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Supplement of

Technical note: Flow cytometry assays for the detection, counting and cell sorting of polyphosphate-accumulating bacteria

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Table S1 : Control of potential interference between SYTO®62 fluorochrome and isotonic buffers (HEPES, PBS, McIlvaine) in the absence of cells.

For each condition, three replicates were performed and the number of events counted by flow cytometry was recorded. The mean and SD are the average values between replicates and the standard deviation of the mean, respectively.

Buffer	replicate	Number of events	Mean	SD
HEPES	R1	5	7	2
	R2	9		
	R3	7		
PBS	R1	2	1	1
	R2	0		
	R3	1		
McIlvaine	R1	2	1	1
	R2	1		
	R3	0		

Table S2 : Control of potential interference between DAPI fluorochrome and isotonic buffers (HEPES, PBS, McIlvaine) in the absence of cells.

For each condition, three replicates were performed and the number of events counted by flow cytometry was recorded. The mean and SD are the average values between replicates and the standard deviation of the mean, respectively.

Buffer	replicate	Number of events	Mean	SD
HEPES	R1	0	0.7	1.2
	R2	0		
	R3	2		
PBS	R1	6	2.3	3.2
	R2	1		
	R3	0		
McIlvaine	R1	5204	4055.7	2885.3
	R2	6190		
	R3	773		

Table S3 : Staining buffer (HEPES, PBS) and labelling performance of SYTO®62 fluorochrome.

For each condition and for each strain (the Gram negative RX and the Gram-positive TE (*T. elongata*)), three replicates were performed and the number of events counted by FCM was recorded. The mean and SD are the average values between replicates and the standard deviation of the mean, respectively.

Strain	Buffer	replicate	Number of events	Mean	SD
TE	HEPES	R1	4515	4512.7	133.5
		R2	4645		
		R3	4378		
	PBS	R1	3687	3593.0	89.8
		R2	3508		
		R3	3584		
RX	HEPES	R1	4837	4779.3	50.7
		R2	4742		
		R3	4759		
	PBS	R1	4429	4427.0	7.2
		R2	4433		
		R3	4419		

Table S4 : Flow cytometry analysis of staining buffer (HEPES, PBS) versus labelling performance of DAPI fluorochrome for the strain *T. elongata*.
For each condition, three replicates were performed. The total number of cells (Total cells) counted by FCM using the fluorescence of SYTO®62 was recorded. The number of polyP+ cells (i.e. positive green fluorescence signal regarding the fluorescence intensity limit defined by the controls) as well as the polyP- cells (i.e. negative green fluorescence signal regarding the fluorescence intensity limit defined by the controls) were counted by FCM using DAPI-green fluorescence. PolyP+ and polyP- cells were expressed as proportion (in %) of total cells. The mean and SD are the average values of the proportion of polyP+ cells between replicates and the standard deviation of the mean, respectively.

Buffer	Replicate N°	Total cells	Cell polyP+	% cell polyP+	Cell polyP-	% cell polyP-	Mean % cell polyP+	SD % cell polyP+
HEPES	R1	16946	14796	87.3	2150	12.69	87.5	0.1
	R2	17998	15758	87.6	2240	12.45		
	R3	18040	15800	87.6	2240	12.42		
PBS	R1	10014	8225	82.1	1789	17.86	82.3	0.2
	R2	11351	9349	82.4	2002	17.64		
	R3	9822	8098	82.4	1724	17.55		

Table S5 : Epifluorescence microscopy of polyP+ cell counts after DAPI labelling for the *T. elongata* strain.

For each condition, three replicates were performed. For each sample, 500 cells were counted. The number of polyP+ cells were counted using DAPI-green fluorescence. PolyP+ cells were expressed as proportion (in %) of total cells. The mean and SD are the average values of the proportion of polyP+ cells between replicates and the standard deviation of the mean, respectively.

Buffer	Replicate N°	Number of cell count/sample	Cell polyP+	% cell polyP+	Mean % cell polyP+	SD % cell polyP+
HEPES	R1	500	478	95.6	92.5	2.9
	R2	500	450	90.0		
	R3	500	459	91.8		
PBS	R1	500	430	86.0	87.2	5.5
	R2	500	412	82,4		
	R3	500	466	93,2		

Table S6: Comparisons of the proportions of polyP+ cells counted by FCM or epifluorescence microscopy (Epi) for the *T. elongata* strain, as a function of buffer (HEPES or PBS).

After Shapiro Wilk normality and Brown-Forsythe homoscedasticity tests, the similarities between treatments were evaluated using a one-way ANOVA, with Tukey's post-hoc test to make multiple comparisons between the groups. A p value of less than 0.05 ($p < 0.05$) was considered as statistically significant. ns: not significant, * $p < 0.05$.

Comparison test	Summary	Adjusted p Value
HEPES FCM vs. PBS FCM	ns	0.2463
HEPES FCM vs. HEPES Epi	ns	0.2775
HEPES FCM vs. PBS Epi	ns	0.9994
PBS FCM vs. HEPES Epi	*	0.0163
PBS FCM vs. PBS Epi	ns	0.287
HEPES Epi vs. PBS Epi	ns	0.2379

Table S7 : Flow cytometry analysis of staining buffer (HEPES, PBS) versus labelling performance of DAPI fluorochrome for the strain RX.
For each condition, three replicates were performed. The total number of cells (Total cells) counted by FCM using the fluorescence of SYTO®62 was recorded. The number of polyP+ cells (i.e. positive green fluorescence signal regarding the fluorescence intensity limit defined by the controls) as well as the polyP- cells (i.e. negative green fluorescence signal regarding the fluorescence intensity limit defined by the controls) were counted by FCM using DAPI-green fluorescence. PolyP+ and polyP- cells were expressed as proportion (in %) of total cells. The mean and SD are the average values of the proportion of polyP+ cells between replicates and the standard deviation of the mean, respectively.

