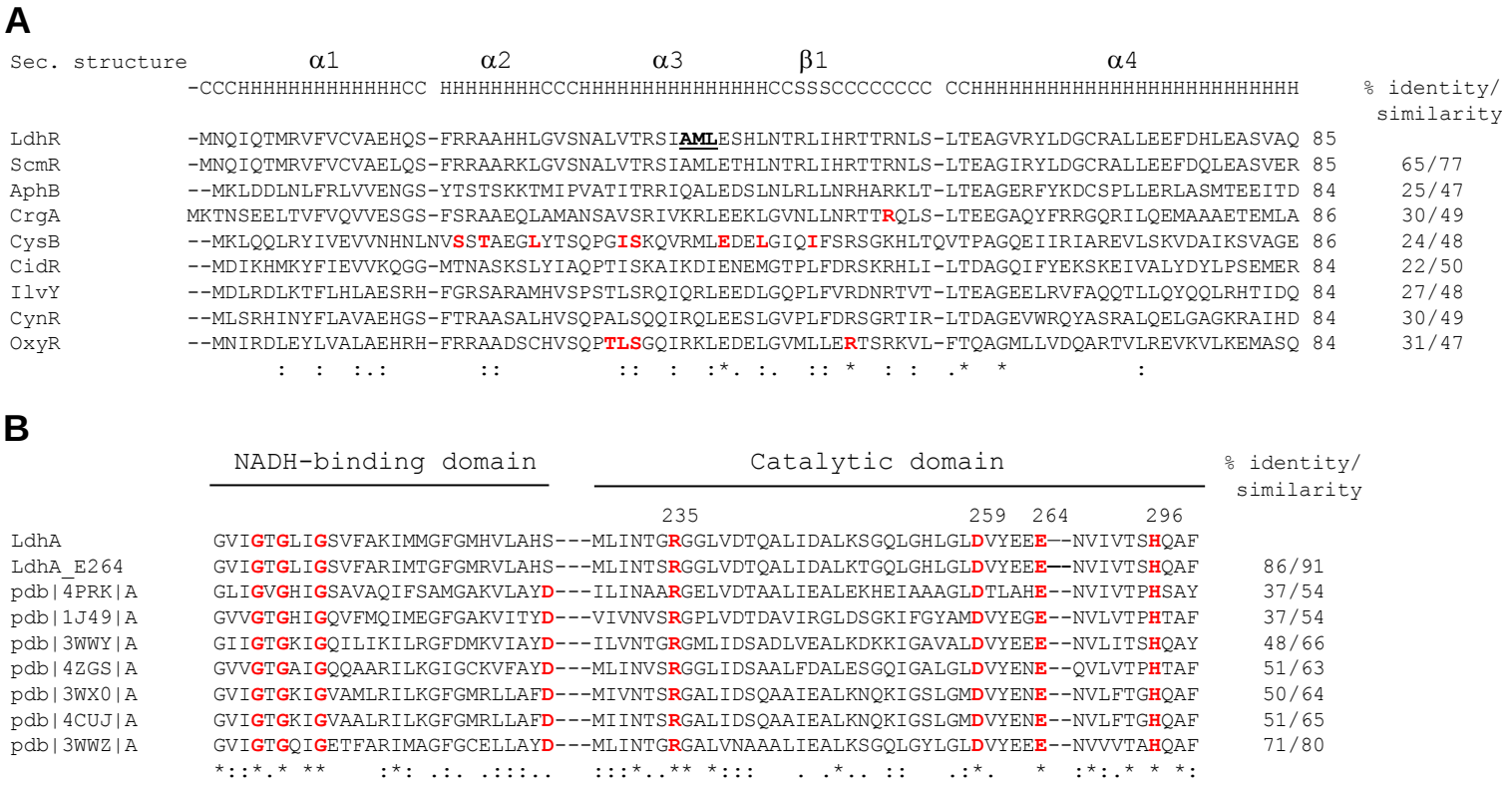

Fig. S1- Proteins LdhR and LdhA display conserved motifs of LTTRs and D-lactate dehydrogenases, respectively. (A) Amino acid sequence alignment of the N-terminal helix-turn-helix (HTH) DNA-binding domain (between amino acid 1 and 85 of LdhR) of a few characterized bacterial LTTRs. The secondary structure is shown above the alignment (H, α-helix; S, β-strand; C, coils). Secondary structure elements were labeled following the predicted LdhR structure. Residues important for DNA binding identified by mutational analysis in CysB, OxyR and CrgA are shown in red. The LdhR homologues included are: *Burkholderia thailandensis* ScmR (ABC37687.1); *Vibrio cholerae* AphB (AAD45271.1); *Neisseria meningitidis* CrgA (AAF37819.1); *Escherichia coli* CysB (AJF44912.1), IlvY (AAA67576.1), CynR (AAA23628.1), and OxyR (CDU40066.1); and *Staphylococcus aureus* CidR (AAS89978.1). Amino acids in bold and underlined represent mutations in the *ldhR* gene of *B. cenocepacia* HI2424 