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Data set: autotrophic growth rates of *Galdieria sulphuraria* (Galdieri) Merola strains isolated from Italian acidic sites

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Abstract

Intraspecific variability (strain differences) in *Galdieria sulphuraria* growth rate was assessed in laboratory growth tests carried out under autotrophic conditions. Here a complete data set of experiments is presented . 23 *G. sulphuraria* strains presently maintained in the ACUF collection of cyanobacteria and microalgae were selected on the basis of the geochemical characteristic of their sampling sites, scattered over Center-South Italy and Sicily. The results have shown that the growth rate values obtained ranged from 0.437 to 0.070, and fail to highlight any relationship between growth rates and geochemical features of sampling sites . Our results also raise the question if the observed differences in growth rates of G. *sulphuraria* strains were independent of the long-term preservation in the ACUF facilities.The marked differences in growth rate observed in this set of experiments could drive a more effective selection of *G. sulphuraria* strains amenable for biotechnological applications.

Keywords: *Galdieria sulphuraria*, strain growth rate, acidic sites, Italy

Riassunto

E' stata valutata la variabilità intraspecifica (differenze di ceppo) nel tasso di crescita di *Galdieria sulphuraria* per mezzo di test di crescita eseguiti in laboratorio in condizioni autotrofe. Qui viene presentato un set completo di dati sperimentali. 23 ceppi di *G. sulphuraria* attualmente mantenuti in ACUF, collezione di cianobatteri e microalghe, sono stati selezionati sulla base delle caratteristiche geochimiche dei loro siti di campionamento, sparsi nel Centro-Sud Italia e in Sicilia. I risultati hanno mostrato che i valori del tasso di crescita ottenuti variano da 0,437 a 0,070 e che non è stata trovata alcuna relazione tra tassi di crescita e caratteristiche geochimiche dei siti di campionamento. I nostri risultati pongono la questione se le differenze nei tassi di crescita osservati tra ceppi di *G. sulphuraria* siano indipendenti dall'acclimatazione nella collezione ACUF, nella quale sono coltivati da oltre 25 anni.I ceppi di *G. sulphuraria* che hanno mostrato un maggiore tasso di crescita potrebbero essere impiegati, dunque, per future applicazioni biotecnologiche.

Parole chiave: *Galdieria sulphuraria*, ceppi, tasso di crescita, siti acidi, Italia

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Introduction

The genus *Galdieria* (Galdieriaceae, Cyanidiophytina) encompasses five species of microalgae, thriving in hydrothermal and acidic sites worldwide (Albertano et al., 2000, Pinto et al., 2007). *Galdieria* has been defined as a polyextremophile (Capece et al., 2013), being able to grow at very low pH values, and within a temperature range from 20°C to about 50 °C (Hirooka et al., 2020), as well as under different nitrogen regimes (Hirooka and Myiagishima, 2016), in autotrophic, mixotrophic and heterotrophic conditions (Curien et al. 2021).

The biotechnological potential of *Galdieria* has been largely studied in recent years: wastewater treatments, recovery of rare elements, phycocyanin and glycogen production and nutritional applications represent only some of the promising fields for its exploitation (Čížková et al., 2019, Čížková et al., 2020, Carbone et al., 2020). However, it has infrequently been investigated if different strains assigned to the same *Galdieria* species exhibit different growth performances, especially in terms of growth rate, a feature that plays a fundamental role in the selection of a strain for biotechnological use.

In 1970s' Taddei and Pinto (1976) performed an extensive sampling campaign, collecting populations of Cyanidiophytina in more than 120 acid sites of Italy. Four main kinds of acidic sites were described by the authors: fumaroles, putizzes, sulphur springs, and sulphur mines. Most of *Galdieria sulphuraria* strains collected by Taddei and Pinto, were subsequently isolated and are still maintained as a part of the ACUF phycobank of living strains (D'Elia et al. 2018, www.acuf.net). To ascertain if *G. sulphuraria* strains from different sites show peculiar growth rates, we selected 23 strains isolated from Central and South Italy acid sites with different environmental conditions. For these strains, data were available on growth experiments performed under controlled light, temperature and stirring conditions (Di Cioccio, 2009). A complete dataset related to these experiments is presented here, along with variance-based analysis, with the aim of identifying possible promising strains for biotechnological applications.

Materials and Methods

The data are taken from Di Cioccio (2009), and concerns axenic cultures of 23 *G. sulphuraria* strains. In Table 1 geographic coordinates and characteristics of sampling sites are reported for the considered strains.