Buffer	Replicate N°	Total cells	Cell polyP+	% cell polyP+	Cell polyP-	% cell polyP-	Mean % cell polyP+	SD % cell polyP+
HEPES	R1	34258	34230	99.9	28	0.1	99.9	0.0
	R2	60437	60372	99.9	65	0.1		
	R3	67443	67382	99.9	61	0.1		
PBS	R1	23428	2103	9	21325	90.9	7.2	1.7
	R2	12787	716	5.6	12071	94.3		
	R3	37143	2601	7	34542	92.9		

Table S8 : Epifluorescence microscopy of polyP+ cell counts after DAPI labelling for the RX strain.

For each condition, three replicates were performed. For each sample, 500 cells were counted. The number of polyP+ cells were counted using DAPI-green fluorescence. PolyP+ cells were expressed as proportion (in %) of total cells. The mean and SD are the average values of the proportion of polyP+ cells between replicates and the standard deviation of the mean, respectively.

Buffer	Replicate N°	Number of cell count/sample	Cell polyP+	% cell polyP+	Mean % cell polyP+	SD % cell polyP+
HEPES	R1	500	10	2.0	1.9	1.0
	R2	500	14	2.8		
	R3	500	4	0.8		
PBS	R1	500	2	0.4	0.9	0.5
	R2	500	6	1.2		
	R3	500	6	1.2		

Table S9: Comparisons of the proportions of polyP+ cells counted by FCM or epifluorescence microscopy (Epi) for the RX strain, as a function of buffer (HEPES or PBS).

After Shapiro Wilk normality and Brown-Forsythe homoscedasticity tests, the similarities between treatments were evaluated using a one-way ANOVA, with Tukey's post-hoc test to make multiple comparisons between the groups. A *p* value of less than 0.05 ($p < 0.05$) was considered as statistically significant. ns: not significant, ***$p < 0.0005$, and ****$p < 0.0001$.

Comparison test	Summary	Adjusted P Value
HEPES FCM vs. PBS FCM	****	<0.0001
HEPES FCM vs. HEPES Epi	****	<0.0001
HEPES FCM vs. PBS Epi	****	<0.0001
PBS FCM vs. HEPES Epi	***	0.0009
PBS FCM vs. PBS Epi	***	0.0003
HEPES Epi vs. PBS Epi	ns	0.6869

Table S10 : Number of cells counted by FCM after SYTO®62 labelling according to whether or not treated with a detergent, Triton X100, for *T. elongata* (TE) and RX culture strains.

For each condition, three replicates were performed. The total number of cells counted by FCM using the fluorescence of SYTO®62 was recorded. The mean and SD are the average values of the number of cells between replicates and the standard deviation of the mean, respectively.

Strain	Buffer	replicate N°	Triton X100	Number of cells	Mean	SD
TE	PBS	1	0 %	80751	77583	9165
TE	PBS	2	0 %	84744		
TE	PBS	3	0 %	67254		
TE	PBS	1	0.3 %	46794	43538	3000
TE	PBS	2	0.3 %	42933		
TE	PBS	3	0.3 %	40887		
RX	PBS	1	0 %	17289	17423	202
RX	PBS	2	0 %	17655		
RX	PBS	3	0 %	17325		
RX	PBS	1	0.3 %	7002	6496	440
RX	PBS	2	0.3 %	6278		
RX	PBS	3	0.3 %	6208		

Table S11 : Effect of 2 % or 4 % formaldehyde fixation on the FCM count of total cells (after SYTO®62 labelling) and proportion of polyP+ cells (after DAPI labelling) of the RX strain as a function of storage temperature (4 °C, -20 °C, -80 °C) and storage duration (0, 2, 7 and 14 days). The mean and SD are the average values of the number of cells between replicates and the standard deviation of the mean, respectively.

Day	Storage Temperature	Formaldehyde concentration		Total cell	Proportion PolyP+ cell	Mean total cell	SD total cell	Mean Polyp+ cell	SD PolyP+ cell
0		0 %	R1	43042	13.7	41879.7	1985.6	14.2	0.6
			R2	39587	14.1				
			R3	43010	14.9				
		2 %	R1	54031	8.2	64634.3	9343.9	7.5	0.7
			R2	68208	7.4				
			R3	71664	6.8				
		4 %	R1	42442	8.9	42043.3	1306.4	8.9	0.0
			R2	43104	8.9				
			R3	40584	8.9				
2	4 °C	2 %	R1	50938	7.3	56979.0	8597.5	6.9	0.6
			R2	66822	6.3				
			R3	53177	7.2				
		4 %	R1	25209	8	20312.7	4573.8	8.8	0.7
			R2	19579	9				
			R3	16150	9.4				
	-20 °C	2 %	R1	59572	4.5	61609.3	5960.1	4.7	0.9
			R2	56935	5.7				
			R3	68321	3.9				
		4 %	R1	27593	5.6	24269.0	2900.4	6.9	1.2
			R2	22961	7.3				
			R3	22253	7.9				
	-80 °C	2 %	R1	47789	0.5	49866.3	2498.0	0.7	0.5
			R2	49172	0.4				
			R3	52638	1.3				
		4 %	R1	23645	1.2	23829.7	1709.5	1.1	0.3
			R2	25624	1.3				
			R3	22220	0.8				
7	4 °C	2 %	R1	47140	7.4	49258.0	1921.1	7.1	0.3
			R2	49746	7.1				
			R3	50888	6.8				
		4 %	R1	21974	8.6	21161.7	1680.8	8.2	0.3
			R2	22282	8				
			R3	19229	8				
	-20 °C	2 %	R1	57275	2.5	52431.3	4487.2	2.4	0.1
			R2	48416	2.4				
			R3	51603	2.4				
		4 %	R1	21598	2	19598.0	2340.4	3.7	2.4
			R2	17024	2.6				
			R3	20172	6.4				
	-80 °C	2 %	R1	51861	0	50767.0	1141.7	0.0	0.0
			R2	50857	0				
			R3	49583	0				
		4 %	R1	27205	1.1	27086.0	524.7	0.8	0.2
			R2	27541	0.7				
			R3	26512	0.7				
14	4°C	2 %	R1	82800	5.2	61929.0	20558.9	5.7	0.5
			R2	61290	6.2				
			R3	41697	5.8				
		4 %	R1	14788	6.1	14779.7	196.6	5.9	0.2
			R2	14972	6				
			R3	14579	5.7				
	-20°C	2 %	R1	61175	2.4	63893.0	2363.7	1.6	0.8
			R2	65036	0.8				
			R3	65468	1.7				
		4 %	R1	13444	3.4	9375.0	3644.4	5.1	1.8
			R2	8270	7				
			R3	6411	5				
	-80°C	2 %	R1	67649	0	63707.0	3828.6	0.0	0.0
			R2	63469	0				
			R3	60003	0				
		4 %	R1	12239	0.9	9954.3	2029.1	0.9	0.1
			R2	9262	0.8				
			R3	8362	0.9				

