

Suppl. Materials

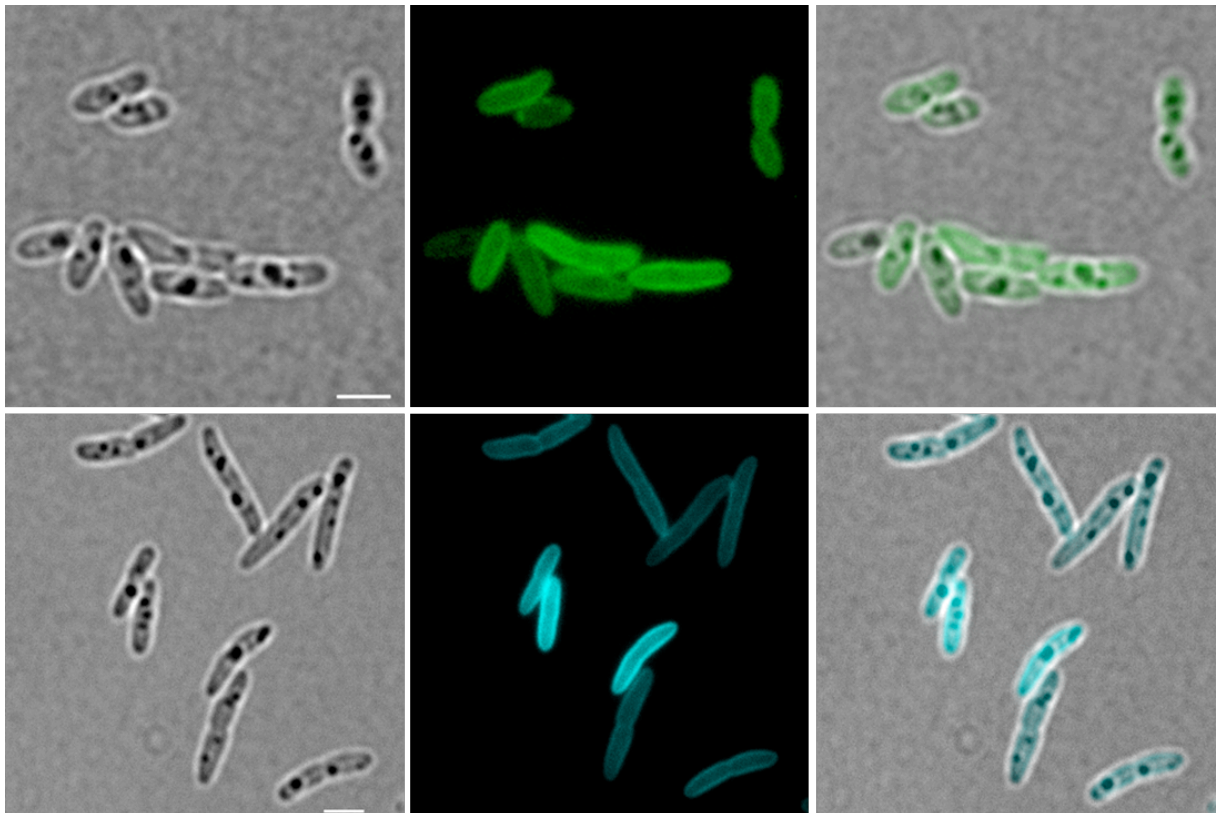
Polyhydroxyalkanoate (PHA) Granules Have no Phospholipids

