TEST OF THE TPA TOXICITY, experiment of the 11/08/15

The toxicity of the TPA was tested. Three LB media were used. Two of it contained 10mM of a mixture of TPANa2+TPAOH and two others one 10mM of Sucrose for the positive control.
The last one was just a classical LB, containing per liter :

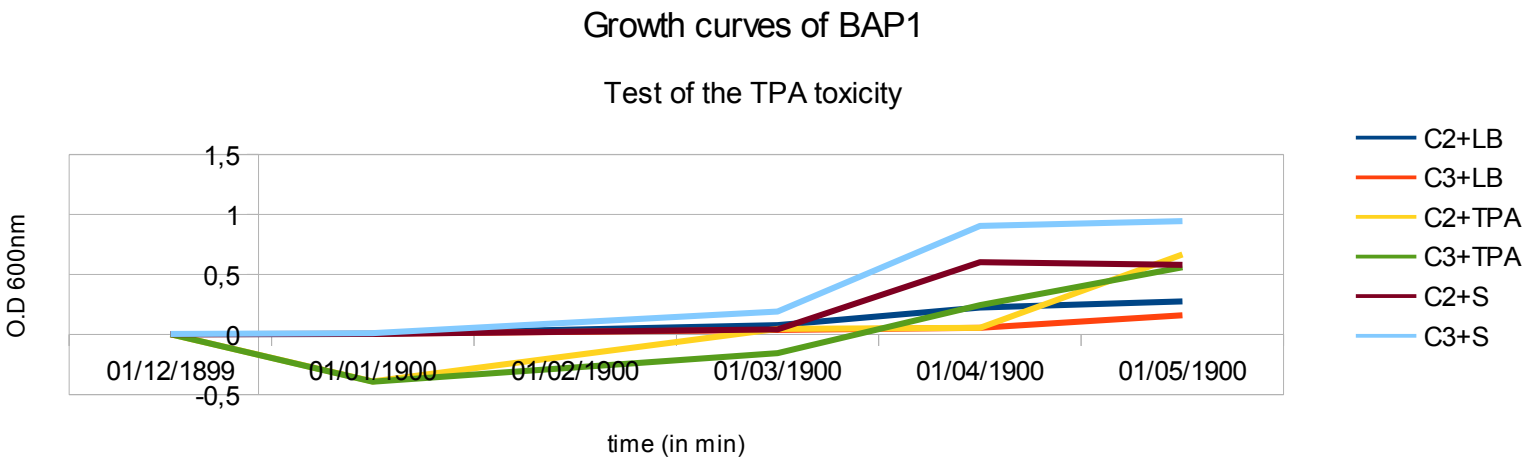
5g of yeast extract
10g of tryptone
5g of NaCl

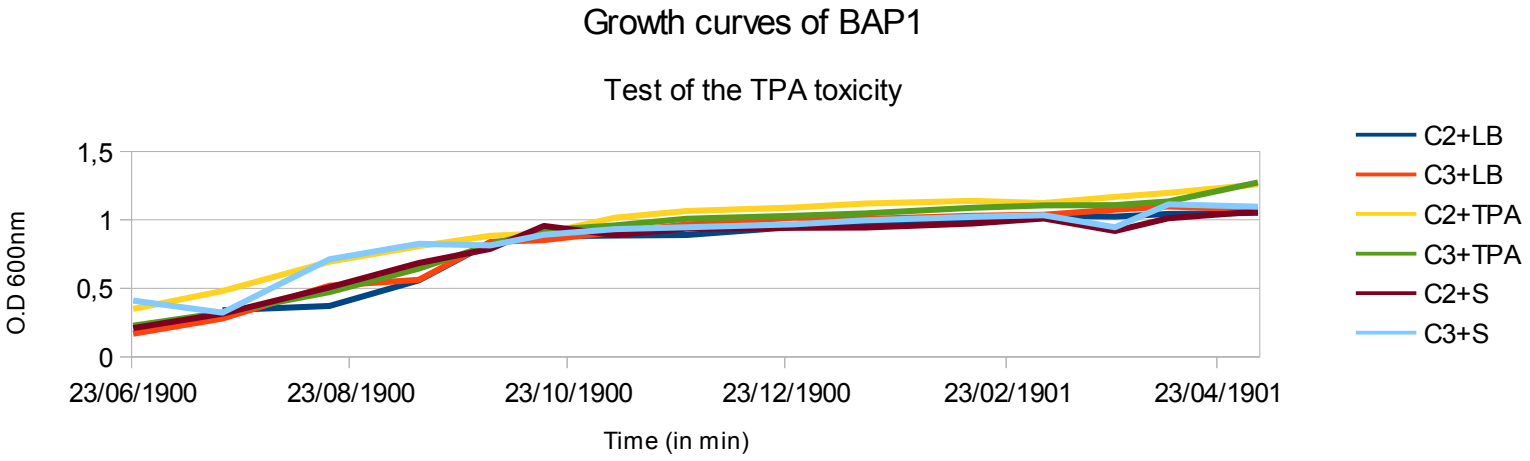
Media were put into FALCON tubes of 15mL and put into incubator at 300rpm and 37°C after being sow with 100uL of BAP1.
The O.D at 600nm was relieved.