The strains were grown in 100 ml Erlenmayer flasks (50 ml culture volume) in sterile modified Allen medium (see recipe at www.acuf.net) acidified at pH 1.5, and at a controlled temperature of 35 ± 1°C. The flasks were placed on a plexiglass shaking apparatus rotating at 62 rpm, with a continuous irradiance of 100 μmol photons m^{-2} s^{-1} , provided by a daylight fluorescent lamps (Osram Lumilux T5 FC). Triplicate flasks were used for each strain, with a starting inoculum of 0.5 ml from an exponential growing culture (ranging from 0.2 to 0.4 O.D.). Growth was monitored by measuring the optical density at 550 nm with a Secoman 250 Spectrophotometer.

The exponential phase was assessed for each strain based on the logarithmic shape of its growth curve, after logarithmic conversion of optical densities, and the growth rate was calculated according to the equation:

$$\frac{Ln(Nt)-Ln(N0)}{(t-t0)}$$

Where:

- Nt is the optical density at the final time
- N_0 is the optical density at the initial time
- T is the final time (days)
- T_0 is the initial time (days)

The growth rates obtained for each strain were compared using the analysis of variance (ANOVA) followed by Tukey test for multiple comparisons. Values of $p \leq 0.05$ were considered statistically significant and are reported in Table S2.

All the analyses were performed using the GraphPad Prism 8.00 software for Windows (GraphPad Software, San Diego, CA, USA).

A principal component analysis (PCA) was employed to evaluate the existence of gradients between growth rates and environmental factors . In addition, the types of sampling acid sites were used as supplementary qualitative variables. The analysis was performed using XLSTAT-Sensory version 2015.6.01 (Addinsoft).

Table 1 - List of the sampling sites of *G. sulphuraria* strains included in this study. fm = fumaroles; pz = putizze ; ss = sulphur springs; sm = sulphur mines.

Strain n°	site	habitat	longitude	latitude	pH	Temperature (°C)
2	Pozzuoli, Pisciarelli (NA)	fm	1°41'47''E	40°49'46''N	1.0	36
4	Castellamare del Golfo, Terme Segestane (TP)	ss	0°26'23''E	37°58'18''N	1.0	35
5	Veiano, acque minerali (VT)	ss	0°20'29''W	42°13'05''N	1.0	38
6	Acquasanta Terme, terme (AP)	ss	0°57'29''E	42°46'19''N	1.6	35
7	Viterbo, Zitelle (VT)	ss	0°23'29''W	42°25'32''N	1.5	21
9	Nepi, terme dei Gracchi (VT)	ss	0°06'38''W	42°12'58''N	0.8	12
10	Tivoli, Acque Albule (RM)	ss	0°16'00''E	41°58'02''N	1.0	24
13	Alì Terme, Granata Cassibile (ME)	ss	2°58'39''E	38°00'32''N	1.0	32
16	Cerchiara di Cal., piscina di Ninfe (CS)	ss	3°57'01''E	39°50'26''N	1.5	20
17	Contursi, bagni Forlenza (NA)	ss	2°46'32''E	40°39'06''N	1.5	31
21	Lipari, isola Vulcano (ME)	ss	2°30'27''E	38°24'50''N	1.0	38
22	Casamicciola Terme, Montecito (NA)	fm	1°26'37''E	40°44'24''N	1.5	34
63	Comitini, Comitini Solfare (AG)	sm	13°29'28''E	37°24'23''N	1.4	25
64	Favara, Ciavolotta (AG)	sm	13°39'00''E	37°16'15''N	1.5	25
70	Rocca San Felice, Mefite (Ansanto) (AV)	pz	2°41'36''E	40°58'25''N	0.9	18
75	Casteltermini, Cozzo Disi (AG)	sm	13°41'07''E	37°30'42''N	1.5	25
79	Palazzo al Piano (SI)	pz	11°09'44"E	43°16'96"N	1.5	32
80	Raddusa, Destricella (CT)	sm	14°32'36''E	37°31'03''N	2.0	22
101	Aidone, Baccarata (EN)	sm	14°27'30''E	37°23'00''N	1.0	25
133	Acireale, Santa Venera (CT)	ss	2°34'00''E	37°36'14''N	2.5	21
162	Strongoli, Comero (CZ)	ss	4°34'03''E	39°16'28''N	1.3	18
215	Vico Equense, Scraio (NA)	ss	1°58'57''E	40°40'17''N	1.5	19
216	Monterotondo, Lago Boracifero (GR)	fm	10°48'43"E	43°09'04" N	1.5	34

Results and Discussion

In Table S1 are reported the original experimental data obtained from growth experiments carried out by Di Cioccio (2009) on 23 *G. sulphuraria* strains, and in Table S2 are presented the results of ANOVA test containing only the significant scores.

The growth rates obtained for each strain are shown in figure 1A.

