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1 A minimal growth medium for *Pseudomonas azotoformans* associated with insect gut

- 2 Ismaela Cavallin, Bessem Chouaia*
- 3 Department of Environmental Sciences, Informatics and Statistics, Ca' Foscari University of Venice.
- 4 30171 Venice, Italy.
- 5 For correspondence: <u>bessem.chouaia@unive.it</u>
- 6

8 Abstract

9 Pseudomonas spp. can be found associated with several insect hosts. Some of these strains play 10 a crucial role in helping their hosts overcome biotic challenges such as plant defence effectors. 11 Defined media and optimally minimal media are instrumental in studying the physiology and metabolic pathways of microorganisms. In the case of the insect-associated Pseudomonas 12 azotoformans isolated from Galeruca laticollis' gut, we tested six previously designed 13 14 defined/minimal media. To determine the optimal medium, we compared the bacterium's growth performances on the different defined/minimal media to its growth performance on a 15 16 rich medium using the Dynamic Time Warping Algorithm. The growth results at both 25°C 17 and 30°C indicated the SMM as the optimal medium, with bacterial growth performances being the closest to a rich medium. Therefore, this medium would be highly suitable to study the 18 19 catabolic pathways for the degradation of some xenobiotics in *P. azotoformans*.

21 Introduction

Bacteria of the genus *Pseudomonas* can be found in many habitats, including host-associated ones [1]. Several *Pseudomonas* strains have been found in plants [2–4] and animals [5–7] with a variety of phenotypes ranging from pathogenicity [3,8] to mutualism [5,9,10]. During recent years, some *Pseudomonas* strains were discovered to play a crucial role in helping different insects overcome biotic challenges such as pesticides or plant defence effectors [5,9,11]. Although the bacterium's role in these symbioses is established, the molecular and biochemical mechanisms by which the symbionts are able to protect their respective hosts remain unknown.

29 Understanding how Pseudomonas species interact with their environment, host-associated or not, is of interest from a fundamental and applied perspective. For example, understanding how 30 31 these bacteria are able to degrade a wide range of xenobiotics (such as pesticides) could help 32 develop more ecologically-friendly approaches to deal with contaminations due to these 33 xenobiotics. Since such metabolic investigations would ideally be carried out outside of the 34 insect host, the development of suitable bacterial growth media that support good growth and produce consistently high viable cell counts is crucial. Such media are instrumental in studying 35 certain aspects of bacterial metabolism at the biochemical and molecular level (i.e. 36 transcriptomics). 37

However, for physiological and metabolical studies of these strains, defined/minimal media (liquid and agar) that support good growth and gives consistently high viable cell counts are a staple to unveil the mechanisms of action of metabolical processes and clarify the effects of changes in medium composition on cell growth and product formation. Defined media are culture media whose chemical composition is well known. A defined medium with only the ingredients necessary for the growth of a microorganism is called a minimal medium. Nevertheless, the number of ingredients that compose a minimal medium varies enormously

and depends mainly on which microorganism is cultivated. Chemically defined minimal mediaallow for a finer metabolic profiling of the microorganism [12,13].

Although several defined or minimal nutritional media have been used for the genus *Pseudomonas*, most of the media were developed for the most common species (e.g., *P. syringae*, *P. aeruginosa*, *P. fluorescens*;) [14–16] and the growth performance of other strains on these media have rarely been investigated. Therefore, there is a need to test the already available minimal defined media and possibly develop more suitable ones for other *Pseudomonas* species.

Here, we aimed to determine an ideal minimal defined medium to grow a strain of *P*. *azotoformans*, isolated from an insect gut, in the laboratory. To this end, we compared the growth performance of this strain on several media to a rich medium (i.e. Luria Bertani broth).

56

57 Materials and methods

58 Strain:

The bacterial strain was isolated from the gut of the beetle *Galeruca laticollis* plated on LB agar media and incubated at 30°C. The strain was initially identified based on its 16S rRNA gene sequence, and the taxonomic assignment was later confirmed through a phylogenomic analysis (data subject of a separate publication). The strain was identified as *Pseudomonas azotoformans* strain GL93.