Media	LB	LB	TPA	TPA	S	S
Clones	C2	C3	C2	C3	C2	C3
Time (min)	C2+LB	C3+LB	C2+TPA	C3+TPA	C2+S	C3+S
0	0,002	0,002	0,002	0,002	0,003	0,004
30	0,005	0,007	-0,390	-0,393	0,005	0,011
62	0,079	0,038	0,046	-0,156	0,043	0,191
115	0,224	0,056	0,056	0,246	0,602	0,906
130	0,275	0,160	0,667	0,560	0,580	0,944
	The spectrometer was changed					
175	/	0,169	0,351	0,228	0,210	0,410
200	0,340	0,280	0,480	0,323	0,316	0,323
230	0,371	0,519	0,694	0,473	0,507	0,712
255	0,559	0,562	0,808	0,644	0,686	0,825
275	0,836	0,839	0,884	0,819	0,788	0,814
290	0,880	0,853	0,901	0,925	0,956	0,892
310	0,886	0,911	1,016	0,959	0,890	0,932
330	0,891	0,966	1,066	1,009	0,937	0,947
360	0,948	1,017	1,091	1,031	0,942	0,968
380	0,982	1,006	1,120	1,047	0,945	0,997
410	1,000	1,030	1,139	1,089	0,974	1,023
430	1,032	1,037	1,123	1,107	1,011	1,033
450	1,025	1,075	1,169	1,108	0,919	0,947
465	1,044	1,098	1,198	1,135	1,012	1,114
490	1,051	1,078	1,260	1,274	1,061	1,096

For this experiment :
S = sucrose 10mmol/L + LB
TPA = (terephtalic acid disodium + 2-hydroxy-terephtalate) 10mmol/L + LB
LB = LB medium
C2 and C3 are two differents clones of BAP1.

The following growth curves were obtained :





Because of a material problem, the spectrophotometer was changed during the experiment. It is why two graphics are presented here.

The pH at the end of the manipulation was :

LB+C2	7,040
LB+C3	7,200
LB+TPA+C2	8,730
LB+TPA+C3	8,700
LB+S+C2	7,050
LB+S+C3	7,020

Analysis and results :
Clones of BAP1 seem to have the same behavior in the differents conditions.
BAP1 grows in the three media. It reaches the exponential phase at differents moments.
The exponential phase of the growth of BAP1 in a media of LB+Sucrose begins between 40 and 65 min, 80 and 85min in TPA+LB media and 100 and 110min in LB media.

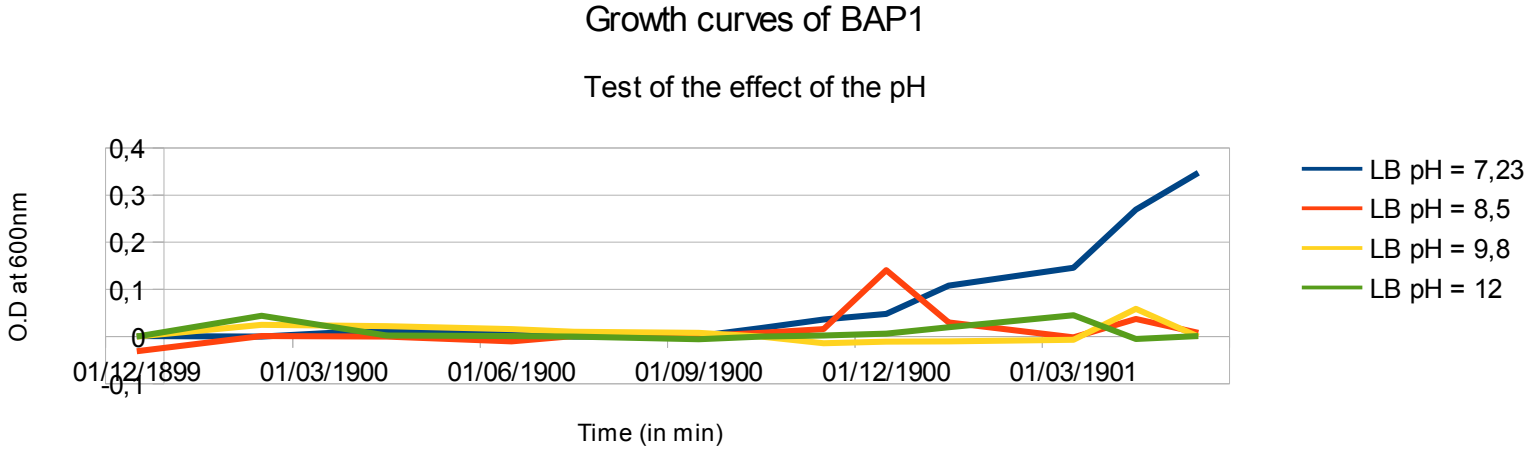
This experiment demonstrates that the TPA was not toxic at this concentration for E.coli BAP1.
The LB+TPANa2+TPAOH appears to be a culture medium for BAP1 more effective than the LB medium.
The TPANa2 or the 2-hydroxy-terephtalate may be some carbone sources for BAP1, or maybe a pH more basic help the growth of BAP1.
The next experiment will give us a beginning of the answer.

Effect of the pH on BAP1

The effect of the pH on the growth of BAP1 in LB was controlled using the O.D at 600nm.
Differents LB medium were used and pH was adjusted by adding more or less NaOH.

Media	LB pH = 7,23	LB pH = 8,5	LB pH = 9,8	LB pH = 12
Time (min)/O.D at 600nm				
0,0000	0,0010	-0,0310	0,0000	0,0000
45,0000	0,0000	0,0010	0,0250	0,0440
120,0000	0,0140	0,0000	0,0220	0,0020
160,0000	0,0060	-0,0100	0,0160	0,0010
200,0000	0,0000	0,0010	0,0100	0,0000
245,0000	0,0010	0,0010	0,0080	-0,0060
275,0000	0,0180	0,0080	0,0000	0,0000
320,0000	0,0360	0,0160	-0,0140	0,0020
355,0000	0,0480	0,1410	-0,0110	0,0060
395,0000	0,1080	0,0300	-0,0100	0,0200
430,0000	0,1460	-0,0020	-0,0070	0,0450
480,0000	0,2690	0,0370	0,0590	-0,0050
505,0000	0,3470	0,0080	0,0000	0,0013

pH at the end of the experiment :	6,5000	10,0000	11,5000	13,0000



Results :
Bap1 grows preferentially in a LB medium where the pH is around 7.
The pH cannot explain the growth of BAP1 in a LB+TPAOH+Na2TPA at pH 8,8.