The values obtained ranged from 0.437 for the strain 162 to 0.070 for strain 117.

Overall, statistical analyses seem to suggest significant yet not definitive differences between growth rates of selected *G.sulphuraria* strains, due to the reduced sample size. The ordination of strains with respect to growth rates and geochemical characteristics of sampling sites was evaluated by PCA, (Fig. 1B). The percentage of variance explained by PC1 was 39.86%, while PC2 explained 34.90% of the variance.

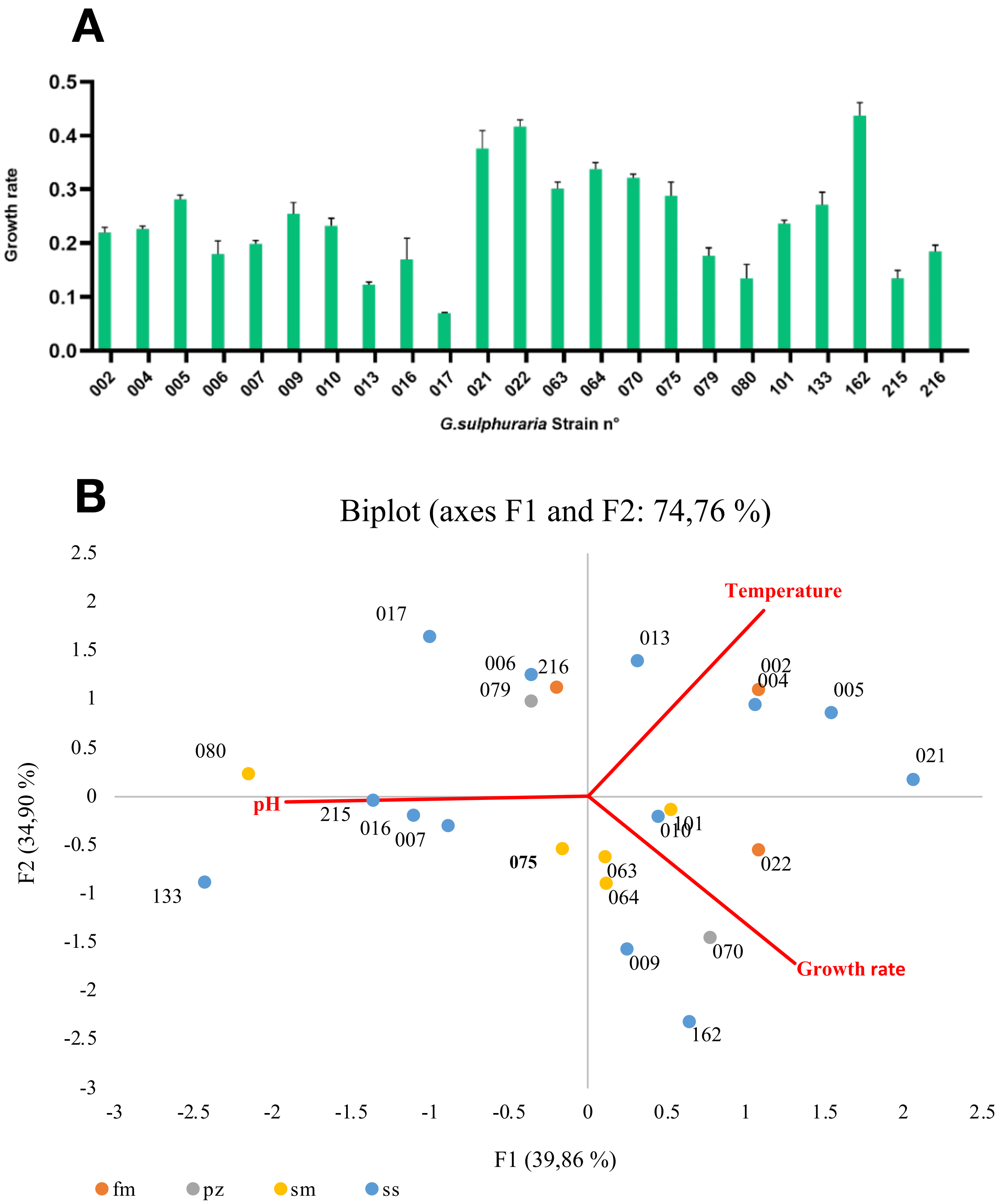

Figure 1: A) Growth rates of the 23 *G. sulphuraria* strains presented in Table S1. Data are shown as the means ± SD of three independent experiments. B) Principal component analysis to test the relationships between *G.sulphuraria* and environmental factors (red arrows). For the abbreviations “fm”, “pz”, “sm”, “ss” see Table 1.