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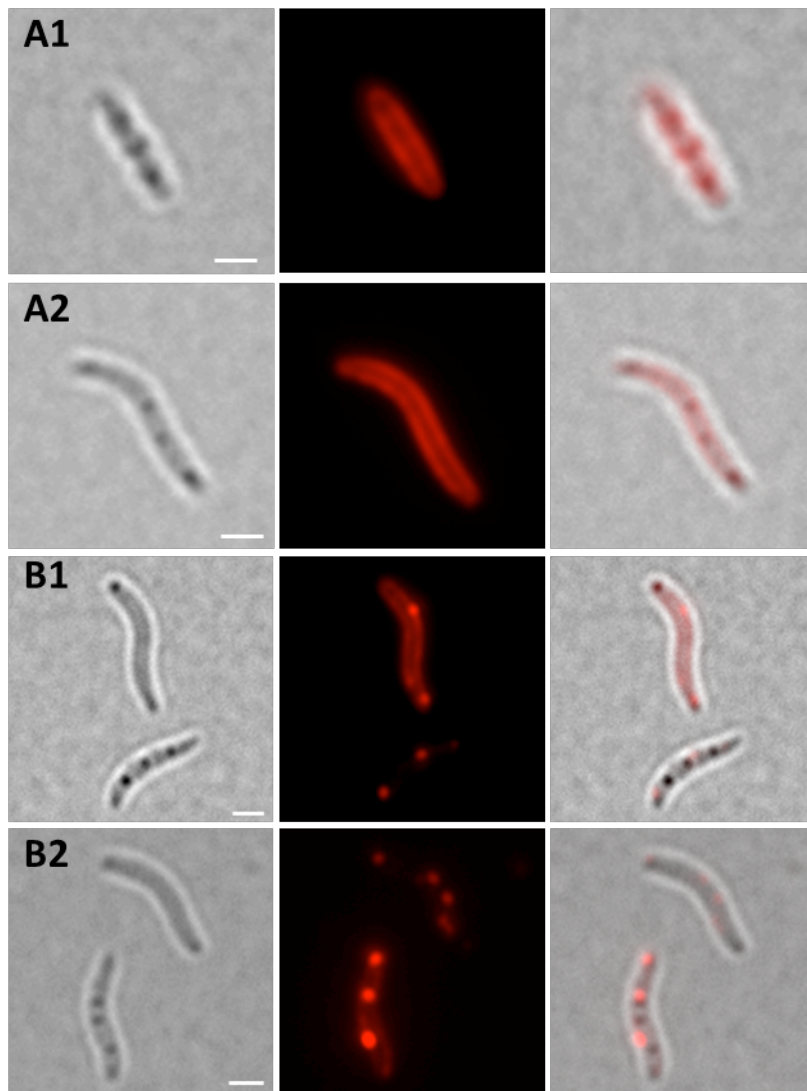
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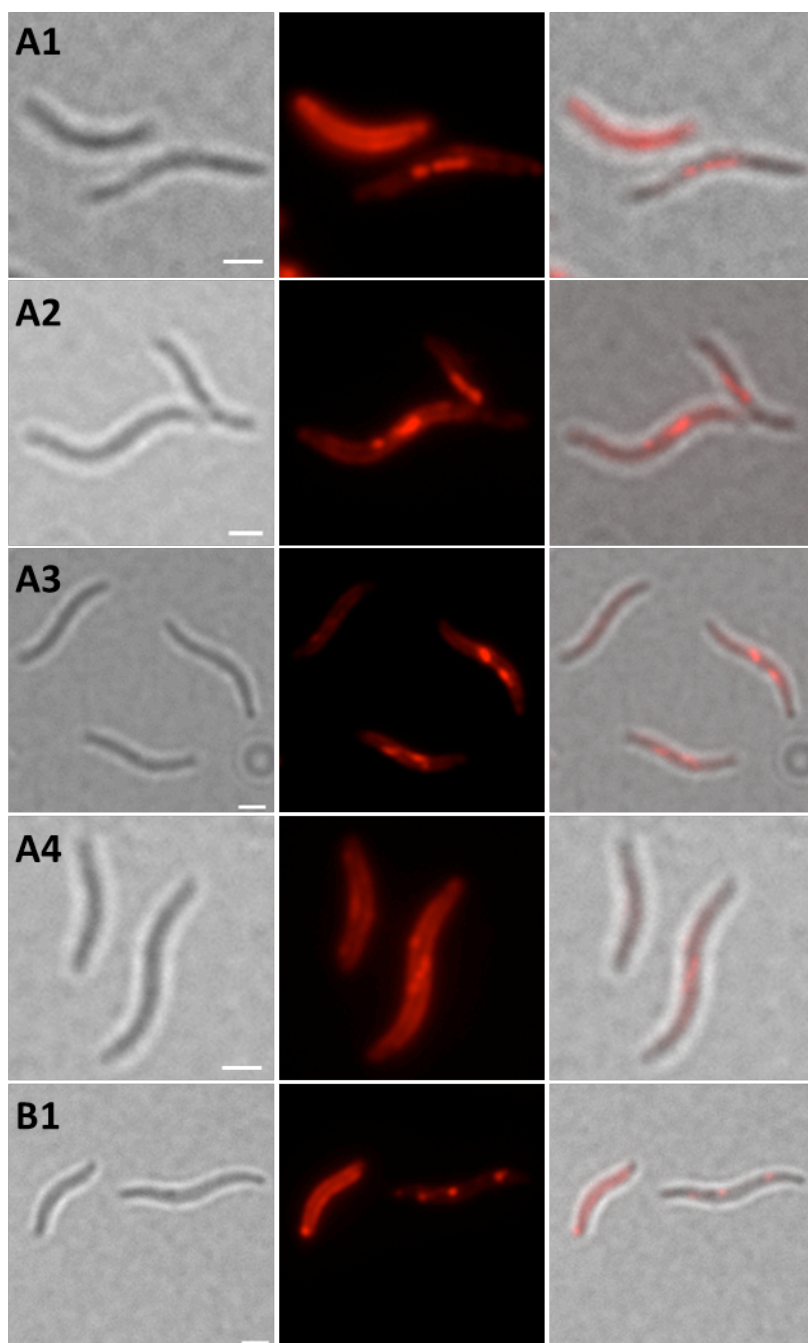