Test of the toxicity of the TPA and EG on solid medium and creation of a medium of selection.

Effects of TPA and EG on a solid medium of LB : Petri's boxes were used.
The M9 is a medium of selection for E.coli. It was tested here for BAP1.

The constitution of a M9 medium is :

Sodium phosphate	6g
Potassium phosphate	3g
sodium chloride	0,5g
ammonium chloride	1g
AGAR	16g
1M Magnésium sulfate	1mL
0,1M calcium chloride	1mL

Strain used : BAP1 Clone 2 O.D at 600nm before seeding = 0,612

Results of the Petri's boxes :	pH	Concentration of Na+ added	Presence of colony with	Presence of colony diluat	Presence of colony diluat	Presence of colony	Durée
LB	7,04	0	yes	yes	Yes	Yes	24h
LB + TPA 6umol/L	/	0	yes	yes	Yes	Yes	24h
LB + TPANa2+TPAOH 6mmol/L	/	2,75mmol/L	yes	yes	Yes	Yes	24h
LB + TPANa2+TPAOH 12mmol/L	7,83 ~ 8,73	13,75mmol/l	yes	yes	Yes	Yes	24h
LB + TPANa2+TPAOH 20mmol/L	/	27,5 mmol/L	/	/	Yes	Yes	24h
LB + Sucrose 10 mmol/L	7,02	0	yes	yes	Yes	Yes	24h
M9	6,89	0	No	No	No	No	72h
M9 + Sucrose 1,2mmol/L	/	0	No	No	No	No	72h
M9 + Sucrose 6mmol/L	/	0	No	No	No	No	72h
M9 + Sucrose + 12 mmol/L	/	0	No	No	No	No	72h
M9 + TPANa2+TPAOH 1,2mmol/L	7,23	2,75mmol/L	No	No	No	No	72h
M9 + TPANa2 +TPAOH 6mmol/L	7,81	13,75mmol/l	No	No	No	No	72h
M9 + TPANa2+TPAOH 12mmol/L	7,87	27,5 mmol/L	No	No	No	No	72h
M9 + Sucrose + TPANa2+TPAOH	/	2,75mmol/L	No	No	No	No	72h
M9 + Sucrose + TPANa2+TPAOH 6	/	13,75mmol/l	No	No	No	No	72h
M9 + Sucrose + TPANa2+TPAOH 1	/	27,5 mmol/L	No	No	No	No	72h
LB + EG 12mmol/L	/	0	yes	yes	/	/	24h
LB + EG 1,2mmol/L	/	0	yes	yes	/	/	24h
LB + EG 6mmol/L	/	0	yes	yes	/	/	24h
LB + EG 20mmol/L	/	0	yes	yes	/	/	24h

Results and discussions :

The most important part of the media are affected by the TPANa2 + TPAOH, and have a basic pH.
The M9 medium doesn't allow the growth of BAP1. It's not a selective medium.
The TPANa2 +TPAOH becomes toxic for a concentration superior to 12mmol/L. It's possible that only the pH can explain the non-growth of BAP1 for a concentration of TPA of 20mmol/L.
The EG was not toxic for a concentration of 20mmol/L. It's really easy to suppose that it can be used in medium in higher concentration.

Detection of 2-hydroxy-Terephtalate

It's necessary to proove that TPA are solubilised.
So, after the reaction of TPA in water with NaOH and FeSO4, the fluorescence at 480nm was measured for differents excitation waves to find if TPAOH was producted.

Protocole of the TECAN system :

