

## FIGURE LEGENDS

Fig. 1 (A) Two-dimensional image of crude extracts of *P. aeruginosa* PAO1 incubated under pyruvate fermentation conditions for seven days. Outlined areas in the gel indicate zones of the 2D gel that are represented in Fig. 1B.

5 (B) Enlarged 2D gel images showing protein extracts of the *P. aeruginosa* PAO1 aerobic culture immediately before the shift to anaerobic pyruvate fermentation conditions and the pyruvate fermentation culture shown in Fig. 1A. Numbers of the boxed spots indicate identified proteins that are synthesized at higher levels during pyruvate fermentation, the numbers correlate with the numbers given in Table 2.

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Fig. 2. Anaerobic survival of *P. aeruginosa* wild type (■), the PAO6251 ( $\Delta arcDABC$ ) mutant (×), the  $\Delta PA4352$  mutant (▲), the  $\Delta PA3309$  mutant (●) and the complemented  $\Delta PA3309$  mutant (○) in the presence of 40 mM pyruvate. CFUs of *P. aeruginosa* wild type without pyruvate served as a control (□). *P. aeruginosa* wild type and mutants were grown  
 15 aerobically in phosphate buffered LB medium. At an  $OD_{578}$  of 0.3, cultures were transferred to rubber-stoppered bottles and 40 mM pyruvate was added. Survival under anaerobic conditions without alternative electron acceptors was determined with viable cell counts on agar plates. Graphs represent the results of at least three independent experiments. Standard deviations were 44 % until day five and below 5 % between day 10 to 20 and are omitted for  
 20 the sake of clarity.

Fig. 3 (A) Diagram showing the expression pattern of the *P. aeruginosa* wild type containing the  $P_{PA3309}$ -*lacZ* reporter gene fusion (strain KS06, grey bars) and the *anr* mutant (PAO6261) containing the  $P_{PA3309}$ -*lacZ* reporter gene fusion (KS08, white bars) during the first four days  
 25 of a pyruvate fermentation experiment. Cells were grown in phosphate buffered LB at 37 °C,

for details see Materials and Methods. Time point “0” is the aerobic culture shortly before the transfer to an anaerobic flask.

(B) Diagram showing the expression pattern of the *P. aeruginosa* wild type containing the

$P_{PA3309}$ -*lacZ* reporter gene fusion (strain KS06, grey bars) and the *anr* mutant (PAO6261)

5 containing the  $P_{PA3309}$ -*lacZ* reporter gene fusion (KS08, white bars) in a shift experiment.

Cells were grown at 37 °C in LB + 50 mM KNO<sub>3</sub> up to an OD<sub>578</sub> of 0.4 (time point “0”) and

transferred to rubber-stoppered flask for a following four hour incubation (time point “4”). β-

galactosidase activities were determined at the indicated time points. Experiments were

repeated three times.

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Fig. 4 (A) 2D gel image of crude extracts of *P. aeruginosa* PAO1 incubated under pyruvate

fermentation conditions for seven days. Boxed area contains the one or two protein spots

marked by an ellipse, which were identified to be PA3309 and represented in detail in Fig.

4B-F.

15 Enlarged 2D gel images showing the area outlined in Fig. 4A of protein extracts of *P.*

*aeruginosa* PAO1 aerobic culture (B) growing exponentially at an OD<sub>578</sub> of 0.4; (C) 16 h after

entering stationary phase; (D) after seven days under pyruvate fermentation; (E) of a six day

old biofilm (*P. aeruginosa* PAO1) and (F) a protein extract of the *anr* mutant PAO6261

grown as a biofilm for six days. Growth conditions of the biofilm experiments are outlined in

20 Materials and Methods.

Fig. 5. Vertical sections showing spatial structures of six day old *P. aeruginosa* KS15

expressing a  $P_{PA3309}$ -*gfp* transcriptional fusion. Biofilms were grown in AB minimal medium

containing 300 μM glucose (A) without and (B) supplemented with 50 mM KNO<sub>3</sub>. Scale bars

25 represent 50 μm. The biofilms were stained with Syto62 (Molecular Probes) to visualize the

biofilm matrix. Promoter activity is visualized by GFP fluorescence, represented as yellow

areas in the red colored biofilm matrix. Using the COMSTAT software (22), GFP was calculated to be expressed in 13.9 % of the total biomass in the absence of nitrate and 29.8 % when medium contained 50 mM nitrate.