Suppl. Fig. S1: Expression of sfGFP-LactC2 and mTurquoise2-LactC2 in *R. eutropha*.

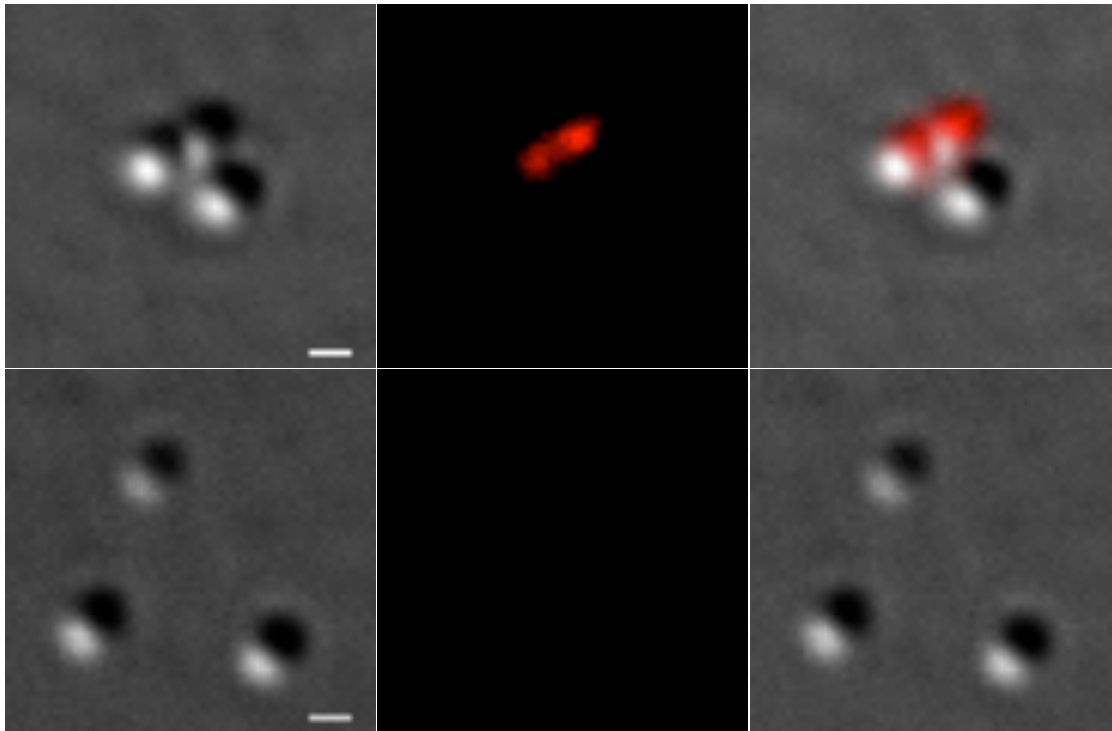
Cells were grown under PHB permissive conditions. From left to right: bright field, green (top middle) or turquoise (bottom middle) channel, merge. Scale bars correspond to 2 μ m. No co-localization of sfGFP-LactC2 or mTurquoise2-LactC2 with PHB granules that are visible in bright field.