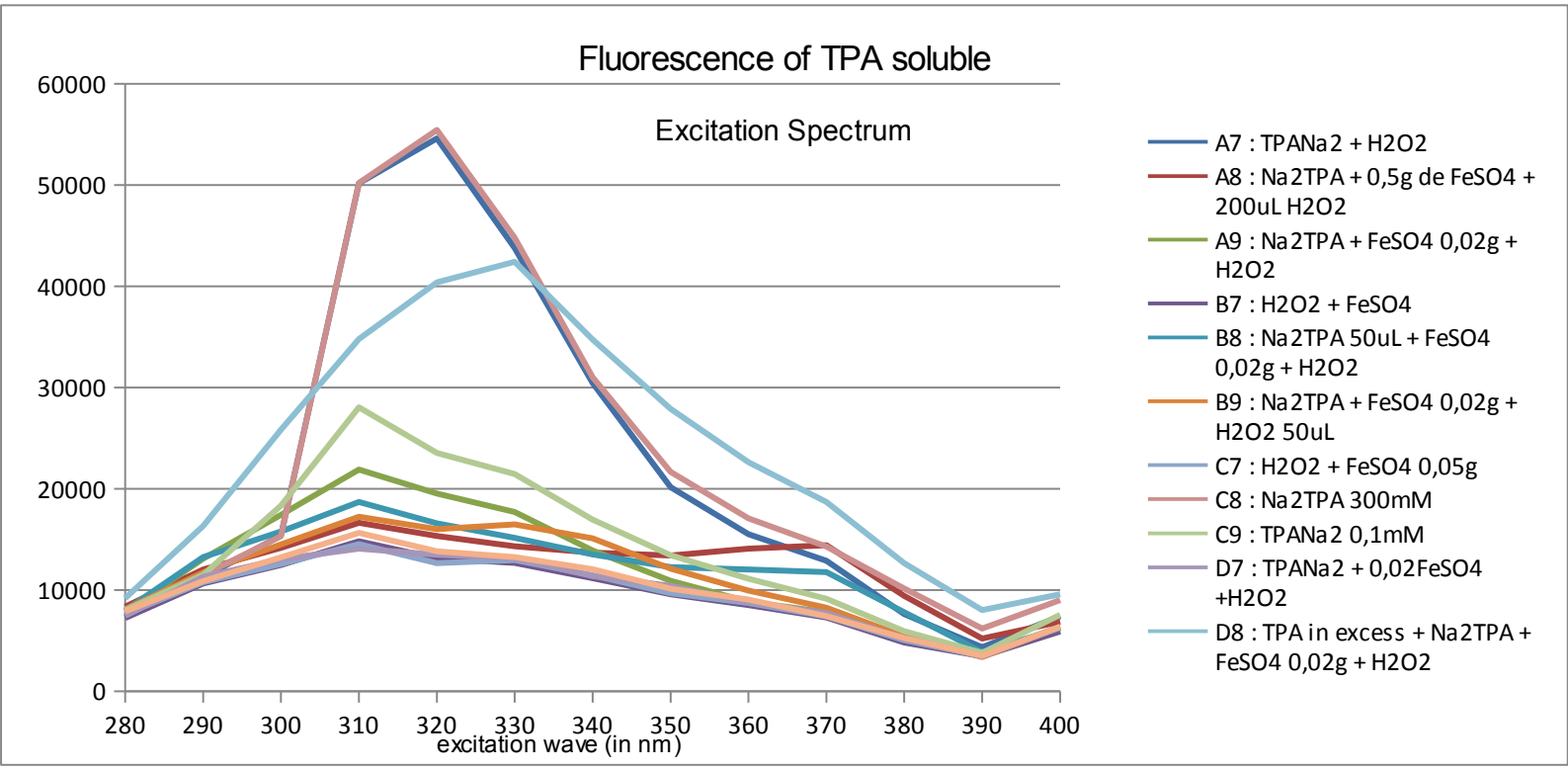
Date:	31/08/2015
Time:	16:18:54

System	AJACCIO-PMM
User	AJACCIO-PMM\Administrateur
Plate	Greiner 96 Flat Bottom Transparent Polystyrol [GRE96ft.pdfx]
Plate-ID (Stacker)	

Shaking (Orbital) Duration:	3 s
Shaking (Orbital) Amplitude:	4 mm

Label: Label1

Mode	Fluorescence Bottom Reading
Excitation Wavelength Start	280 nm
Excitation Wavelength End	400 nm
Excitation Wavelength Step Size	10 nm
Excitation Scan Number	13
Emission Wavelength	430 nm
Bandwidth (Em)	280...850: 20 nm
Bandwidth (Ex) (Range 1)	230...315: 5 nm
Bandwidth (Ex) (Range 2)	316...850: 10 nm
Gain	150 Manual
Number of Flashes	25
Integration Time	20 µs
Lag Time	0 µs
Settle Time	0 ms
Part of Plate	A7-D9
Start Time:	31/08/2015 16:18:58



Results and discussions :

Theses results show that ours solutions contains some TPAOH before adding FeSO4.
The addition of FeSO4 decrease the quantity of TPAOH in our solution. A precipitate was observed.
The soluble TPA contains TPAOH.

Orientation of the reaction of solubilisation of TPA to produce more Na2TPA

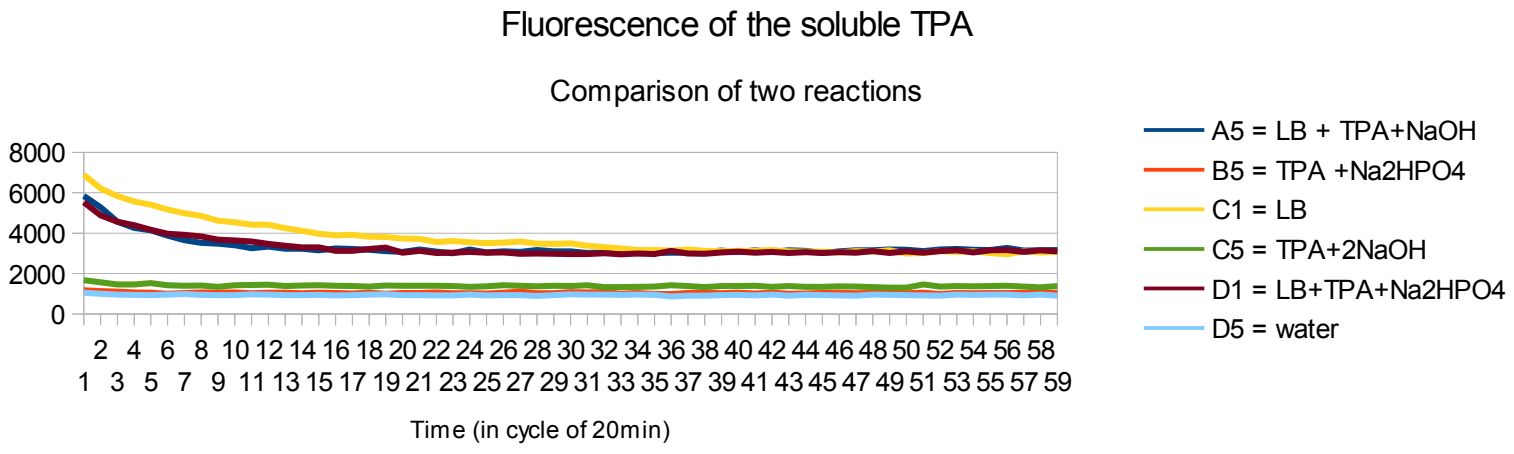
The reaction between the NaOH and the TPA produces TPAOH and Na2TPA, but they is no proof of the existence of Na2TPA except by or reference.
We choose to use Na2HPO4 in place of NaOH.
The fluorescence of the TPAOH was measured like described before.