Author contributions

Conceptualization, M.P. and A.D.M.; methodology, M.P.; software, M.P. ; A.D.M. and N.M.; validation, A.D.M., and N.M.; data curation, M.P.; writing–original draft preparation, M.P.; writing–review and editing,M.P.; N.M.; A.D.M.; supervision, A.D.M. All authors have read and agreed to the published version of the manuscript.

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Table S1: Experimental data obtained from growth experiments carried out by Di Cioccio (2009) on 23 *G. sulphuraria* strains. A, B, C - OD at 550 nm.

	ACUF strain N°			
Starting date 11/12/07	**75**	**64**		
A	0.136	0.06		
B	0.121	0.059		
C	0.09	0.07		
average value (AV)	0.116	0.063		
AV x 100	11.6	6.3		
Log AV x 100	1.06	0.8		
12/12/2007				
A	0.19	0.082		
B	0.156	0.091		
C	0.135	0.082		
average value (AV)	0.16	0.085		
AV x 100	16	8.5		
Log AV x 100	1.2	0.93		
13/12/2007				
A	0.289	0.11		
B	0.277	0.121		
C	0.202	0.135		
average value (AV)	0.256	0.122		
AV x 100	25.6	12.2		
Log AV x 100	1.4	1.08		
17/12/2007				
A	0.692	0.544		
B	0.687	0.533		
C	0.643	0.542		
average value (AV)	0.674	0.54		
AV x 100	67.4	54		
Log AV x 100	1.82	1.73		
Starting date 21/01/08	**63**	**17**		
A	0.073	0.133		
B	0.069	0.126		

C	0.073	0.126
average value (AV)	0.072	0.128
AV x 100	7.2	12.8
Log AV x 100	0.86	1.1
22/01/2008		
A	0.063	0.126
B	0.059	0.111
C	0.062	0.112
average value (AV)	0.061	0.116
AV x 100	6.1	11.6
Log AV x 100	0.78	1.06
23/01/2008		
A	0.077	0.26
B	0.076	0.124
C	0.074	0.125
average value (AV)	0.076	0.17
AV x 100	7.6	17
Log AV x 100	0.88	1.23
24/01/2008		
A	0.095	0.178
B	0.11	0.18
C	0.105	0.172
average value (AV)	0.103	0.177
AV x 100	10.3	17.7
Log AV x 100	1.01	1.25
25/01/2008		
A	0.14	0.208
B	0.145	0.216
C	0.133	0.228
average value (AV)	0.139	0.217
AV x 100	13.9	21.7
Log AV x 100	1.14	1.33
28/01/2008		
A	0.402	0.467
B	0.42	0.444

C	0.4	0.466
average value (AV)	0.407	0.459
AV x 100	40.7	45.9
Log AV x 100	1.6	1.66
Starting date 25/01/08	**70**	
A	0.056	
B	0.054	
C	0.058	
average value (AV)	0.056	
AV x 100	5.6	
Log AV x 100	0.748	
28/01/2008		
A	0.136	
B	0.131	
C	0.128	
average value (AV)	0.132	
AV x 100	13.2	
Log AV x 100	1.12	
29/01/2008		
A	0.239	
B	0.22	
C	0.214	
average value (AV)	0.224	
AV x 100	22.4	
Log AV x 100	1.35	
30/01/2008		
A	0.301	
B	0.287	
C	0.271	
average value (AV)	0.286	
AV x 100	28.6	
Log AV x 100	1.45	
31/01/2008		
A	0.436	
B	0.399	

C	0.405			
average value (AV)	0.413			
AV x 100	41.3			
Log AV x 100	1.61			
01/02/2008				
A	0.502			
B	0.511			
C	0.494			
average value (AV)	0.502			
AV x 100	50.2			
Log AV x 100	1.7			
Starting date 01/02/08	**6**	**7**	**9**	**10**
A	0.101	0.115	0.071	0.076
B	0.12	0.113	0.65	0.069
C	0.1	0.112	0.059	0.088
average value (AV)	0.107	0.113	0.26	0.078
AV x 100	10.7	11.3	26	7.8
Log AV x 100	1.03	1.05	1.41	0.89
04/02/2008				
A	0.115	0.192	0.117	0.143
B	0.116	0.189	0.109	0.136
C	0.119	0.19	0.08	0.14
average value (AV)	0.117	0.19	0.102	0.14
AV x 100	11.7	19	10.2	14
Log AV x 100	1.07	1.28	1.01	1.14
05/02/2008				
A	0.184	0.248	0.156	0.198
B	0.169	0.237	0.155	0.203
C	0.127	0.233	0.137	0.214
average value (AV)	0.16	0.239	0.149	0.205
AV x 100	16	23.9	14.9	20.5
Log AV x 100	1.2	1.38	1.17	1.31
06/02/2008				
A	0.279	0.342	0.208	0.285
B	0.268	0.35	0.181	0.289