64 *Culture media and growth conditions*:

In addition to LB media, which was used as a growth reference, five minimal media (namely
SMM, M9, MOPS, MMV, and MMP) were tested (see supplementary file S1 for details).
During the first test, bacteria grown on MMP produced fluorescent siderophores that could

potentially interfere with the optical density (OD) readings. In order to minimize the production
of these siderophores, the medium was supplemented with calcium chloride (CaCl₂;
supplementary figure S1).

71 The bacterium was incubated overnight at 30°C in a fresh LB medium prior to the experiments. Log-phase cells were collected by centrifugation at 2000 g for 10 minutes and washed three 72 times in a sterile NaCl 9‰ solution to remove any remnants of the previous growth medium. 73 Finally, the cells were concentrated to the desired concentration (10^8 cells/ml) , and 1 ml was 74 used for the inoculation of different growth media. To test if the bacterium could grow on the 75 different media, 100 ml of each medium were inoculated with 1 ml of a NaCl 9‰ solution 76 containing 10⁸ bacterial cells. Bacterial growth at 30°C was monitored for seven days, taking 77 OD readings at 24h intervals using an ONDA V-10 plus spectrophotometer. 78

79 Three minimal media (SMM, M9 and MMP+CaCl₂) as well as LB proved suitable to grow the *Pseudomonas* strain (i.e. $OD_{medium} > OD_{LB}*0.2$ after seven days) and were used in a second 80 81 experiment. A 24-well plate was prepared with 200 µl of a given medium per well, one medium 82 per row (see supplementary figure S2 for an illustration of the experimental design). Five out of the six wells for each medium were inoculated with 10 µl of the bacterial inoculate prepared 83 as outlined above. The last well was used as blank. Bacterial growth was monitored for six 84 85 days on a BioTek Synergy H1 multi-plate reader with OD readings at 600 nm every 15 minutes, 86 preceded by 5 seconds of orbital shaking to homogenize the cultures. The experiment was carried out twice, once at 30°C and a second time at 25 °C. 87

88 Data analyses:

89 the R (https://cran.r-project.org) package growthcurver 90 (<u>https://github.com/sprouffske/growthcurver</u> [17]) was used to infer the different terms of each 91 logistic curve equation Nt = $\frac{N0 K}{N0 + (K - N0) e^{(-rt)}}$, where Nt is the number of cells (or the

92 absorbance reading) at time t, N0 is the initial cell count (or absorbance reading), K is the 93 carrying capacity, and r is the growth rate. The Dynamic Time Warping (DTW; [18]) 94 Algorithm from the package **SimilarityMeasures** (https://cran.r-95 project.org/web/packages/SimilarityMeasures/) was then used to determine the similarity between the different growth curves. First, we compared the growth curves between the 96 replicates of each growth medium to exclude outliers. To this end, the values generated by 97 98 pairwise comparisons of all growth curves were aggregated in a similarity distance matrix and visualized after hierarchical clustering. Secondly, we compared the averaged growth curves 99 100 (i.e., curve resulting from the averaged values of the single curves) of the different media using 101 the same approach used for the intra-medium comparisons.

102 *Metabolic pathway assessment*:

The metabolic capacities of P. azotoformans were checked using the four complete genomes available on the NCBI database (accession numbers: LT629702, CP041236, CP014546 and CP019856). In particular, the protein sequences of each genome were downloaded and submitted to GhostKOALA (ver 2.2, [19]) for metabolic pathway reconstruction. The pathways related to the central metabolism (e.g., glycolysis, pentose phosphate, TCA cycle, purine and pyrimidine synthesis, amino acid synthesis) were checked visually for completeness.