Protocole of the TECAN :

Application: Tecan i-control	Tecan i-control , 1.10.4.0
Device: infinite 200Pro	Serial number: 1304000213
Firmware: V_3.37_07/12_Infinite (Jul 20 2012/13.56.47)	MAI, V_3.37_07/12_Infinite (Jul 20 2012/13.56.47)
Date: 09/09/2015	
Time: 17:49:57	
System	AJACCIO-PMM
User	AJACCIO-PMM\Administrateur
Plate	Corning 96 Flat Bottom white, clear bottom Polystyrol [COR96fw clear bottom.pdfx]
Plate-ID (Stacker)	

List of actions in this measurement script:	
Kinetic	
Shaking (Orbital) Duration:	60 s
Shaking (Orbital) Amplitude:	4 mm
Fluorescence	
Shaking (Orbital) Duration:	600 s
Shaking (Orbital) Amplitude:	4 mm

2 Labels	
Kinetic Measurement	
Kinetic duration	20:01:00
Interval Time	00:20:00
Mode	Fluorescence Bottom Reading
Excitation Wavelength	310 nm
Emission Wavelength	430 nm
Excitation Bandwidth	5 nm
Emission Bandwidth	20 nm
Gain	150 Manual
Number of Flashes	25
Integration Time	20 µs
Lag Time	0 µs
Settle Time	0 ms
Part of Plate	A1-D5
Start Time: 09/09/2015 17:49:57	



Results :

The LB doesn't allow the measurement of the fluorescence of the soluble TPA because of it own fluorescence.

The reaction between the TPA and the Na2HPO4, by comparison with the reaction between the TPA and the NaOH, produces less TPAOH.

So, we can conclude that more Na2TPA must be produced because the HPO4 doesn't react with the TPA.

Test of the efficacity of the M9 medium with vitamins and rare salts as a selective medium.

Here, we test the capacity of the M9 with vitamin medium to be a selective medium. The aim is to proove that the soluble TPA is, or not, a carbon source for BAP1.

Media	Time (in hours)	O.D at 600nm	Strains	pH at the end of the expe
M9 without sugar et without bacteri	60h	0	BAP1	7
M9 without sugar	60h	0,3	BAP1	7
M9 + EG 10mmol/L	60h	0,25	BAP1	7
M9 + TPA 10 mmol/L	60h	0,05	BAP1	8,6
M9 + sucrose 10mmol/L	60h	0,35	BAP1	7
M9 + sucrose 120mmol/L	60h	0,6	BAP1	7

Results :
The cultures grow in the medium but also with no carbon source. That means that our medium contains, because of the Supradyn, some carbon sources.
The ethylene glycol decreases a little bit the number of bacteria but it isn't toxic at this concentration. It isn't a carbon source.
The TPANa2+NaOH was not used as a carbon source, but the pH wasn't the same that the others media, so it's not prooved that it is toxic in M9 media.
The sucrose increases the O.D at 600nm. BAP1 can use this simple source of carbon.

Because of thoses results, a medium was created to avoid all traces of sugar, insipiring of the experiment of Pfeiffer.

Pfeiffer Medium composition :			
M traces 100X		M9 salts 4X :	
sodium molybdène	0,015g/L	Na2HPO4	28g/L
manganèse chloride	0,002g/L	KH2PO4	12g/L
zinc sulfate	0,175g/L	NaCl	2g/L
cuivre sulfate	0,013g/L	NH4Cl	4g/L
cobalt nitrate	0,013g/L	0,1M CaCl2	1mL
acide borique	0,012g/L	0,2% B1/Thiamin	1mL

The two part of the medium were filtred by a 0,22um MILIPORE
Some new media were realised with differents carbon sources like soluble TPA and EG.
A control of the fluorescence in the medium was used to see if the TPAOH was used as a carbon source by BAP1.
A measurement of the O.D at 600nm was used to control the growth or not of our bacteria in the new M9 with only rare salts.

Protocole of the TECAN system :

