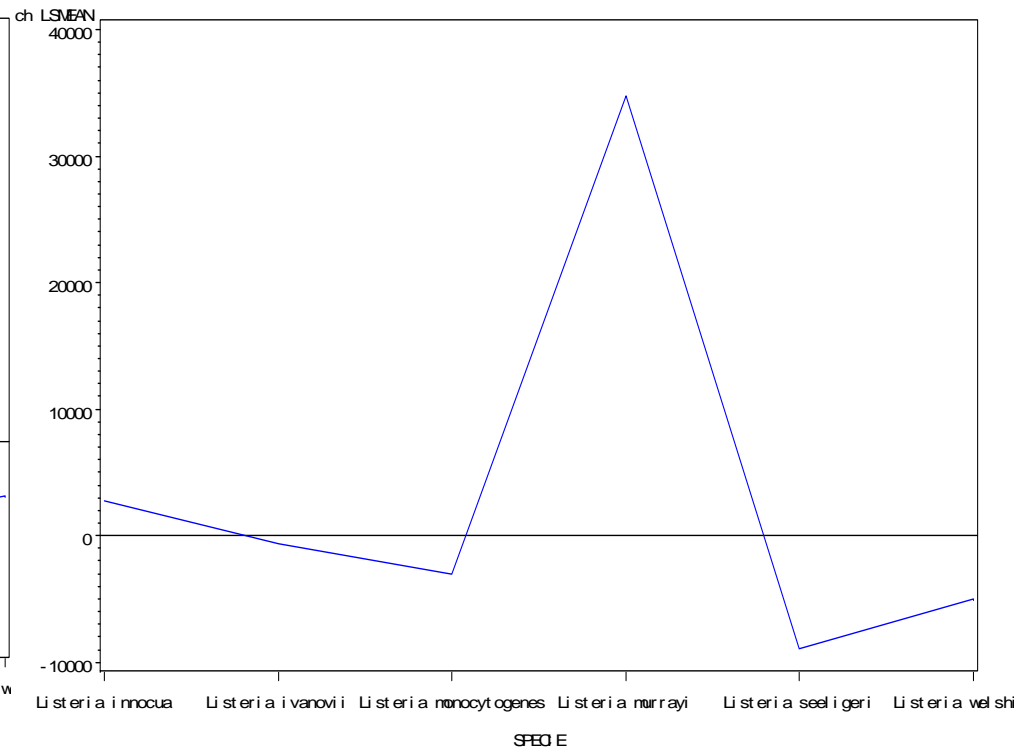


Listeria murrayi

none-*Listeria grayi*_ssp_*grayi*_NCTC10815



none-*Listeria grayi*_ssp_*grayi*_NCTC10815_2



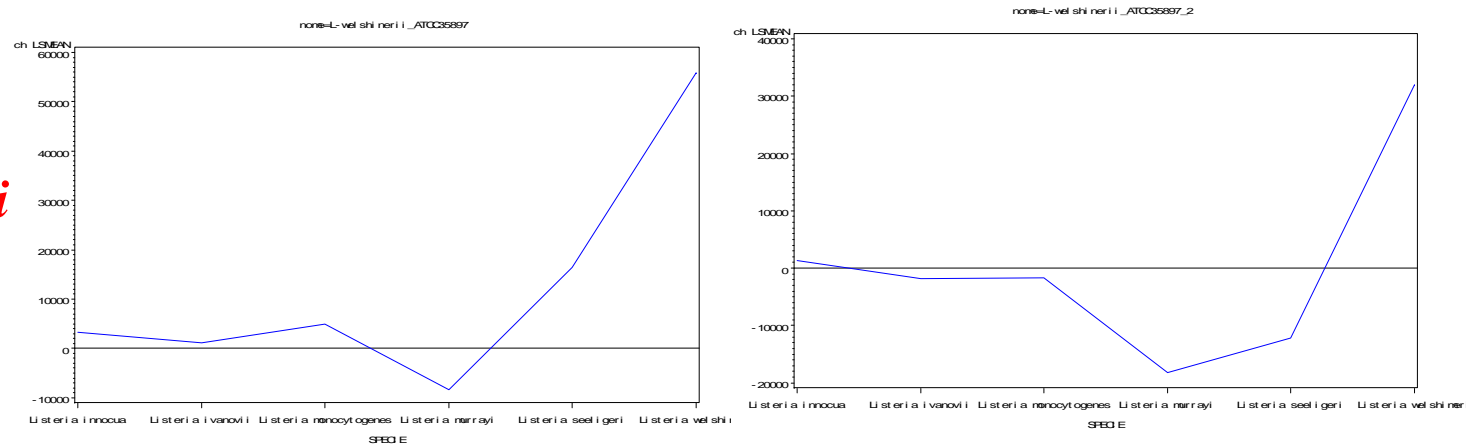
L. ivanovii ?