C	0.216	0.338	0.173	0.3
average value (AV)	0.254	0.343	0.187	0.291
AV x 100	25.4	34.3	18.7	29.1
Log AV x 100	1.4	1.53	1.27	1.46
07/02/2008				
A	0.41	0.45	0.28	0.377
B	0.389	0.477	0.258	0.399
C	0.326	0.486	0.248	0.413
average value (AV)	0.375	0.471	0.262	0.396
AV x 100	37.5	47.1	26.2	39.6
Log AV x 100	1.57	1.67	1.42	1.6
Starting date 08/02/08	**2**	**4**	**5**	
A	0.108	0.06	0.068	
B	0.118	0.058	0.078	
C	0.125	0.06	0.072	
average value (AV)	0.117	0.059	0.073	
AV x 100	11.7	5.9	7.3	
Log AV x 100	1.06	0.77	0.86	
11/02/2008				
A	0.289	0.075	0.16	
B	0.31	0.081	0.165	
C	0.317	0.09	0.173	
average value (AV)	0.305	0.082	0.166	
AV x 100	30.5	8.2	16.6	
Log AV x 100	1.48	0.91	1.22	
12/02/2008				
A	0.397	0.14	0.186	
B	0.412	0.15	0.191	
C	0.48	0.14	0.202	
average value (AV)	0.43	0.143	0.193	
AV x 100	4.3	14.3	19.3	
Log AV x 100	0.63	1.15	1.28	
13/02/2008				
A	0.551	0.205	0.263	
B	0.546	0.21	0.279	

C	0.538	0.208	0.298		
average value (AV)	0.545	0.208	0.28		
AV x 100	54.5	20.8	28		
Log AV x 100	1.73	1.31	1.46		
14/02/2008					
A	0.632	0.259	0.366		
B	0.651	0.266	0.371		
C	0.646	0.253	0.387		
average value (AV)	0.643	0.259	0.375		
AV x 100	6.4	25.9	37.5		
Log AV x 100	0.8	1.41	1.57		
15/02/2008					
A	0.775	0.305	0.471		
B	0.801	0.311	0.512		
C	0.805	0.315	0.529		
average value (AV)	0.794	0.31	0.504		
AV x 100	7.94	31	50.4		
Log AV x 100	0.89	1.49	1.7		
Starting date 15/02/08	**162**	**21**	**22**		
A	0.02	0.045	0.033		
B	0.028	0.031	0.04		
C	0.026	0.035	0.035		
average value (AV)	0.025	0.037	0.036		
AV x 100	2.5	3.7	3.6		
Log AV x 100	0.4	0.57	0.55		
18/02/2008					
A	0.093	0.133	0.149		
B	0.089	0.142	0.15		
C	0.093	0.147	0.158		
average value (AV)	0.092	0.141	0.152		
AV x 100	9.2	14.1	15.2		
Log AV x 100	0.96	1.15	1.18		
19/02/2008					
A	0.148	0.191	0.212		
B	0.137	0.192	0.208		

C	0.123	0.189	0.204		
average value (AV)	0.136	0.191	0.208		
AV x 100	13.6	19.1	20.8		
Log AV x 100	1.13	1.28	1.31		
20/02/2008					
A	0.226	0.263	0.336		
B	0.239	0.271	0.34		
C	0.243	0.272	0.335		
average value (AV)	0.236	0.269	0.337		
AV x 100	23.6	26.9	33.7		
Log AV x 100	1.37	1.49	1.52		
21/02/2008					
A	0.331	0.333	0.44		
B	0.337	0.34	0.451		
C	0.318	0.336	0.439		
average value (AV)	0.329	0.336	0.443		
AV x 100	32.9	33.6	44.3		
Log AV x 100	1.5	1.5	1.6		
Starting date 11/04/08	**13**	**133**	**215**	**216**	**79**
A	0.18	0.106	0.175	0.218	0.098
B	0.169	0.11	0.17	0.217	0.1
C	0.17	0.092	0.156	0.226	0.087
average value (AV)	0.173	0.1027	0.167	0.2203	0.095
AV x 100	17.3	10.3	16.7	22	9.5
Log AV x 100	1.24	1.01	1.22	1.34	0.98
14/04/2008					
A	0.252	0.145	0.252	0.396	0.152
B	0.29	0.152	0.261	0.376	0.129
C	0.288	0.186	0.256	0.379	0.073
average value (AV)	0.277	0.161	0.2563	0.3837	0.118
AV x 100	27.7	16.2	25.6	38.4	11.8
Log AV x 100	1.44	1.21	1.41	1.58	1.07
16/04/2008					
A	0.34	0.335	0.306	0.576	0.241
B	0.331	0.361	0.31	0.548	0.211