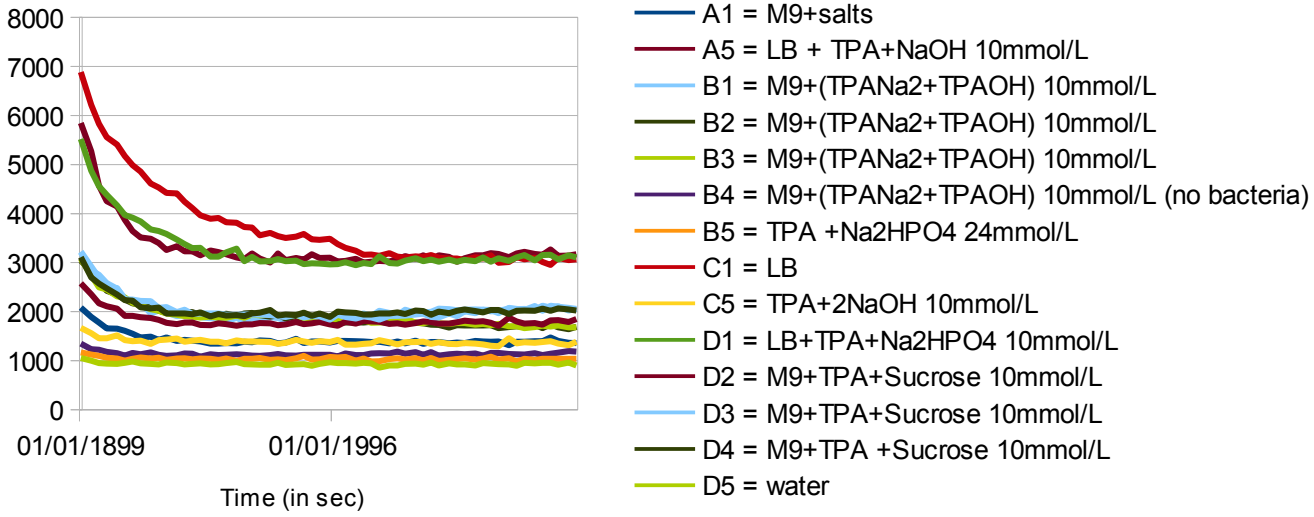
Application: Tecan i-control	Tecan i-control , 1.10.4.0
Device: infinite 200Pro	Serial number: 1304000213
Firmware: V_3.37_07/12_Infinite (Jul 20 2012/13.56.47)	MAI, V_3.37_07/12_Infinite (Jul 20 2012/13.56.47)