Hybridization patterns with mono-specific samples

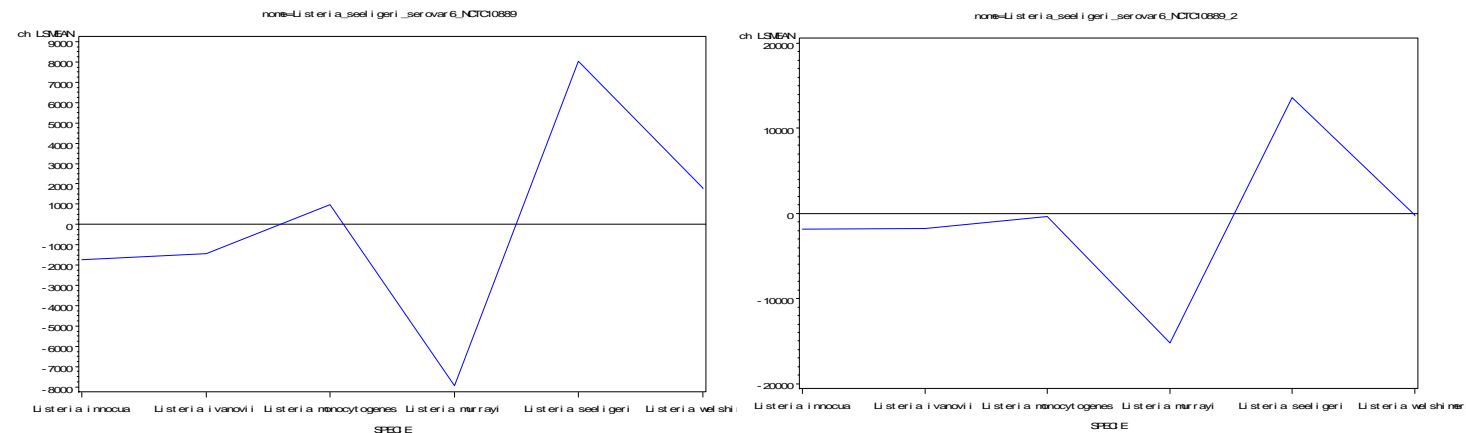
RESULTS

Array	Dupliato
L-welshinerii_ATCC35897	L-welshinerii_ATCC35897_2
L_seeligeri_serovar6_NCTC10889	L_seeligeri_serovar6_NCTC10889_2