C	0.352	0.457	0.308	0.531	0.14
average value (AV)	0.341	0.384	0.308	0.5517	0.1973
AV x 100	34.1	38.4	30.8	5.5	19.7
Log AV x 100	1.53	1.58	1.49	0.74	1.29
17/04/2008					
A	0.426	0.479	0.354	0.648	0.322
B	0.418	0.459	0.37	0.655	0.298
C	0.428	0.483	0.378	0.6	0.195
average value (AV)	0.424	0.477	0.367	0.6343	0.2717
AV x 100	42.4	47.4	36.7	6.3	27.2
Log AV x 100	1.62	1.67	1.56	0.8	1.43
23/05/2008	16	101	80		
A	0.134	0.13	0.12		
B	0.154	0.13	0.19		
C	0.166	0.117	0.1		
average value (AV)	0.151	0.124	0.137		
AV x 100	15.1	12.4	13.7		
Log AV x 100	1.79	1.09	1.13		
26/05/2008					
A	0.195	0.219	0.152		
B	0.21	0.198	0.143		
C	0.207	0.188	0.125		
average value (AV)	0.204	0.202	0.14		
AV x 100	20.4	20.2	14		
Log AV x 100	1.3	1.3	1.14		
28/05/2008					
A	0.323	0.394	0.254		
B	0.301	0.367	0.234		
C	0.242	0.306	0.185		
average value (AV)	0.289	0.356	0.224		
AV x 100	28.9	35.6	22.4		
Log AV x 100	1.46	1.55	1.35		
29/05/2008					
A	0.405	0.474	0.333		
B	0.389	0.459	0.299		

C	0.29	0.405	0.253
average value (AV)	0.361	0.446	0.295
AV x 100	36.1	44.6	29.5
Log AV x 100	1.55	1.65	1.47
30/05/2008			
A	0.416	0.496	0.328
B	0.4	0.488	0.394
C	0.369	0.417	0.22
average value (AV)	0.395	0.467	0.314
AV x 100	39.5	46.7	31.4
Log AV x 100	1.6	1.67	1.5

Table S2: Results of ANOVA test containing only the significant scores. Data shown are means ± SD of three independent values. * indicates $p < 0.05$, ** indicates $p < 0.005$, and **** indicates $p < 0.0001$.

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Summary	p value
Strain002 vs. Strain005	-0.0624	-0.1204 to -0.004387	*	0.023
Strain002 vs. Strain 013	0.09637	0.03835 to 0.1544	****	<0.0001
Strain002 vs. Strain017	0.1492	0.09119 to 0.2072	****	<0.0001
Strain002 vs. Strain 021	-0.1567	-0.2147 to -0.09865	****	<0.0001
Strain002 vs. Strain 022	-0.1974	-0.2554 to -0.1394	****	<0.0001
Strain002 vs. Strain063	-0.08163	-0.1396 to -0.02362	***	0.0005
Strain002 vs. Strain064	-0.1185	-0.1765 to -0.06049	****	<0.0001
Strain002 vs. Strain 070	-0.1019	-0.1599 to -0.04389	****	<0.0001
Strain002 vs. Strain075	-0.06863	-0.1266 to -0.01062	**	0.007
Strain002 vs. Strain 080	0.08477	0.02675 to 0.1428	***	0.0002
Strain002 vs. Strain 162	-0.2178	-0.2758 to -0.1598	****	<0.0001
Strain002 vs. Strain 215	0.08483	0.02682 to 0.1428	***	0.0002
Strain004 vs. Strain 013	0.1035	0.04552 to 0.1615	****	<0.0001
Strain004 vs. Strain017	0.1564	0.09835 to 0.2144	****	<0.0001
Strain004 vs. Strain 021	-0.1495	-0.2075 to -0.09149	****	<0.0001
Strain004 vs. Strain 022	-0.1903	-0.2483 to -0.1323	****	<0.0001
Strain004 vs. Strain063	-0.07447	-0.1325 to -0.01645	**	0.0022
Strain004 vs. Strain064	-0.1113	-0.1693 to -0.05332	****	<0.0001
Strain004 vs. Strain 070	-0.09473	-0.1527 to -0.03672	****	<0.0001
Strain004 vs. Strain075	-0.06147	-0.1195 to -0.003453	*	0.0273
Strain004 vs. Strain 080	0.09193	0.03392 to 0.1499	****	<0.0001
Strain004 vs. Strain 162	-0.2107	-0.2687 to -0.1527	****	<0.0001
Strain004 vs. Strain 215	0.092	0.03399 to 0.1500	****	<0.0001
Strain005 vs. Strain 006	0.1019	0.04392 to 0.1599	****	<0.0001
Strain005 vs. Strain 007	0.08277	0.02475 to 0.1408	***	0.0004
Strain005 vs. Strain 013	0.1588	0.1008 to 0.2168	****	<0.0001
Strain005 vs. Strain016	0.1123	0.05425 to 0.1703	****	<0.0001