109

110 **Results and discussion**

111 Growth performance of P. azotoformans GL93 on different media

P. azotoformans was able to grow on all tested media but with very different growth performances (Table 1, Supplementary figure S3). As such, the bacterium reached the stationary phase on LB medium in less than 24h (mean = 18h), but it took longer to reach this phase on other media. It took ca 24h for the bacteria to reach the stationary phase on MMV, ca

116	36h on M9 and more than 48h on the other media. MOPS was the medium with the longest lag
117	phase (mean = 36h). Similarly, the shortest generation time was observed on LB (ca. 1h) and
118	the longest on both MMP and SMM (ca. 20h). However, P. azotoformans reached higher
119	bacterial densities in MMP (OD ₆₀₀ = 1.18 ± 0.04) and SMM (OD ₆₀₀ = 1.3 ± 0.01) than in all
120	other media, including LB (OD ₆₀₀ \approx 1 ± 0.01). In the other media, the maximum value of OD ₆₀₀
121	remained inferior to one (namely M9: 0.3 \pm 0.01, MMP + CaCl ₂ : 0.2 \pm 0.004, MMW: 0.12 \pm
122	0.005, and MOPS: 0.03 \pm 0.004). It is noteworthy that the addition of CaCl ₂ to the MMP
123	medium reduced the production of siderophores (see Supplementary figure S1), but also had a
124	notable effect on both the optical density (which decreased from an OD_{600} of 1.18 ± 0.04 to 0.2
125	\pm 0.004) and on the generation time, lowering it from 20h to 12h. A possible explanation could
126	be that, since the bacterium no longer produced siderophores, more resources could be invested
127	into growth, resulting in a shortening of the time needed for cellular division. Considering the
128	low bacterial density achieved on MMW and MOPS compared to LB (OD_{600} of 0.12 and 0.03,
129	respectively), these two media were not included in subsequent experiments. The low growth
130	performance on these two media could be due to a chemical being present at a highly limiting
131	concentration or a growth-inhibiting factor for our strain.

132

Medium\time (h) 0 24 48 72 144 168 LB 0.03 ± 0.01 1.19 ± 0.04 0.98 ± 0.04 0.91±0.02 0.97 ± 0.04 0.94 ± 0.02 SMM 0.02 ± 0.02 0.37 ± 0.01 0.53 ± 0.02 0.66 ± 0.02 1.16 ± 0.01 1.3±0.01 M9 0.02 ± 0.02 0.07 ± 0.01 0.42 ± 0.02 0.39 ± 0.03 $0.24{\pm}0.01$ 0.3 ± 0.01 MOPS 0 0.02 ± 0 0.03 ± 0.01 0.04 ± 0.01 0.03 ± 0 0.03 ± 0 MMW 0 ± 0 0.16 ± 0 0.18 ± 0 0.15±0 0.1 ± 0 0.12 ± 0 MMP 0 0.32 ± 0.01 0.49 ± 0.02 0.65 ± 0.02 1.08 ± 0.03 1.18 ± 0.04 $MMP + CaCl_2$ 0 ± 0 0.12±0 0.18 ± 0 0.2±0.01 0.21 ± 0.01 0.2 ± 0

133 Table 1: Optical density (OD) readings at 600 nm for each medium.

134 The reported values are the average of three readings and the standard error



136 Identification of the best minimal medium for P. azotoformans



Figure 1: Growth curves obtained from the absorbance readings of the different media incubated at 25°C. The experimental curves (grey dots) correspond to the absorbance values recorded every 15 minutes for seven days. Each column corresponds to a medium, and each graph shows the growth performance in a single well (N=5 per medium). The red line represents the theoretical curve fitted over the experimental points.

The growth of *P. azotoformans* on the selected media (SMM, M9, and MMP + CaCl2, hereafter MMP) was monitored every 15 minutes at two constant temperatures (i.e., 25°C and 30°C). The data obtained from 5 replicate cultures for each medium at both temperatures was used to infer the theoretical growth curve for each culture (Figs 1, 3). Comparing the growth curves of the five replicate cultures for each medium at both temperatures (Fig. 2, 4) revealed a certain degree of variability between replicates for some conditions. In particular, there was some

variability in the exponential growth phase for M9 at both temperatures and for MMP at 30°C





153 **Figure 2**: The theoretical growth curves of the 5 replicate cultures per medium at 25°C.