Table S12 : Comparisons of the proportions of polyP+ cells counted by FCM for the RX strain, as a function of formaldéhyde concentration (2 % or 4 %) after 1h of incubation (day 0).

After Shapiro Wilk normality and Brown- Forsythe homoscedasticity tests, the similarities between treatments were evaluated using a one-way ANOVA with Tukey's post-hoc test to make multiple comparisons between the groups. A p value of less than 0.05 ($p < 0.05$) was considered as statistically significant: *$p < 0.05$, ****$p < 0.0001$.

Comparison test	Summary	Adjusted P Value
0 % vs. 2 %	****	<0.0001
0 % vs. 4 %	****	<0.0001
2 % vs. 4 %	*	0,0393

Table S13 : Effect of 2 % or 4 % formaldehyde fixation on the FCM count of total cells (after SYTO®62 labelling) and proportion of polyP+ cells (after DAPI labelling) of the *T. elongata* strain as a function of storage temperature (4 °C, -20 °C, -80 °C) and storage duration (0, 2, 7 and 14 days). The mean and SD are the average values of the number of cells between replicates and the standard deviation of the mean, respectively.

Day	Storage Temperature	Formaldehyde concentration		Total cell	Proportion PolyP+ cell	Mean total cell	SD total cell	Mean Polyp+ cell	SD PolyP+ cell
0		0 %	R1	30893	99.9	32963.3	2061.6	99.8	0.2
			R2	32981	99.6				
			R3	35016	99.8				
		2 %	R1	27492	99.9	26664.0	803.7	99.9	0.1
			R2	25887	99.9				
			R3	26613	100				
		4 %	R1	22716	99.8	22235.3	594.2	99.9	0.1
			R2	22419	100				
			R3	21571	100				
2	**4 °C**	2 %	R1	26090	100	25308.0	2136.2	100.0	0.0
			R2	22891	100				
			R3	26943	100				
		4 %	R1	24569	100	19954.7	4114.4	99.9	0.1
			R2	16668	99.9				
			R3	18627	99.9				
	-20 °C	2 %	R1	28097	99.9	23267.3	4183.4	99.9	0.1
			R2	20769	99.9				
			R3	20936	100				
		4 %	R1	20571	100	21258.3	603.4	100.0	0.0
			R2	21701	100				
			R3	21503	100				
	-80 °C	2 %	R1	32515	100	28515.0	3472.8	100.0	0.1
			R2	26269	100				
			R3	26761	99.9				
		4 %	R1	20737	100	20834.7	879.6	100.0	0.0
			R2	20008	100				
			R3	21759	100				
7	**4 °C**	2 %	R1	24763	100	23809.0	839.0	100.0	0.1
			R2	23478	99.9				
			R3	23186	100				
		4 %	R1	21056	99.9	20997.0	424.6	99.9	0.1
			R2	21389	99.9				
			R3	20546	100				
	-20 °C	2 %	R1	25989	100	21013.7	5925.7	100.0	0.0
			R2	22594	100				
			R3	14458	100				
		4 %	R1	12194	100	14729.0	4993.9	100.0	0.1
			R2	20482	100				
			R3	11511	99.9				
	-80 °C	2 %	R1	25500	99.9	24924.3	1463.1	99.9	0.0
			R2	23261	99.9				
			R3	26012	99.9				
		4 %	R1	19327	99.8	20358.7	2374.0	99.8	0.0
			R2	18675	99.8				
			R3	23074	99.8				
14	**4 °C**	2 %	R1	26665	99.9	23495.3	2753.0	99.9	0.1
			R2	21701	100				
			R3	22120	99.9				
		4 %	R1	21575	100	20357.3	1056.3	100.0	0.1
			R2	19809	99.9				
			R3	19688	100				
	-20 °C	2 %	R1	10429	99.9	11354.0	823.9	99.9	0.1
			R2	11624	99.9				
			R3	12009	100				
		4 %	R1	16583	100	15514.0	7654.7	99.9	0.1
			R2	7381	99.9				
			R3	22578	99.9				
	-80 °C	2 %	R1	24925	100	26944.7	1767.5	100.0	0.1
			R2	27700	100				
			R3	28209	99.9				
		4 %	R1	23471	100	23100.7	916.5	99.9	0.1
			R2	23774	99.9				
			R3	22057	99.8				

Table S14 : Comparisons of the proportions of polyP+ cells counted by FCM for the *T. elongata* strain, as a function of formaldéhyde concentration (2% or 4%) after 1h of incubation (day 0).