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Summary	p value
Strain005 vs. Strain017	0.2116	0.1536 to 0.2696	****	<0.0001
Strain005 vs. Strain 021	-0.09427	-0.1523 to -0.03625	****	<0.0001
Strain005 vs. Strain 022	-0.135	-0.1930 to -0.07702	****	<0.0001
Strain005 vs. Strain 079	0.1056	0.04755 to 0.1636	****	<0.0001
Strain005 vs. Strain 080	0.1472	0.08915 to 0.2052	****	<0.0001
Strain005 vs. Strain 162	-0.1554	-0.2134 to -0.09742	****	<0.0001
Strain005 vs. Strain 215	0.1472	0.08922 to 0.2052	****	<0.0001
Strain005 vs. Strain 216	0.09743	0.03942 to 0.1554	****	<0.0001
Strain006 vs. Strain 009	-0.07463	-0.1326 to -0.01662	**	0.0021
Strain006 vs. Strain 017	0.1097	0.05165 to 0.1677	****	<0.0001
Strain006 vs. Strain 021	-0.1962	-0.2542 to -0.1382	****	<0.0001
Strain006 vs. Strain 022	-0.237	-0.2950 to -0.1790	****	<0.0001
Strain006 vs Strain063	-0.1212	-0.1792 to -0.06315	****	<0.0001
Strain006 vs. Strain 064	-0.158	-0.2160 to -0.1000	****	<0.0001
Strain006 vs. Strain 070	-0.1414	-0.1994 to -0.08342	****	<0.0001
Strain006 vs. Strain075	-0.1082	-0.1662 to -0.05015	****	<0.0001
Strain006 vs. Strain 133	-0.0914	-0.1494 to -0.03339	****	<0.0001
Strain006 vs. Strain 162	-0.2574	-0.3154 to -0.1994	****	<0.0001
Strain007 vs. Strain 013	0.076	0.01799 to 0.1340	**	0.0016
Strain007 vs. Strain 017	0.1288	0.07082 to 0.1868	****	<0.0001
Strain007 vs. Strain 021	-0.177	-0.2350 to -0.1190	****	<0.0001
Strain007 vs. Strain 022	-0.2178	-0.2758 to -0.1598	****	<0.0001
Strain007 vs. Strain063	-0.102	-0.1600 to -0.04399	****	<0.0001
Strain007 vs. Strain064	-0.1389	-0.1969 to -0.08085	****	<0.0001
Strain007 vs. Strain 070	-0.1223	-0.1803 to -0.06425	****	<0.0001
Strain007 vs. Strain 075	-0.089	-0.1470 to -0.03099	****	<0.0001
Strain007 vs. Strain 080	0.0644	0.006387 to 0.1224	*	0.0159
Strain007 vs. Strain 133	-0.07223	-0.1302 to -0.01422	**	0.0034
Strain007 vs. Strain 162	-0.2382	-0.2962 to -0.1802	****	<0.0001
Strain007 vs. Strain 215	0.06447	0.006453 to 0.1225	*	0.0157