154

152

In order to determine which replicates represented significant deviations from the general trend of each medium, we applied the DTW algorithm on a pairwise distance matrix between the replicates. Using this approach, several replicates were indeed determined to be outliers: 1 LB replicate, 1 SMM replicate and 2 M9 replicates at 25°C (Supplementary Figure S4), as well as 1 SMM and 1 M9 replicate at 30°C (Supplementary Figure S6). No outliers were detected among the different replicates of MMP at both temperatures. The replicates determined to be outliers were removed and the remaining replicates were used to calculate the average

theoretical growth curve for each medium at both temperatures (Supplementary Figures S5,





164

Figure 3: Growth curves obtained from the absorbance readings of the different media incubated at 30°C. The experimental curves (grey dots) correspond to the absorbance values recorded every 15 minutes for seven days. Each column corresponds to a medium, and each graph describes the growth performance in a single well (N=5 per medium). The red line represents the theoretical curve fitted over the experimental points.

Comparing the average growth curves of the three minimal media to that of the rich LB medium revealed that the SMM medium performed best and even surpassed the LB medium in terms of bacterial density (i.e. higher OD_{600}) in the stationary phase (Fig. 5A, C). In contrast, the other two minimal media M9 and MMP could not sustain the same growth and vitality performance (Fig. 5). MMP was the most different from bacterial growth on the LB medium.

- 176 This was also evident from the inferred generation time at each medium based on the average
- absorbance values: generation time at 25°C was 99 min, 432 min, 560 min and 609 min on LB,
- 178 SMM, M9 and MMP, respectively (Supplementary figure S5; tables 2, 3).
- 179



181 **Figure 4**: The theoretical growth curves of the 5 replicate cultures per medium at 30°C.

182

When comparing the growth performances on the different media at 25° and 30°C, it emerged that *Pseudomonas* generally grew more slowly at 30°C than at 25°C. Hence, generation time at 30°C was 162 min, 446 min, 684 min and 1100 min on LB, SMM, M9 and MMP, respectively (Supplementary figure S7; tables 2, 3). SMM represents the exception to this trend, since bacteria growing on SMM had similar growth rates at both temperatures (average

188 generation time at 25°C: 432 min *vs* average generation time at 30°C: 446). Despite growing

189 at a similar rate, bacterial density remained lower at 30°C than at 25°C (Fig. 5 A.C).

	30°C				25°C			
Sample	K	N0	r	Generation time	K	N0	r	Generation time
LB1	1.811	0.101	0.004	168	1.979	0.007	0.007	93
LB2	1.956	0.109	0.004	171	2.094	0.011	0.007	103
LB3	1.924	0.091	0.004	157	2.161	0.014	0.006	110
LB4	1.882	0.094	0.004	159	2.075	0.013	0.006	107
LB5	1.914	0.094	0.004	158	1.964	0.006	0.007	93
SMM1	1.896	0.006	0.002	363	2.232	0.015	0.002	412
SMM2	2.008	0.015	0.001	448	2.368	0.02	0.002	444
SMM3	1.994	0.022	0.001	497	2.408	0.02	0.002	436
SMM4	1.978	0.017	0.001	490	2.415	0.017	0.002	433
SMM5	1.770	0.020	0.001	474	2.277	0.013	0.002	445
M91	0.911	0.035	0.001	536	0.996	0.076	0.001	736
M92	1.298	0.080	0.001	831	0.98	0.048	0.001	530
M93	1.257	0.069	0.001	739	0.989	0.039	0.002	462
M94	1.172	0.070	0.001	700	1.061	0.033	0.002	379
M95	1.016	0.072	0.001	670	1.05	0.032	0.002	395
MMP1*	0.397	0.022	0.001	643	0.261	0.027	0.001	663
MMP2*	0.733	0.050	0.001	1391	0.243	0.026	0.001	599
MMP3*	1.144	0.059	0.001	1670	0.236	0.024	0.001	592
MMP4*	0.601	0.055	0.001	1247	0.218	0.023	0.001	561
MMP5*	0.459	0.025	0.001	784	0.25	0.028	0.001	626

190 Table 2: Logistic curve parameters estimated for each sample both at 30°C and 25°C

191 The medium contains CaCl₂ at the concentration reported in the supplementary file

192 Table 3: Logistic curve parameters estimated for the averaged curve for each medium both at

193 **30°C and 25°C**

	30°C					25°C			
sample	K	N0	r	Generation time	K	N0	r	Generation time	
LB	1.897	0.098	0.004	163	2.028	0.009	0.007	99	
SMM	1.963	0.014	0.002	446	2.355	0.018	0.002	432	
M9	1.063	0.062	0.001	684	0.981	0.054	0.001	560	
MMP*	0.557	0.043	0.001	1100	0.241	0.025	0.001	610	

194 The medium contains CaCl₂ at the concentration reported in the supplementary file



Figure 5: Comparison of the growth performance of *P. azotoformans* on the different media at 25°C
(A, B) and 30°C (C, D). A and C: Averaged theoretical growth curves on the different media. B and D:
Dendrograms of the curves' similarity using the DTW metric.