After Shapiro Wilk normality and Brown-Forsythe homoscedasticity tests, the similarities between treatments were evaluated using a one-way ANOVA with Tukey's post-hoc test to make multiple comparisons between the groups. A *p* value of less than 0.05 ($p < 0.05$) was considered as statistically significant. ns: not significant.

Anova Summary	F	P. value	P. value
ns	**2.083**	0.2056	0.0625

Table S15 : Comparisons of the proportions of polyP+ cells counted by FCM for the *T. elongata* strain, as a function of storage duration (2, 7, 17 days) and temperature (4 °C, -20 °C, -80 °C).

After Shapiro Wilk normality and Brown-Forsythe homoscedasticity tests, the similarities between treatments were evaluated using two-way repeated measures Anova test, with Tukey's post-hoc test to make multiple comparisons between the groups. A *p* value of less than 0.05 ($p < 0.05$) was considered as statistically significant. ns: not significant.

Anova source of variation	Summary	Adjusted P Value
2 % formaldéhyde		
Interaction	ns	0.0625
Duration	ns	0.4854
Temperature	ns	0.7023

Table S16 : Comparisons of the proportions of polyP+ cells counted by FCM for the RX strain, as a function of storage temperature (4 °C, -20 °C, -80 °C).

After Shapiro Wilk normality and Brown-Forsythe homoscedasticity tests, the similarities between treatments were evaluated using two-way repeated measures Anova test, with Tukey's post-hoc test to make multiple comparisons between the groups. A p value of less than 0.05 ($p < 0.05$) was considered as statistically significant. ns: not significant, $^{*}p < 0.05$, $^{**}p < 0.001$, $^{***}p < 0.0005$, and $^{****}p < 0.0001$.

Comparison test	Summary	Adjusted P Value
After 2 days- 2% formaldéhyde		
4 °C vs. -20 °C	ns	0.0637
4 °C vs. -80 °C	***	0.0003
-20 °C vs. -80 °C	*	0.0135
After 7 days- 2 % formaldéhyde		
4 °C vs. -20 °C	**	0.0012
4 °C vs. -80 °C	***	0.0001
-20 °C vs. -80 °C	****	<0.0001
After 14 days- 2 % formaldéhyde		
4 °C vs. -20 °C	**	0.0069
4 °C vs. -80 °C	**	0.0044
-20 °C vs. -80 °C	ns	0.128

Comparison test	Summary	Adjusted P Value
After 2 days- 4 % formaldéhyde		
4 °C vs. -20 °C	ns	0.1848
4 °C vs. -80 °C	**	0.0022
-20 °C vs. -80 °C	*	0.0201
After 7 days- 4 % formaldéhyde		
4 °C vs. -20 °C	ns	0.1405
4 °C vs. -80 °C	****	<0.0001
-20 °C vs. -80 °C	ns	0.3004
After 14 days- 4 % formaldéhyde		
4 °C vs. -20 °C	ns	0.7566
4 °C vs. -80 °C	****	<0.0001
-20 °C vs. -80 °C	ns	0.0979

Table S17 : Comparisons of the proportions of polyP+ cells counted by FCM for the RX strain, as a function of storage duration (2, 7, 14 days).

After Shapiro Wilk normality and Brown-Forsythe homoscedasticity tests, the similarities between treatments were evaluated using two-way repeated measures Anova test, with Tukey's post-hoc test to make multiple comparisons between the groups. A p value of less than 0.05 ($p < 0.05$) was considered as statistically significant. ns: not significant, $^{**}p < 0.001$.

Comparison test	Summary	Adjusted P Value
4 °C- 2 % formaldéhyde		
2 days vs 7 days	ns	0.8878
2 days vs 14 days	ns	0.303
7 days vs 14 days	ns	0.1456
-20 °C- 2 % formaldéhyde		
2 days vs 7 days	ns	0.0928
2 days vs 14 days	ns	0.1403
7 days vs 14 days	ns	0.3511
-80 °C- 2 % formaldéhyde		
2 days vs 7 days	ns	-
2 days vs 14 days	ns	-
7 days vs 14 days	ns	-

Comparison test	Summary	Adjusted P Value
4 °C- 4 % formaldéhyde		
2 days vs 7 days	ns	0.6535
2 days vs 14 days	ns	0.0575
7 days vs 14 days	**	0.0074
-20 °C- 4 % formaldéhyde		
2 days vs 7 days	ns	0.131
2 days vs 14 days	ns	0.2535
7 days vs 14 days	ns	0.7033
-80 °C- 4 % formaldéhyde		
2 days vs 7 days	ns	0.4157
2 days vs 14 days	ns	0.5099
7 days vs 14 days	ns	0.9593

Table S18 : PolyP+ cell counts by epifluorescence microscopy and FCM after DAPI labelling before and after cell sorting for the mixed RX and *T. elongata* strains.

For each condition, three replicates were counted in epifluorescence microscopy. The total number of cells counted by FCM using the fluorescence of SYTO®62 was recorded. The mean and SD are the average values of the percentage of polyP+ cells between replicates and the standard deviation of the mean, respectively.