selected in an experimentally evolved biofilm, leading to deletion of 38A, 39M and amino acid exchange L40V (Traverse et al., 2013. Proc Natl Acad Sci U S A 110:E250-9). (B) Amino acid sequence alignment of LdhA from *B. multivorans ATCC* 17616 and *B. thailandensis* E264 and other D-isomer specific 2-hydroxyacid dehydrogenases with the 3D-structure determined. LdhA homologues included are: *Pseudomonas aeruginosa* D-lactate dehydrogenase (pdb|3WWZ|A); *Escherichia coli* D-lactate dehydrogenase (pdb|3WX0|A); *Salmonella enterica* D-lactate dehydrogenase (pdb|4CUJ|A); *Fusobacterium nucleatum* D-lactate dehydrogenase (pdb|3WWY|A); *Chlamydomonas reinhardtii* pyruvate reductase (pdb|4ZGS|A); *Lactobacillus jensenii* D-lactate dehydrogenase (pdb|4PRK|A); and *Lactobacillus bulgaricus* D-lactate dehydrogenase (pdb|1J49|A). Asterisks indicate the amino acid residues that are identical in all the proteins; one or two dots indicate semi-conserved or conserved substitutions, respectively. Identity/similarity at the amino acid level between full length LdhR and each homologue is also shown.