199

Taken together, *P. azotoformans* grown on SMM performed best compared to the other minimal media (shorter generation time and higher bacterial density). However, it was not comparable to growth on a rich medium.

Based on the metabolism of *P. azotoformans* inferred from the available genomes on the NCBI database (Table 4), we can hypothesize that the higher growth performance on the SMM medium could be due to minimal composition. SMM contains the necessary glucose as a carbon source, glutamine as a nitrogen source, K_2HPO_4 as a phosphate source and MgSO₄ as a sulfur source. These four ingredients are sufficient in *P. azotoformans* for the biosynthesis of

all the needed building blocks for metabolism and growth. The addition of other nutrients, such
as for the other media tested, will probably trigger other, possibly secondary, metabolic
pathways. These active metabolic pathways could divert resources (e.g., ATP, metabolites)
from the central metabolism, thus slowing the growth and multiplication of the bacterial cells.
This may result in slower performances when *P. azotoformans* is incubated in more complex
media.

214

215 Conclusion

An optimal medium allows for good microbial growth, which can be summarised as short generation time and high cell concentration in solution. In light of the results achieved here, the SMM medium is the most suitable medium for studying the metabolism of *P. azotoformans*. SMM outperformed the other two media (i.e., M9 and MMP), and the bacterial growth performances were consistently closer to those on LB. Furthermore, while the optimal growth temperature is 25°C, the SMM medium was the least susceptible to performance variations at a suboptimal growth temperature.

223 The better growth performance on the SMM medium could be partly explained by the fact that 224 the medium contains the necessary nutrients sufficient for the biosynthesis of all the needed building blocks for metabolism and growth. In addition, P. azotoformans is a phylogenetic 225 closer to P. fluorescens [20], which SMM was originally designed, than other *Pseudomonas* 226 species. In contrast, most of the other minimal media tested here had been designed for P. 227 aeruginosa, except for the more generic MMP. The phylogenetic proximity with P. fluorescens 228 229 may suggest similar metabolic needs between the two species. Thus, a medium developed for one of the two species could offer better growth performances for the second species than 230 231 media developed for more distant relatives.

232 Table 4: Level of completeness of the different metabolic pathways that are part of the central

233 metabolism in four P. azotoformans strains

Strain (accession number)	ssion number) LMG21611 P45A		S4 (CP014546)	F77
	(LT629702)	(CP041236)		(CP019856)
glycolysis	complete	complete	complete	complete
pentose phosphate	complete	complete	complete	complete
TCA	complete	complete	complete	complete
oxidative phosphorylarion	complete	complete	complete	complete
fatty acids synthesis	complete	complete	complete	complete
glycerophospholipid synthesis	complete	complete	complete	complete
purine synthesis	complete	complete	complete	complete
pirimidine synthesis	complete	complete	complete	complete
serine synthesis	complete	complete	complete	complete
cystein synthesis	complete	complete	complete	complete
phenylalanine synthesis	complete	complete	complete	complete
tryptophan synthesis	complete	complete	complete	complete
alanine synthesis	complete	complete	complete	complete
aspartate synthesis	complete	complete	complete	complete
asparagine sythesis	complete	complete	complete	complete
glutamine synthesis	complete	complete	complete	complete
glutamate synthesis	complete	complete	complete	complete
threonine synthesis	complete	complete	complete	complete
glycine synthesis	complete	complete	complete	complete
valine synthesis	complete	complete	complete	complete
leucine synthesis	complete	complete	complete	complete
isoleucine synthesis	complete	complete	complete	complete
lysine synthesis	complete	complete	incomplete	complete
arginine synthesis	complete	complete	complete	complete
tyrosine synthesis	complete	complete	complete	complete
histidine synthesis	complete	complete	complete	complete
methionine synthesis	complete	complete	complete	complete
Proline synthesis	complete	complete	complete	complete
Thiamine synthesis	complete	complete	complete	complete
riboflavin synthesis	complete	complete	complete	complete
vitamin B6 synthesis	complete	complete	complete	complete
NAD+/NADP+ synthesis	complete	complete	incomplete	complete
Coenzyme A synthesis	complete	complete	incomplete	complete
biotin synthesis	incomplete	incomplete	incomplete	incomplete
folate synthesis	complete	complete	complete	complete