Suppl. Fig. S2: Expression of DsRed2EC-LactC2 in magnetosome-free *M. gryphiswaldense* $\Delta mamAB$ mutant. Cells showing membrane-bound DsRed2EC-LactC2 fluorescence but no filament-like fluorescence (**A1 and A2**). Cells focussed to foci of DsRed2EC-LactC2 fluorescence (**B1 and B2**). Note, presence of several globular inclusions in bright field (PHB granules) in all images that do not co-localize with DsRed2EC-LactC2 fluorescence. From left to right: bright field, fluorescence channel, merge. Scale bars correspond to 2 μm .



Suppl. Fig. S3: Expression of DsRed2EC-LactC2 in PHB granule-free *M. gryphiswaldense* Δ *phaCAB* mutant. Cells focussed to filament-like dsRed2EC-LactC2 fluorescence resembling magnetosome chains (**A1-A4**). Cell focussed to cell membrane-bound DsRed2EC-LactC2 fluorescence and foci of DsRed2EC-LactC2 fluorescence (**B1**). Note, absence of any globular inclusions in bright field (PHB granules). From left to right: bright field, fluorescence channel, merge. Scale bars correspond to 2 μ m.



Suppl. Fig. S4: PHB granules isolated from *R. eutropha* H16 cells. PHB granules isolated from *R. eutropha* H16 cells expressing DsRed2EC-LactC2 are shown in the top row. In the bottom row, PHB granules isolated from *R. eutropha* H16 cells expressing DsRed2EC alone are shown. DIC (left), red channel (middle), overlay (right). Scale bars correspond to 0.5 μm .

Suppl. Table S5: Primers used for construction of gene fusions

| plasmid | primer for amplification of inserts |
|--|--|
| pBBR1MCS-2- <i>P_{phaC}-eyfp-c1-psd</i> | eYFP-Psd-for: ccgctcgaggcatgaactatcctcatccgctgatcgcc eYFP-Psd-rev: cgggatcctcacttcacgtcgagttcggcgaggatg |
| pCM62- <i>P_{phaC}-dsRed2-c1</i> | DsRed2EC-for: ggggaattccatagggcgagcagtgagaacatcatcacc DsRed2EC-rev: ggactagttgatcagttatctagatccgggtggatcc |
| pCM62- <i>P_{phaC}-dsRed2EC-c1-lactC2</i> | LactC2-for: cccaagcttcatgcactgaacccctaggcc LactC2-rev: ggactagtttaacagcccagcagctcc |
| pETDuet- <i>phaCcrAB</i> <i>venlactC2</i> | PhaA-for: gggatcactctcggcatggacgagctgtacaagtgacgcttgcatgagtgccgg PhaB-rev: gaattcggatcctggctgtggtgatgatggatggctgctgctcagccatgtgcaggccg PhaC- Cerulean-for: ccctctagaataatTTTgTTTaaactttaagaaggagatataccatggcgaccggcaaagg PhaC- Cerulean-rev: gctaccgctgccacttctgatccgctacctgcttggctttgacgtatcgcc Venus-Lact-for: cccatcttagtatattagtttaagtataagaaggagatatacatatggtgagcaagggcgagg Venus-Lact-rev: cttcaggcctaggggttcagtgcaattgtacagctcgtccatgccgag ReCr-for: gtcaaagccaaggcaggttagcggatcaggaagtgccagcggttagcgtgagcaagggcgaggagctgttc ReCr-rev: ccggcactcatcaagcgtcacttgtacagctcgtccatg LactC2-for: ctcgcatggacgagctgtacaagtgcaactgaacccctaggcctgaag LactC2-rev: gtcagcgatcgctggccggccgatccaattgagatctgcttaacagcccagcagctccac |
| pCM62- <i>P_{phaC}-mTurquoise2-c1-lactC2</i> | mmTurq2-LactC2-for: ccatgcaaagtgccggccaggccaatgcccggagccggttcgaatagtgacggcagagagacaatcaacatagagcaagggcgaggagctg mTurq2-LactC2-rev: gggatggtattatccttcaggcctaggggttcagtgcatgaagcttgagctcgagatctttatatactgctccataccaag |
| pCM62- <i>P_{phaC}-sfGFP-c1-lactC2</i> | sfGFP-LactC2-for: ccatgcaaagtgccggccaggccaatgcccggagccggttcgaatagtgacggcagagagacaatcaacatagggatcaaaaggtgaag aattatttac sfGFP-LactC2-rev: tggatattatccttcaggcctaggggttcagtgcatgaagcttgagctcgagatctttatataattcatccataccatgtg |
| pBAM- <i>P_{mamDC}-dsRed2EC-c1-lactC2</i> | DsRed2EC-LactC2-for: ggggaattccatagggcgagcagtgagaacatcatcacc DsRed2EC-LactC2-rev: ggggaattcgctagcttaacagcccagcagctccactcg |