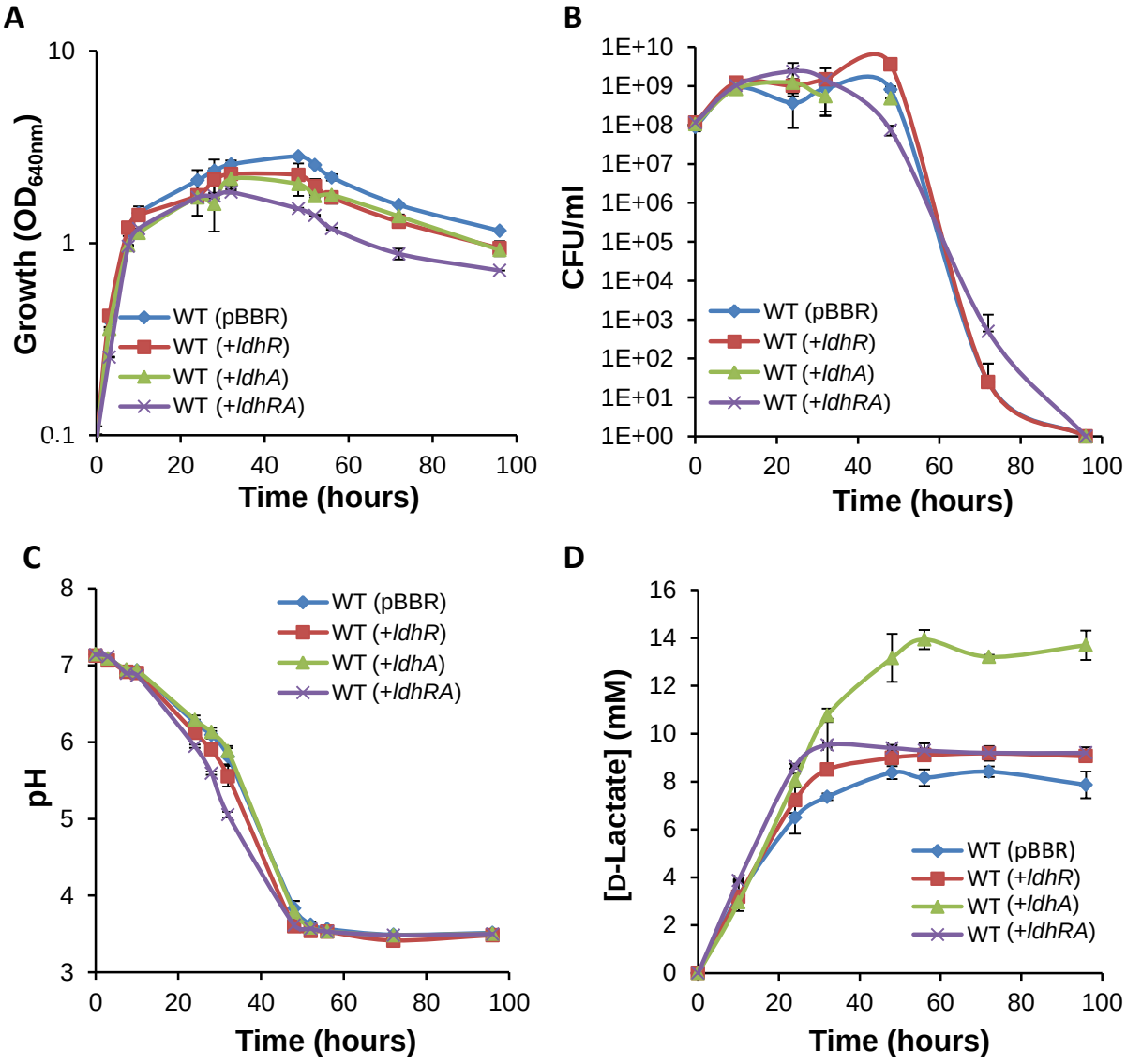

Fig. S2- Overexpression of genes *ldhR* and *ldhA* in *B. multivorans* ATCC 17616 accelerates the negative effect on cell viability. Culture growth as measured by turbidity (OD_{640nm}) (A) and colony forming units plating (B) of *B. multivorans* ATCC 17616 complemented with the empty vector pBBR1MCS, pMM137-2 expressing *ldhR* gene from the bce promoter, pLM016-2 expressing *ldhA* from the bce promoter, and pARG015-1 expressing *ldhRA* genes from their own promoter. (C) Culture medium pH measured for the indicated strains. (D) Concentration of D-lactate in the supernatants of *B. multivorans* ATCC 17616 wild-type complemented with the empty vector, or the *ldhR*, *ldhA*, and *ldhRA* genes as measured by HPLC. Genotype symbols are consistent for each panel. Cells were grown in medium supplemented with 2% D-glucose. Error bars indicate the standard deviation.