234 Acknowledgements

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236 **References**

- Peix A, Ramírez-Bahena MH, Velázquez E. Historical evolution and current status of the taxonomy of genus *Pseudomonas*. Infect Genet Evol. 2009;9: 1132–1147. doi:10.1016/j.meegid.2009.08.001
- Anderson AJ, Kim YC. Insights into plant-beneficial traits of probiotic *Pseudomonas chlororaphis* isolates. J Med Microbiol. 2020;69: 361–371. doi:10.1099/jmm.0.001157
- Xin XF, Kvitko B, He SY. *Pseudomonas syringae*: What it takes to be a pathogen. Nat
 Rev Microbiol. 2018;16: 316–328. doi:10.1038/nrmicro.2018.17
- Flury P, Vesga P, Dominguez-Ferreras A, Tinguely C, Ullrich CI, Kleespies RG, et al.
 Persistence of root-colonising *Pseudomonas protegens* in herbivorous insects
 throughout different developmental stages and dispersal to new host plants. ISME J.
 2019;13: 860–872. doi:10.1038/s41396-018-0317-4
- 5. Shukla SP, Beran F. Gut microbiota degrades toxic isothiocyanates in a flea beetle pest.
 Mol Ecol. 2020;29: 4692–4705. doi:10.1111/mec.15657
- 250 6. Zhukova M, Sapountzis P, Schiøtt M, Boomsma JJ. Diversity and Transmission of Gut
 251 Bacteria in *Atta* and *Acromyrmex* Leaf-Cutting Ants during Development. Frontiers in
 252 Microbiology. 2017. p. 1942. doi:10.3389/fmicb.2017.01942
- 7. Montagna M, Gómez-Zurita J, Giorgi A, Epis S, Lozzia GC, Bandi C.
 Metamicrobiomics in herbivore beetles of the genus *Cryptocephalus* (Chrysomelidae):
 toward the understanding of ecological determinants in insect symbiosis. Insect Sci.
 2015;22: 340–352. doi:10.1111/1744-7917.12143
- B. Dolan SK, Kohlstedt M, Trigg S, Ramirez PV, Kaminski CF, Wittmann C, et al.
 Contextual flexibility in *Pseudomonas aeruginosa* central carbon metabolism during
 growth in single carbon sources. MBio. 2019;11: e02684-19. doi:10.1128/mBio.02684 19
- 261 9. Ceja-Navarro JA, Vega FE, Karaoz U, Hao Z, Jenkins S, Lim HC, et al. Gut microbiota
 262 mediate caffeine detoxification in the primary insect pest of coffee. Nat Commun.
 263 2015;6: 7618. doi:10.1038/ncomms8618
- Pieterse CMJ, Zamioudis C, Berendsen RL, Weller DM, Van Wees SCM, Bakker
 PAHM. Induced systemic resistance by beneficial microbes. Annu Rev Phytopathol.
 2014;52: 347–375. doi:10.1146/annurev-phyto-082712-102340
- Wang GH, Berdy BM, Velasquez O, Jovanovic N, Alkhalifa S, Minbiole KPC, et al.
 Changes in Microbiome Confer Multigenerational Host Resistance after Sub-toxic
 Pesticide Exposure. Cell Host Microbe. 2020;27: 213-224.e7.
 doi:10.1016/j.chom.2020.01.009
- 12. Singleton C, Gilman J, Rollit J, Zhang K, Parker DA, Love J. A design of experiments approach for the rapid formulation of a chemically defined medium for metabolic profiling of industrially important microbes. PLoS One. 2019;14: 7–11. doi:10.1371/journal.pone.0218208