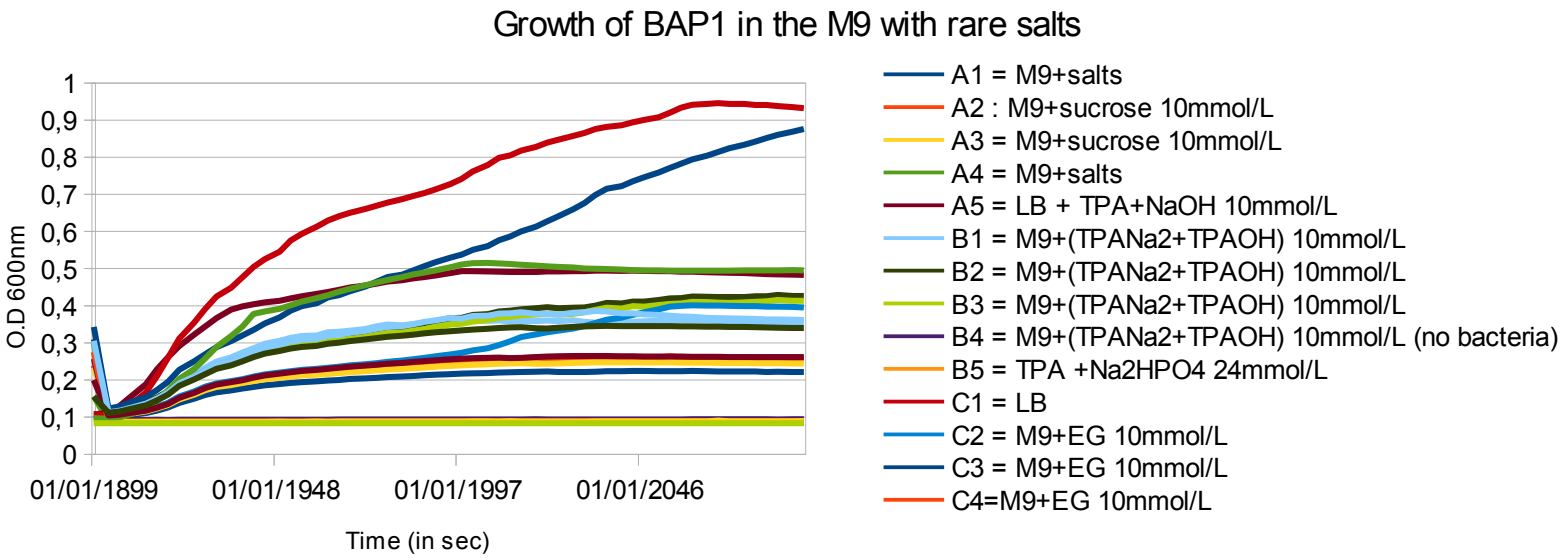
Date:	09/09/2015
Time:	17:49:57

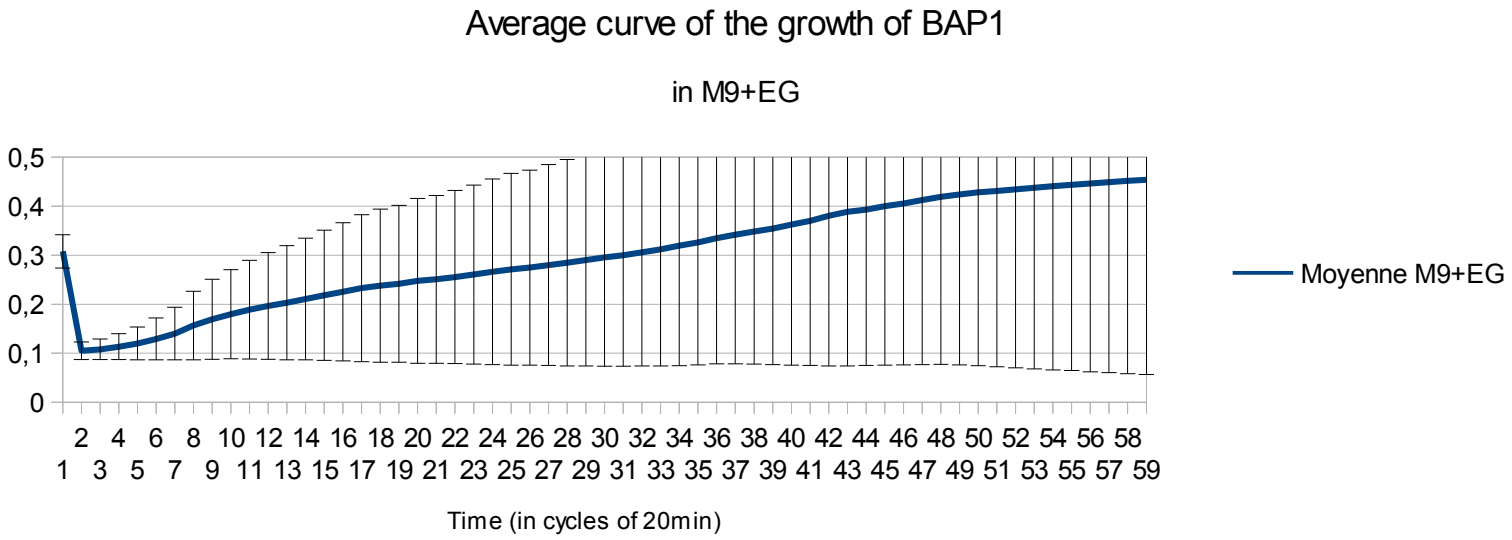
System	AJACCIO-PMM
User	AJACCIO-PMM\Administrateur
Plate	Corning 96 Flat Bottom white, clear bottom Polystyrol [COR96fw clear bottom.pdfx]
Plate-ID (Stacker)	

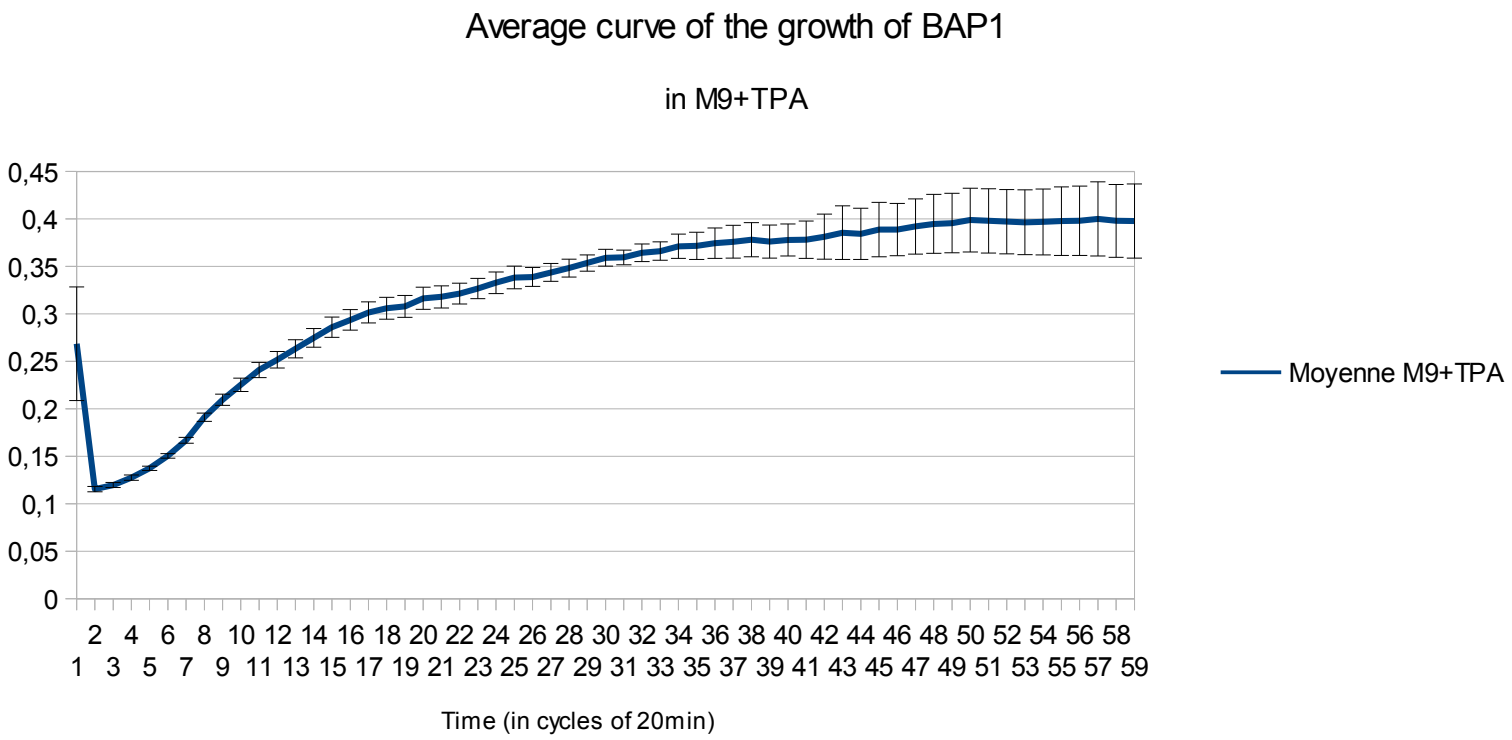
List of actions in this measurement script:	
Kinetic	
Shaking (Orbital) Duration:	60 s
Shaking (Orbital) Amplitude:	4 mm
Absorbance	
Fluorescence	
Shaking (Orbital) Duration:	600 s
Shaking (Orbital) Amplitude:	4 mm