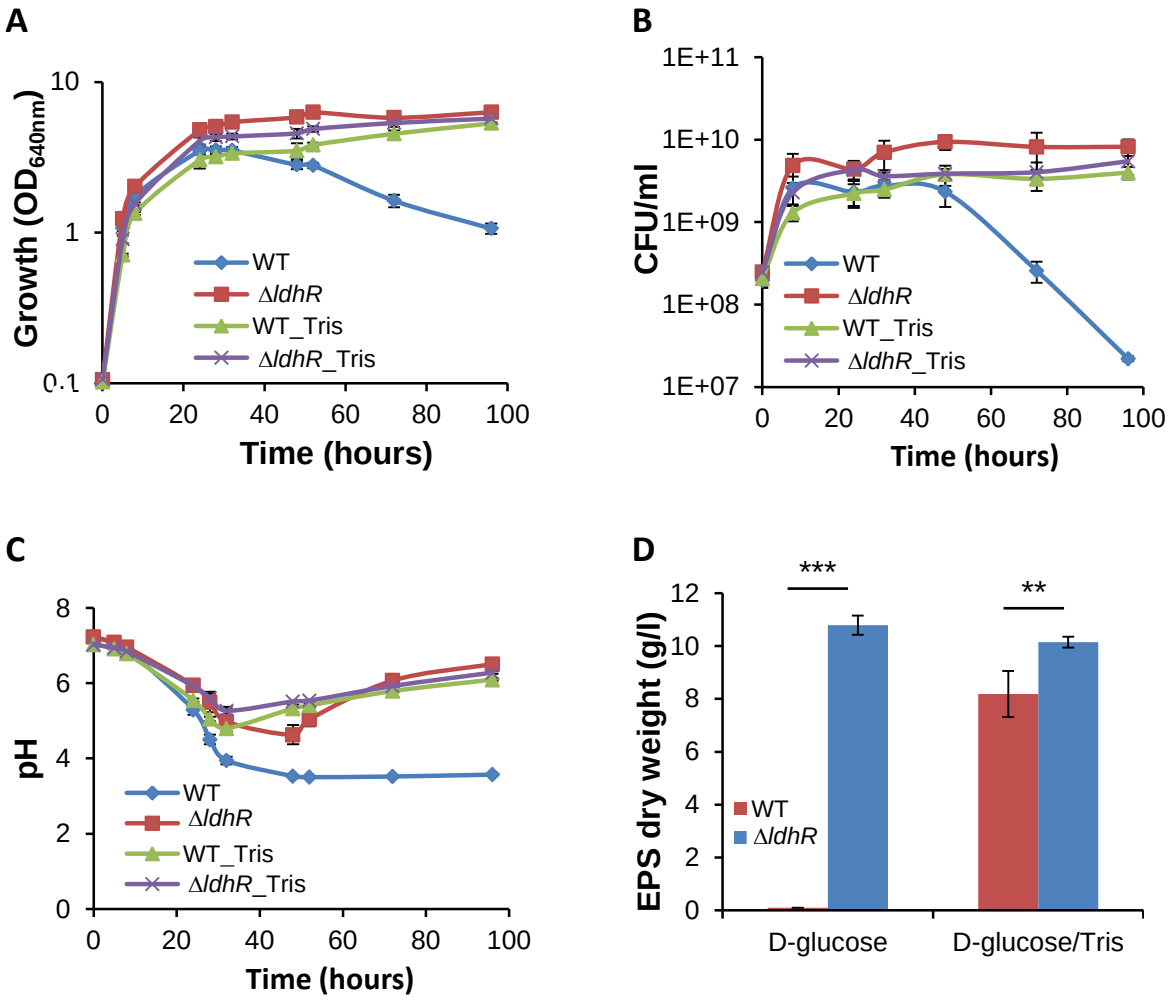

Fig. S3- Buffering glucose-rich medium prevents extreme acidification, loss of cell viability, and restores polysaccharide production by the wild-type *B. multivorans*. Culture growth as measured by turbidity (OD_{640nm}) (A) and colony forming units plating (B), and culture medium pH (C) of *B. multivorans* ATCC 17616 and the Δ*ldhR* mutant in medium containing 2% D-glucose, unbuffered or buffered with 0.2 M Tris.Cl pH 7.2. (D) EPS production in both media after 96 hours of growth, expressed as ethanol precipitate dry weight (g/l). Significantly greater amount of EPS was produced by the Δ*ldhR* mutant (**, $P<0.01$; ***, $P<0.001$), by Tukey´s HSD multiple comparison test. Error bars indicate the standard deviation.