Method	Condition	Replicate N°	Number of cell count/sample	Cell polyP+	% cell polyP+	Mean % cell polyP+	SD % cell polyP+
Epifluorescence microscopy	Before cell sorting	R1	500	93	18.6	12.6	7.5
		R2	500	75	15.0		
		R3	500	21	4.2		
	After cell sorting-fraction polyP+	R1	200	186	93.0	93.3	1.0
		R2	200	189	94.5		
		R3	200	185	92.5		
	After cell sorting-fraction polyP-	R1	200	13	6.5	12.2	6.3
		R2	200	38	19.0		
		R3	200	22	11.0		
FCM	Before cell sorting		57672	21070	36.5		
	After cell sorting-fraction polyP+		7116	5631	79.1		
	After cell sorting-fraction polyP-		689	18	2.6		

Table S19 : PAB cell sorting from lake water sample

Proportion of polyP+ cells, after PAB labelling with DAPI and SYTO®62, in a water sample from Lake Pavin. Cells were counted by epifluorescence microscopy in the sample before fluorescence activated cell sorting and, in the polyP, + and polyP- fractions after cell sorting.

For each condition, three replicates were counted in epifluorescence microscopy. The mean and SD are the average values of the percentage of polyP+ cells between replicates and the standard deviation of the mean, respectively.

For comparison test t-test of Student. A *p* value of less than 0.05 ($p < 0.05$) was considered as statistically significant. ns: not significant, ****$p < 0.0001$.

Condition	Replicate N°	Number of cell count/sample	Cell polyP+	% cell polyP+	Mean % cell polyP+	SD % cell polyP+
Before cell sorting	R1	500	57	11.4	9.7	1.5
	R2	500	44	8.8		
	R3	500	44	8.8		
After cell sorting-fraction polyP+	R1	200	105	52.5	52.0	1.8
	R2	200	100	50.0		
	R3	200	107	53.5		
After cell sorting-fraction polyP-	R1	200	11	5.5	5.5	3.0
	R2	200	5	2,5		
	R3	200	17	8,5		

Comparison test	Summary	Adjusted P Value
Before cell sorting vs polyP+ fraction	****	<0.0001
Before cell sorting vs polyP- fraction	ns	0.0978

Table S20 : PolyP+ cell counts after DAPI or JC-D7 labelling for the *T. elongata* strain.

For each condition, three replicates were counted in FCM or epifluorescence microscopy. The mean and SD are the average values of the percentage of polyP+ cells between replicates and the standard deviation of the mean, respectively.

For comparison test: after Shapiro Wilk normality and Brown-Forsythe homoscedasticity tests, the similarities between treatments were evaluated using a one-way ANOVA with Tukey's post-hoc test to make multiple comparisons between the groups. A p value of less than 0.05 ($p < 0.05$) was considered as statistically significant. ns: not significant, *$p < 0.05$, **$p < 0.001$.

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	Replicate N°	Number of cell count/sample	Cell polyP+	% cell polyP+	Mean % cell polyP+	SD % cell polyP+
Epifluorescence microscopy counts	R1	1000	947	94.70	96.3	1.9
	R2	1000	984	98.40		
	R3	1000	958	95.80		
FCM counts-DAPI labelling	R1	11107	11096	99.90	99.9	0.0
	R2	10719	10712	99.93		
	R3	10832	10823	99.92		
FCM counts-JC-D7 labelling	R1	9274	8809	94.99	93.7	1.5
	R2	8441	7772	92.07		
	R3	9434	8862	93.94		

Comparison test	Summary	Adjusted P Value
Epifluorescence counts vs FCM DAPI	*	0.0433
Epifluorescence counts vs FCM JC-D7	ns	0.1284
FCM DAPI vs FCM JC-D7	**	0.0036

Table S21 : PolyP+ cell counts after DAPI or JC-D7 labelling for the RX strain.
For each condition, three replicates were counted in FCM or epifluorescence microscopy. The mean and SD are the average values of the percentage of polyP+ cells between replicates and the standard deviation of the mean, respectively.
For comparison test: after Shapiro Wilk normality and Brown-Forsythe homoscedasticity tests, the similarities between treatments were evaluated using a one-way ANOVA with Tukey's post-hoc test to make multiple comparisons between the groups. A p value of less than 0.05 ($p < 0.05$) was considered as statistically significant. ns: not significant, ***$p < 0.0005$, and ****$p < 0.0001$.

	Replicate N°	Number of cell count/sample	Cell polyP+	% cell polyP+	Mean % cell polyP+	SD % cell polyP+
Epifluorescence microscopy counts	R1	1000	132	13.2	12.3	1.2
	R2	1000	109	10.9		
	R3	1000	127	12.7		
FCM counts-DAPI labelling	R1	19437	1178	6.1	5.8	0.5
	R2	21846	1341	6.1		
	R3	19833	1034	5.2		
FCM counts-JC-D7 labelling	R1	3617	170	4.7	4.8	0.3
	R2	4472	205	4.6		
	R3	3829	198	5.2		

Comparison test	Summary	Adjusted P Value
Epifluorescence counts vs FCM DAPI	***	0.0001
Epifluorescence counts vs FCM JC-D7	****	<0.0001
FCM DAPI vs FCM JC-D7	ns	0.3501

Table S22 : PolyP+ cell counts after DAPI or JC-D7 labelling for the lake sediment sample.
For each condition, three replicates were counted in FCM or epifluorescence microscopy. The mean and SD are the average values of the percentage of polyP+ cells between replicates and the standard deviation of the mean, respectively.
For comparison test: after Shapiro Wilk normality and Brown-Forsythe homoscedasticity tests, the similarities between treatments were evaluated using a one-way ANOVA with Tukey's post-hoc test to make multiple comparisons between the groups. A p value of less than 0.05 ($p < 0.05$) was considered as statistically significant. ns: not significant, ****$p < 0.0001$.