Suppl. Table S6: Amino acid sequences of constructed fusion proteins

DsRed2EC-LactC2:

MASSENIITEFMRFKVRMEGTVNGHEFEIEGEGEGRPYEGHNTVCLKVTKGGPLPFAWDILSPQFQYGSK
VYVKHPADIPDYKLSFPEGFKWERVMNFEDGGVATVTQDSSLQDGCIFYKVKFIGVNFPSDGPVMQKK
TMGWEASTERLYPRDGVKGETHKALKLKDGGHYLVEFKSIYMAKKPVQLPGYYYYDAKLDITSHNEDYT
IVEQYERTEGRHHLFLRSRAQASCTEPLGLKDNTIPNKQITASSYYKTWGLSAFSWFPYYARLDNQGKFNA
WTAQTNASASEWLQIDLGSQKRVTGIITQGARDFGHIQYVAAYRVAYGDDGVTWTEYKDPGASESKIFPG
NMDNNSHKKNIFETPFQARFVRIQPVAWHNRITLRVLLGC

sfGFP-LactC2:

MASKGEELFTGVVPIVVELDGDVNGHKFSVRGEGEGDATNGKLTCLKFICTTGKLPVPWPPTLVTTLYGVQ
CFSRYPDHMKRHDFFKSAMPEGYVQERTISFKDDGTYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHK
LEYNFNSHNVYITADKQKNGIKANFKIRHNVEDGVSQVLADHYQQNTPIGDGPVLLPDNHLYSTQSVLSKD
PNEKRDHMLLEFVTAAGITHGMDELYKRSRAQASCTEPLGLKDNTIPNKQITASSYYKTWGLSAFSWFP
YYARLDNQGKFNAWTAQTNASASEWLQIDLGSQKRVTGIITQGARDFGHIQYVAAYRVAYGDDGVTWTE
YKDPGASESKIFPGNMDNNSHKKNIFETPFQARFVRIQPVAWHNRITLRVLLGC

eYFP-Psd:

MVSKGEELFTGVVPIVVELDGDVNGHKFSVSGEGEGDATYGKLTCLKFICTTGKLPVPWPPTLVTTFGYGLQC
FARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHK
LEYNFNSHNVYIMADKQKNGIKVNFKIRHNIEDGVSQVLADHYQQNTPIGDGPVLLPDNHLYSYQSALS
PNEKRDHMLLEFVTAAGITLGMDELYKSGLRSGMNYPHPLIAREGWPFAGAFVISLLVHASAGFWW
ALPLWIITVFLQFFRDPPRPIPSQPNVAVLAPADGRIVVVEKTQDPYAGREALKISVFMNVFNVHSNRVSV
DGAVEKVEYFPGKFNADMDKASVENERNAVLRRAADGQLVTLVQVAGLVARRILCYTKVGDNLRSRQ
RYGFIRFGSRVDVYLPDARPRVTIGEKVSASSTILAEADV