Listeria Welshinerii

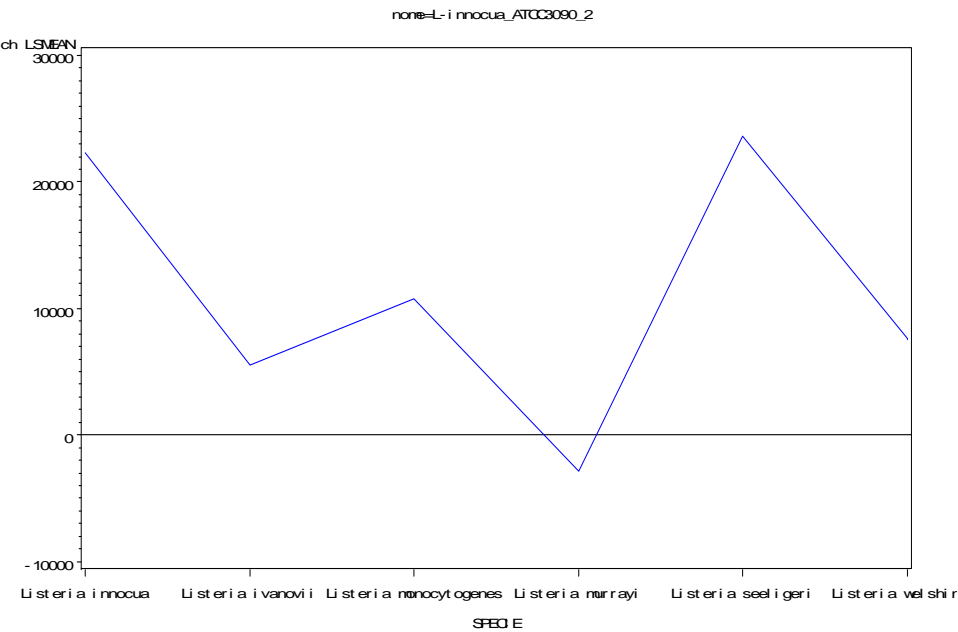


Listeria Seeligeri



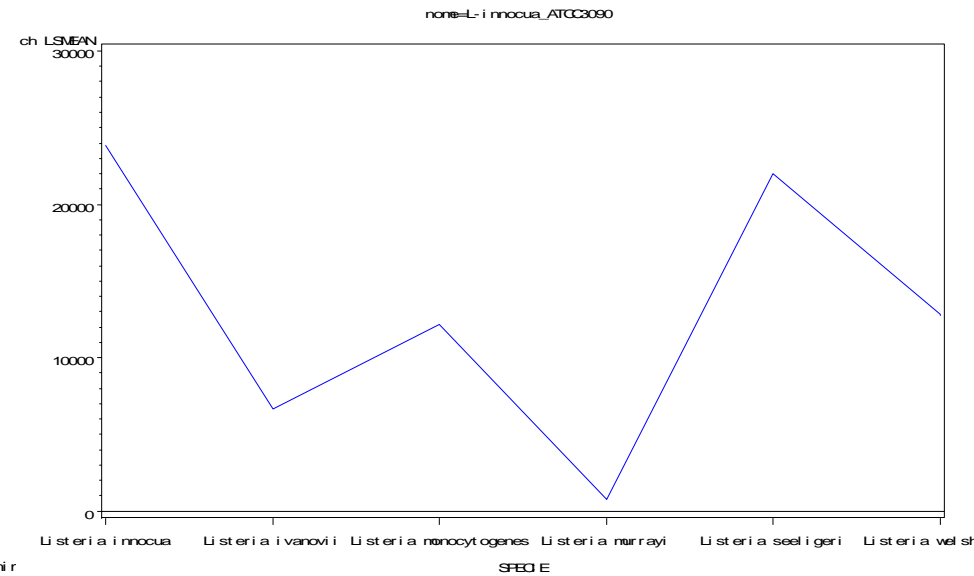
Same results was found for *L. ivanovii*

Listeria innocua



L. innocua

L. seeligerii



Hybridization patterns with mono-specific samples

Hybridization results of the microarray pig-zoonosis V4s with mono-specific samples show

- high discriminating power to level of genus*
- high discriminating power to level of species (limitedly to the *Listeria* sp.)*

We need further examinations to verify if it is possible to discriminate also the organisms with regard to subspecies and serotype level

*further investigations are necessary to establish the response with samples containing more species and/or genus contemporarily
(as meat or environmental samples)*