	Replicate N°	Number of cell count/sample	Cell polyP+	% cell polyP+	Mean % cell polyP+	SD % cell polyP+
Epifluorescence microscopy counts	R1	1000	188	18.8	10.5	7.2
	R2	1000	66	6.6		
	R3	1000	60	6.0		
FCM counts-DAPI labelling	R1	10355	8714	84.2	82.8	2.3
	R2	10301	8660	84.1		
	R3	9203	7381	80.2		
FCM counts-JC-D7 labelling	R1	4436	228	5.1	5.0	0.1
	R2	4713	231	4,9		
	R3	4449	223	5,0		

Comparison test	Summary	Adjusted P Value
Epifluorescence counts vs FCM DAPI	****	<0.0001
Epifluorescence counts vs FCM JC-D7	ns	0.3429
FCM DAPI vs FCM JC-D7	****	<0.0001

Table S23 : PolyP+ and polyP- cell counts after DAPI labelling for different bacterial strains.
For each condition, three replicates were counted in FCM. The mean and SD are the average values of the percentage of polyP+ cells between replicates and the standard deviation of the mean, respectively.

Tube Name	Replicate N°	PolyP - #Events	% PolyP -	PolyP + #Events	% PolyP +	Mean % cell polyP+	SD % cell polyP+
T. elongata	R1	14061	78.5	3911	21.8	19.6	2.4
T. elongata	R2	12851	83.3	2641	17.1		
T. elongata	R3	13329	80.6	3271	19.8		
Flavobacterium sp.	R1	14021	100	8	0.1	0.1	0.1
Flavobacterium sp.	R2	16433	99.9	10	0.1		
Flavobacterium sp.	R3	15249	100	5	0		
Acinetobacter lwoffii	R1	15922	99.9	14	0.1	0.1	0.0
Acinetobacter lwoffii	R2	14569	99.9	12	0.1		
Acinetobacter lwoffii	R3	16564	99.9	9	0.1		
Pseudomonas sp.	R1	15455	99.9	8	0.1	0.0	0.1
Pseudomonas sp.	R2	13806	100	6	0		
Pseudomonas sp.	R3	15251	100	5	0		
Stenotrophomonas rhizophila	R1	11491	100	4	0	0.0	0.0
Stenotrophomonas rhizophila	R2	13349	100	1	0		
Stenotrophomonas rhizophila	R3	12149	100	1	0		
Microbacterium hydrocarbonoxydans	R1	13416	99.9	12	0.1	0.1	0.0
Microbacterium hydrocarbonoxydans	R2	12768	99.9	10	0.1		
Microbacterium hydrocarbonoxydans	R3	13753	99.9	10	0.1		

Table S24 : PolyP+ and polyP- cell counts after JC-D7 labelling for different bacterial strains.
For each condition, three replicates were counted in FCM. The mean and SD are the average values of the percentage of polyP+ cells between replicates and the standard deviation of the mean, respectively.

Tube Name	Replicate N°	PolyP - #Events	% PolyP -	PolyP + #Events	%PolyP +	Mean % cell polyP+	SD % cell polyP+
T. elongata	R1	11753	84.6	2228	16	17.5	2.4
T. elongata	R2	10071	80.3	2540	20.3		
T. elongata	R3	11598	84.5	2222	16.2		
Flavobacterium sp.	R1	15967	99.5	80	0.5	0.5	0.0
Flavobacterium sp.	R2	17611	99.5	88	0.5		
Flavobacterium sp.	R3	17208	99.6	81	0.5		
Acinetobacter lwoffii	R1	18014	99.9	20	0.1	0.1	0.0
Acinetobacter lwoffii	R2	18517	99.9	18	0.1		
Acinetobacter lwoffii	R3	19230	99.9	16	0.1		
Pseudomonas sp.	R1	14598	99.8	31	0.2	0.2	0.0
Pseudomonas sp.	R2	14241	99.8	30	0.2		
Pseudomonas sp.	R3	14434	99.8	27	0.2		
Stenotrophomonas rhizophila	R1	15674	100	0	0	0.0	0.0
Stenotrophomonas rhizophila	R2	14514	100	1	0		
Stenotrophomonas rhizophila	R3	14583	100	0	0		
Microbacterium hydrocarbonoxydans	R1	14950	99.9	15	0.1	0.2	0.1
Microbacterium hydrocarbonoxydans	R2	15144	99.9	26	0.2		
Microbacterium hydrocarbonoxydans	R3	14469	99.8	32	0.2		

Table S25: Proportion of PolyP+ cells after JC-D7 labelling and FCM counting and concentrations of bioavailable orthophosphates (Olsen extraction method) in different soils from conventional and organic plots.

	SAMPLE CODE	BIOLOGICAL REPLICATE	POLYP+ (%)	OLSEN PHOSPHORUS (µg/g SOIL)
CONVENTIONALLY FARMED	Parcel 1	R1	6.6	116
	Parcel 1	R2	4.4	117
	Parcel 1	R3	3.7	112
	Parcel 2	R1	4.9	39.9
	Parcel 2	R2	3.6	39.2
	Parcel 2	R3	3.1	33.3
	Parcel 3	R1	2.2	60.7
	Parcel 3	R2	3.1	63.3
	Parcel 3	R3	2.1	72
ORGANICALLY FARMED	Parcel 4	R1	0.5	23.8
	Parcel 4	R2		27.2
	Parcel 4	R3	0.4	42.9
	Parcel 5	R1	0.2	30.7
	Parcel 5	R2	0.4	35.8
	Parcel 5	R3	0.3	33.6
	Parcel 6	R1	0.5	40.7
	Parcel 6	R2		37.7
	Parcel 6	R3	0.7	41.9

Note : Olsen phosphorus was not available for the replicate 2 (R2) of parcel 4 and 6.