PhaC-Cerulean-PhaAB (sequence of PhaA and PhaB not shown):

MATGKGAAASTQEGKSQPFKVTGPFDPATWLEWSRQWQGTEGNGHAAASGIPGLDALAGVKIAPA
QLGDIQQRMYKDFSAWQAMAEGKAEATGPLHRRFAGDAWRTNLPYRFAAAFYLLNARALTELADA
VEADAKTRQRIRFAISQWVDAMSPANFLATNPEAQRLLES GGESLRAGVRNMMEDLTRGKISQTESA
FEVGRNVAVTEGAVVFENEYFQLLQYKPLTDKVHARPLLMVPPCINKYYILDLPESLVRHVVEQGHTVF
LVSWRNPDASMAGSTWDDYIEHAAIRAIEVARDISGQDKINVLGFCVGGTIVSTALAVLAARGEHPAASV
TLLTLLDFADTGILDVDFVDEGHVQLREATLGGGAGAPCALLRGLANTFSFLRPNDLVWNYVVDNYLK
GNTPVPFDLLFWNGDATNLPGPWYCWYLRHTYLQNELKVPKLTVCVGPVDLASIDVPTYIYGSREDHIV
PWTAAYASTALLANKLRFVLGASGHIAGVINPPAKNKRSHWTNDALPESPQQWLAGAIEHHGSWWPD
WTAWLAGQAGAKRAAPANYGNARYRAIEPAPGRYVKAKAGSGSGSGSVSKGEELFTGVVPIVVELD
DVNGHKFSVSGEGEGDATYGKLTCLKFICTTGKLPVPWPPTLVTTLTWGVQCFARYPDHMKQHDFFKSAM
PEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNASDNVYITADKQKNG
IKANFKIRHNIEDGVSQVLADHYQQNTPIGDGPVLLPDNHLYSTQSKLSKDPNEKRDHMLLEFVTAAGITL
GMDELYK

Venus-LactC2:

MVSKGEELFTGVVPIVVELDGDVNGHKFSVSGEGEGDATYGKLTCLKICTTGKLPVPWPPTLVTTLYGLQC
FARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHK
LEYNFNSHNVYITADKQKNGIKANFKIRHNIEDGGVQVLADHYQQNTPIGDGPVLLPDNHLYSYQSKLSKD
PNEKRDHMLLEFVTAAGITLGMDELYKCTEPLGLKDNTIPNKQITASSYYKTWGLSAFSWFPYYARLDN
QGKFNAWTAQTNASASEWLQIDLGSQKRVTGIITQGARDFGHIQYVAAYRVAYGDDGVTWTEYKDPGA
SESKIFPGNMDNNSHKKNIFETPFQARFVRIQPVAWHNRITLRVLLGC

mTurquoise2-LactC2:

MSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLT~~LFICTTGKLPVPWPTLVTTLSWGVQC~~
FARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHK
LENYFSDNVYITADKQKNGIKANFKIRHNIEDGGVQLADHYQQNTPIGDGPVLLPDNHYLSTQSKLSKDP
NEKRDHMLLEFVTAAGITLGMDELYK*RSRAQASCTEPLGLKDNTIPNKQITASSYYKTWGLSAFSWFPY*
YARLDNQQGKFNAWTAQTNSASEWLQIDLGSQKRVGTGIITQGARDFGHIQYVAAYRVAYGDDGVTWTEY
KDPGASESKIFPGNMDNNSHKKNIFETPFQARFVRIQPVAWHNRITLRVELLGC

Amino acids coding for the fluorescent protein are indicated by coloured letters, amino acids of the linker region are shown in italic letters and amino acids of the protein of interest are indicated by non-italic black letters