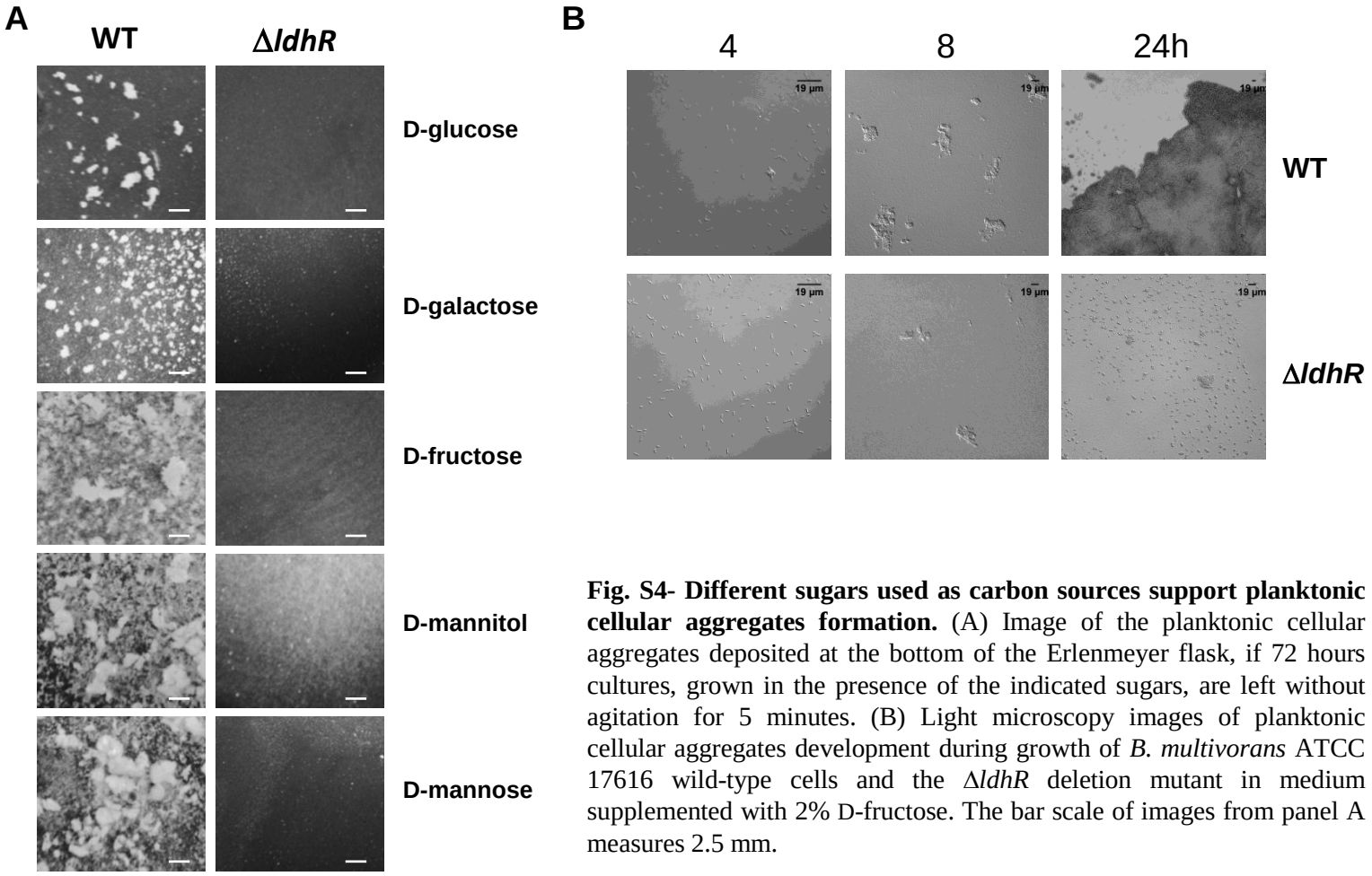

Fig. S4- Different sugars used as carbon sources support planktonic cellular aggregates formation. (A) Image of the planktonic cellular aggregates deposited at the bottom of the Erlenmeyer flask, if 72 hours cultures, grown in the presence of the indicated sugars, are left without agitation for 5 minutes. (B) Light microscopy images of planktonic cellular aggregates development during growth of *B. multivorans* ATCC 17616 wild-type cells and the Δ*ldhR* deletion mutant in medium supplemented with 2% D-fructose. The bar scale of images from panel A measures 2.5 mm.