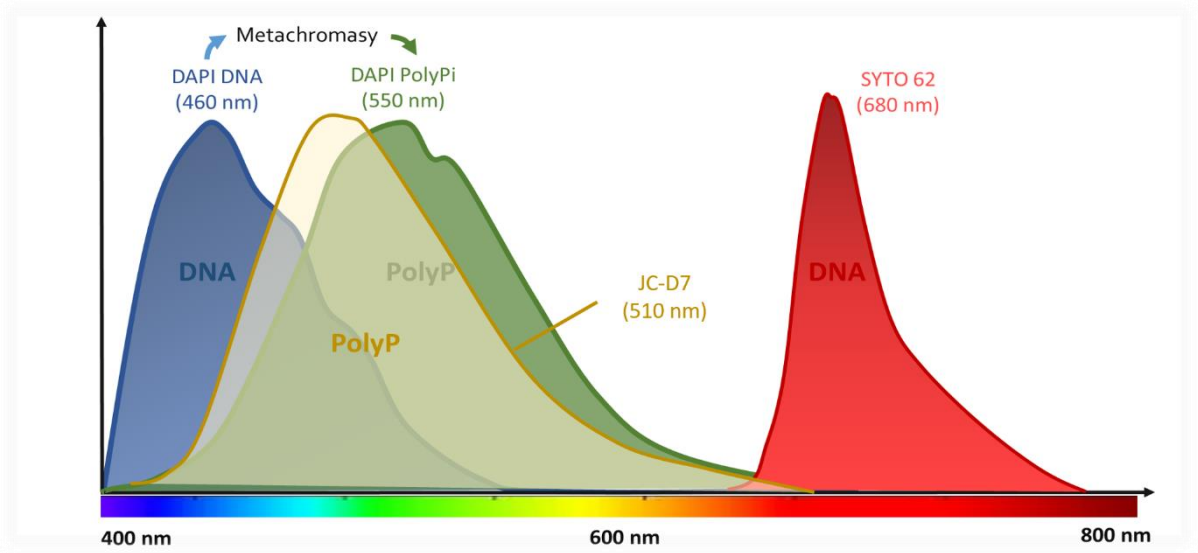

Figure S1 : Emission curves and compatibility of fluorochromes (DAPI, JC-D7, SYTO®62) used for flow cytometry and epifluorescence microscopy analyses.
DAPI is a fluorescent dye with metachromatic properties. DAPI binds strongly to DNA and the DAPI-DNA complex fluoresces blue, with maximum emission at 460 nm. DAPI also forms complexes with polyP that emit a yellow-green fluorescence, with maximum emission at 550 nm

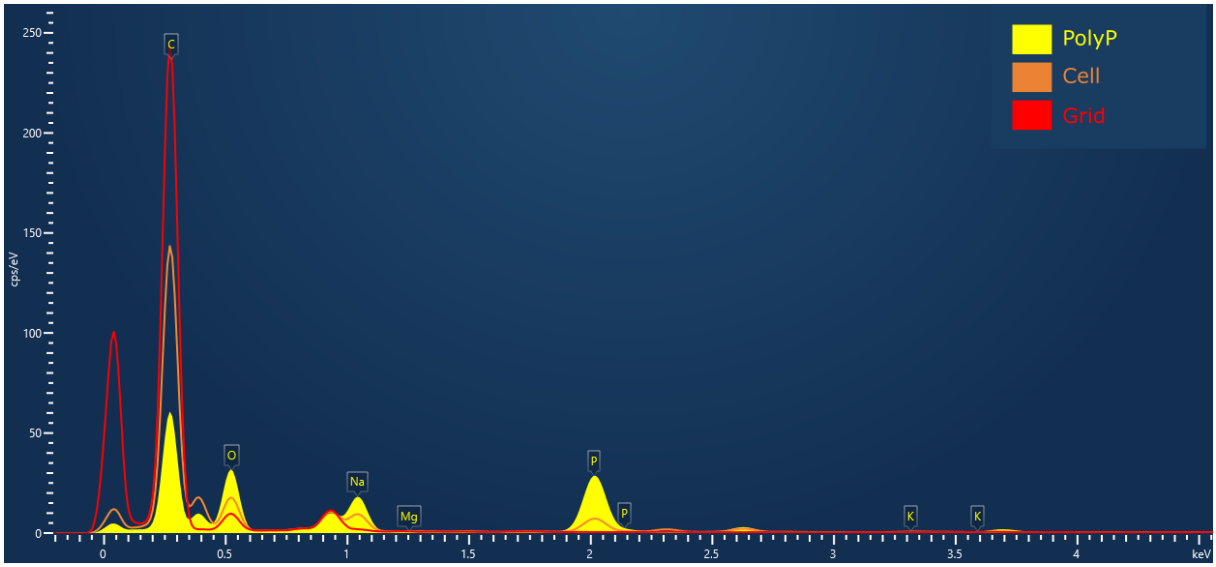

Figure S2 : Representative energy dispersive X-ray (EDX) spectra of polyP granule in *Tetrasphaera elongata* (polyP in yellow) compared to EDX spectra outside polyP granule (cell in orange) and outside the cell (grid in red).