- 13. Roth JR. Genetic Techniques in Studies of Bacterial Metabolism. Methods Enzymol.
 1970;17: 3–35. doi:10.1016/0076-6879(71)17165-0
- 27714.LaBauve AE, Wargo MJ. Growth and laboratory maintenance of *Pseudomonas*278aeruginosa.CurrProtocMicrobiol.2012;1–8.279doi:10.1002/9780471729259.mc06e01s25
- 15. Mishek HP, Stock SA, Florick JDE, Blomberg WR, Franke JD. Development of a
 chemically-defined minimal medium for studies on growth and protein uptake of *Gemmata obscuriglobus*. J Microbiol Methods. 2018;145: 40–46.
 doi:10.1016/j.mimet.2017.12.010
- 16. Sörensen M, Khakimov B, Nurjadi D, Boutin S, Yi B, Dalpke AH, et al. Comparative
 evaluation of the effect of different growth media on in vitro sensitivity to azithromycin
 in multi-drug resistant *Pseudomonas aeruginosa* isolated from cystic fibrosis patients.
 Antimicrob Resist Infect Control. 2020;9: 1–7. doi:10.1186/s13756-020-00859-7
- 288 17. Sprouffske K, Wagner A. Growthcurver: An R package for obtaining interpretable
 289 metrics from microbial growth curves. BMC Bioinformatics. 2016;17: 17–20.
 290 doi:10.1186/s12859-016-1016-7
- 18. Berndt DJ, Clifford J. Using dynamic time warping to find patterns in time series. Work
 Knowl Knowl Discov Databases. 1994;398: 359–370. Available:
 http://www.aaai.org/Papers/Workshops/1994/WS-94-03/WS94-03-031.pdf
- 19. Kanehisa M, Sato Y, Morishima K. BlastKOALA and GhostKOALA: KEGG tools for
 functional characterization of genome and metagenome sequences. J. Mol. Biol.
 2016;428: 726-731. doi:10.1016/j.jmb.2015.11.006
- 20. Anzai Y, Kim H, Park J, Wakabayashi H, Oyaizu H. Phylogenetic affiliation of the
 pseudomonads based on 16S rRNA sequence. Int J Syst Evol Microbiol. 2000;50: 1563–
 1589. doi: 10.1099/00207713-50-4-1563

301 Supplementary materials

302 **Supplementary file S1**: Composition of the different defined media used in this study.

303 **Supplementary figure S1**: The effect of adding CaCl₂ to the MMP medium on the production

304 of siderophores. A: *Pseudomonas* grown in MMP. The yellow colour indicates the production

305 of siderophores. B: *Pseudomonas* grown in MMP + CaCl2. The transparent medium indicates

that no siderophores were produced.

307 Supplementary figure S2: The experimental setup with the media disposition on the plate for
 308 the second part of the experiment (i.e., continuous long time monitoring using a plate reader).

Supplementary figure S3: Comparison of the growth performance of *P. azotoformans* on the six different media at 30°C. The curves represent the averaged theoretical growth curves for each medium.

312 Supplementary figure S4: Dendrograms showing the similarity between the theoretical 313 growth curves of the five replicates for each medium at 25°C. The dotted red line delineates 314 outliers based on the DTW metric (75% similarity threshold).

Supplementary figure S5: Averaged growth curve for each medium at 25°C after excluding outliers. The experimental curves (grey dots) correspond to the absorbance values recorded every 15 minutes for seven days. The red line represents the theoretical curve fitted over the experimental points. The inserts show the generation time inferred from the averaged absorbance values.

Supplementary figure S6: Dendrograms showing the similarity between the theoretical growth curves of the five replicates for each medium at 30°C. The dotted red line delineates outliers based on the DTW metric (75% similarity threshold).

Supplementary figure S7: Averaged growth curve for each medium at 30°C after excluding outliers. The experimental curves (grey dots) correspond to the absorbance values recorded every 15 minutes for seven days. The red line represents the theoretical curve fitted over the experimental points. The inserts show the generation time inferred from the averaged absorbance values.