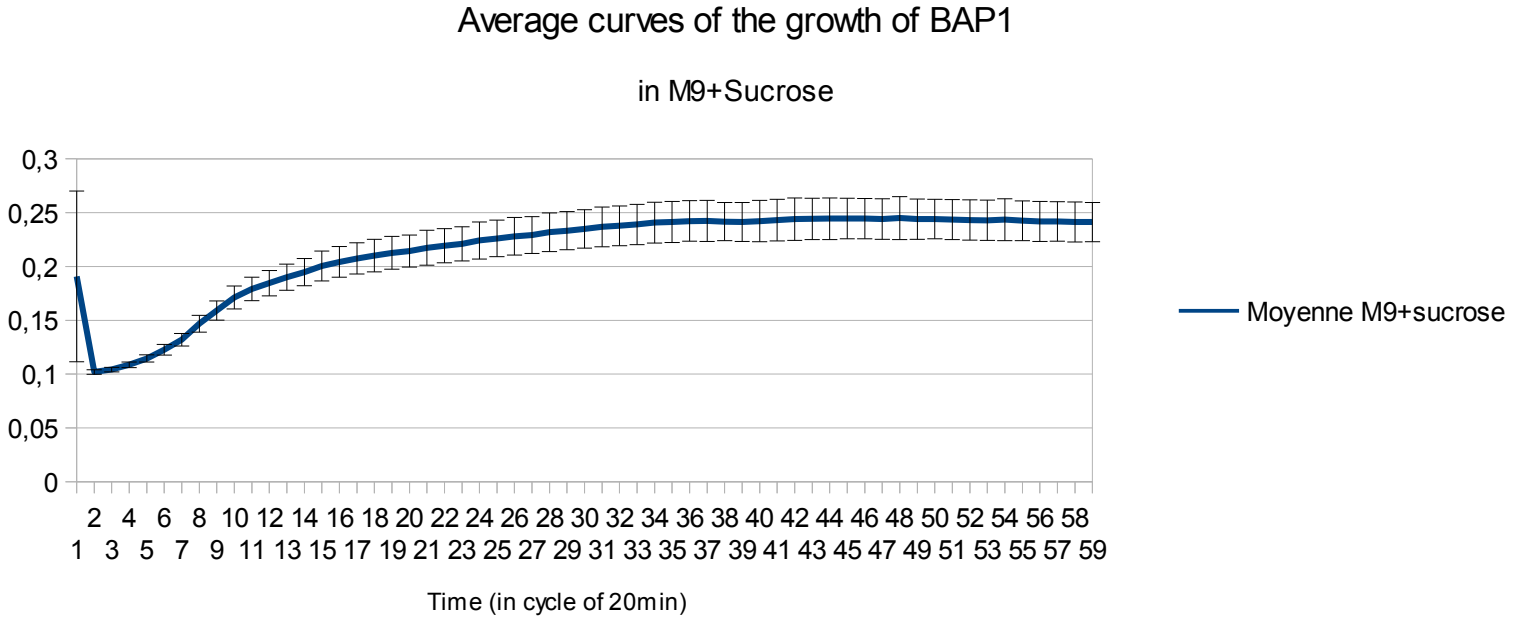
2 Labels		
Kinetic Measurement		
Kinetic duration		20:01:00
Interval Time		00:20:00
Mode		Absorbance
Wavelength		600 nm
Bandwidth		9 nm
Number of Flashes		25
Settle Time		0 ms
Mode		Fluorescence Bottom Reading
Excitation Wavelength		310 nm
Emission Wavelength		430 nm
Excitation Bandwidth		5 nm
Emission Bandwidth		20 nm
Gain		150 Manual
Number of Flashes		25
Integration Time		20 µs
Lag Time		0 µs
Settle Time		0 ms
Part of Plate		A1-D5
Start Time:	09/09/2015 17:49:57	




Growth of BAP1 in the M9 with rare salts
O.D 600nm
1
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
01/01/1899
01/01/1948
01/01/1997
01/01/2046
Time (in sec)
A1 = M9+salts
A2 : M9+sucrose 10mmol/L
A3 = M9+sucrose 10mmol/L
A4 = M9+salts
A5 = LB + TPA+NaOH 10mmol/L
B1 = M9+(TPANa2+TPAOH) 10mmol/L
B2 = M9+(TPANa2+TPAOH) 10mmol/L
B3 = M9+(TPANa2+TPAOH) 10mmol/L
B4 = M9+(TPANa2+TPAOH) 10mmol/L (no bacteria)
B5 = TPA +Na2HPO4 24mmol/L
C1 = LB
C2 = M9+EG 10mmol/L
C3 = M9+EG 10mmol/L
C4=M9+EG 10mmol/L


Average curve of the growth of BAP1
in M9+EG
0,5
0,4
0,3
0,2
0,1
0
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50 52 54 56 58
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59
Time (in cycles of 20min)
Moyenne M9+EG


Average curve of the growth of BAP1
in M9+TPA
0,45
0,4
0,35
0,3
0,25
0,2
0,15
0,1
0,05
0
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50 52 54 56 58
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59
Time (in cycles of 20min)
Moyenne M9+TPA



Results and discussions :

The fluorescent experiment shows a bletching. It was caused by the autofluorescence of the media.
The TPAOH was not consommed by BAP1 because the fluorescence don't decrease after the bletching.

The fluorescence experiment demonstrates the impossibility to measure it in LB.
We found again a better fluorescence at 430nm for the soluble TPA from the reaction between TPA and NaOH. So, it contains more TPAOH.
The TPAOH was not used as a carbon source in our differents media because the fluorescence don't decrease after the bletching.
In comparison with the M9 with rare salts but no carbon source, BAP1 grow in a medium with soluble TPA as a sole source of carbon.
It's not the TPAOH that is consummed by BAP1, so it's the Na2TPA which is used by thoses bacteria.