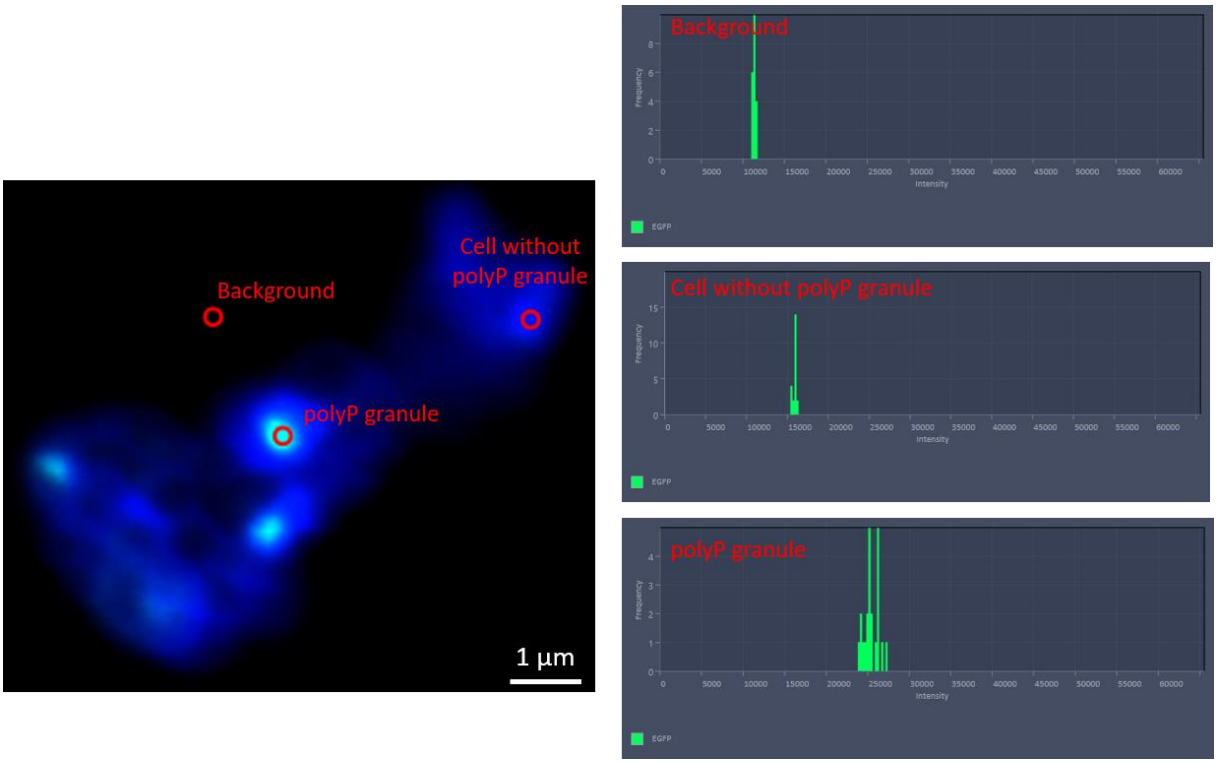

Figure S3 : Green fluorescence values from a photograph of a *T. elongata* culture sample observed by epifluorescence microscopy.
On the left is a photograph of *T. elongata* cells showing the background, a cell without polyP granules and a cell with polyP granules. On the right, histograms show the green intensity values corresponding to the background, the cell without polyP granule and with polyP granule.

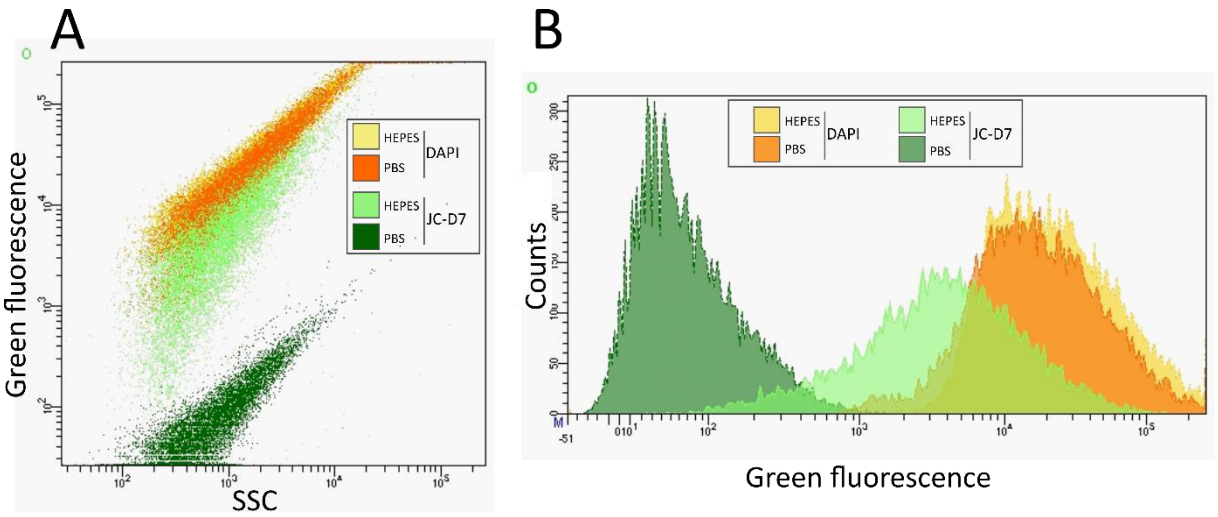

Figure S4 : Overlays of cytograms obtained for *T. elongata* after labelling polyP with JC-D7 or DAPI in HEPES or PBS buffer.

(A) Fluorescence intensity after labelling *T. elongata* with DAPI or JC-D7 (green fluorescence) in HEPES or PBS buffer *versus* side scatter (SSC). **(B)** Number of events (counts) *versus* fluorescence intensity after labelling *T. elongata* with DAPI or JC-D7 (green fluorescence) in HEPES or PBS buffer.