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Summary	p value
Strain009 vs. Strain 013	0.1315	0.07345 to 0.1895	****	<0.0001
Strain009 vs. Strain016	0.08497	0.02695 to 0.1430	***	0.0002
Strain009 vs. Strain017	0.1843	0.1263 to 0.2423	****	<0.0001
Strain009 vs. Strain 021	-0.1216	-0.1796 to -0.06355	****	<0.0001
Strain009 vs. Strain 022	-0.1623	-0.2203 to -0.1043	****	<0.0001
Strain009 vs. Strain064	-0.0834	-0.1414 to -0.02539	***	0.0003
Strain009 vs. Strain 070	-0.0668	-0.1248 to -0.008787	*	0.0101
Strain009 vs. Strain 079	0.07827	0.02025 to 0.1363	***	0.001
Strain009 vs. Strain 080	0.1199	0.06185 to 0.1779	****	<0.0001
Strain009 vs. Strain 162	-0.1827	-0.2407 to -0.1247	****	<0.0001
Strain009 vs. Strain 215	0.1199	0.06192 to 0.1779	****	<0.0001
Strain009 vs. Strain 216	0.07013	0.01212 to 0.1281	**	0.0052
Strain010 vs. Strain 013	0.1092	0.05115 to 0.1672	****	<0.0001
Strain010 vs. Strain016	0.06267	0.004653 to 0.1207	*	0.0219
Strain010 vs. Strain017	0.162	0.1040 to 0.2200	****	<0.0001
Strain010 vs. Strain 021	-0.1439	-0.2019 to -0.08585	****	<0.0001
Strain010 vs. Strain 022	-0.1846	-0.2426 to -0.1266	****	<0.0001
Strain010 vs. Strain063	-0.06883	-0.1268 to -0.01082	**	0.0068
Strain010 vs. Strain 064	-0.1057	-0.1637 to -0.04769	****	<0.0001
Strain010 vs. Strain 070	-0.0891	-0.1471 to -0.03109	****	<0.0001
Strain010 vs. Strain 080	0.09757	0.03955 to 0.1556	****	<0.0001
Strain010 vs. Strain 162	-0.205	-0.2630 to -0.1470	****	<0.0001
Strain010 vs. Strain 215	0.09763	0.03962 to 0.1556	****	<0.0001
Strain013 vs. Strain 021	-0.253	-0.3110 to -0.1950	****	<0.0001
Strain013 vs. Strain 022	-0.2938	-0.3518 to -0.2358	****	<0.0001
Strain013 vs. Strain063	-0.178	-0.2360 to -0.1200	****	<0.0001
Strain013 vs. Strain 064	-0.2149	-0.2729 to -0.1569	****	<0.0001
Strain013 vs. Strain 070	-0.1983	-0.2563 to -0.1403	****	<0.0001
Strain013 vs. Strain075	-0.165	-0.2230 to -0.1070	****	<0.0001
Strain013 vs. Strain 101	-0.1133	-0.1713 to -0.05532	****	<0.0001

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Summary	p value
Strain013 vs. Strain 133	-0.1482	-0.2062 to -0.09022	****	<0.0001
Strain013 vs. Strain 162	-0.3142	-0.3722 to -0.2562	****	<0.0001
Strain013 vs. Strain 216	-0.06133	-0.1193 to -0.003320	*	0.0279
Strain016 vs. Strain017	0.09933	0.04132 to 0.1573	****	<0.0001
Strain016 vs. Strain 021	-0.2065	-0.2645 to -0.1485	****	<0.0001
Strain016 vs. Strain 022	-0.2473	-0.3053 to -0.1893	****	<0.0001
Strain016 vs. Strain063	-0.1315	-0.1895 to -0.07349	****	<0.0001
Strain016 vs. Strain064	-0.1684	-0.2264 to -0.1104	****	<0.0001
Strain016 vs. Strain070	-0.1518	-0.2098 to -0.09375	****	<0.0001
Strain016 vs. Strain075	-0.1185	-0.1765 to -0.06049	****	<0.0001
Strain016 vs. Strain 101	-0.06683	-0.1248 to -0.008820	**	0.01
Strain016 vs. Strain 133	-0.1017	-0.1597 to -0.04372	****	<0.0001
Strain016 vs. Strain 162	-0.2677	-0.3257 to -0.2097	****	<0.0001
Strain017 vs. Strain 021	-0.3059	-0.3639 to -0.2479	****	<0.0001
Strain017 vs. Strain 022	-0.3466	-0.4046 to -0.2886	****	<0.0001
Strain017 vs. Strain063	-0.2308	-0.2888 to -0.1728	****	<0.0001
Strain017 vs. Strain064	-0.2677	-0.3257 to -0.2097	****	<0.0001
Strain017 vs. Strain 070	-0.2511	-0.3091 to -0.1931	****	<0.0001
Strain017 vs. Strain075	-0.2178	-0.2758 to -0.1598	****	<0.0001
Strain017 vs. Strain 079	-0.106	-0.1640 to -0.04802	****	<0.0001
Strain017 vs. Strain 080	-0.06443	-0.1224 to -0.006420	*	0.0158
Strain017 vs. Strain 101	-0.1662	-0.2242 to -0.1082	****	<0.0001
Strain017 vs. Strain 133	-0.2011	-0.2591 to -0.1431	****	<0.0001
Strain017 vs. Strain 162	-0.367	-0.4250 to -0.3090	****	<0.0001
Strain017 vs. Strain 215	-0.06437	-0.1224 to -0.006353	*	0.016
Strain017 vs. Strain 216	-0.1142	-0.1722 to -0.05615	****	<0.0001
Strain021 vs. Strain 063	0.07503	0.01702 to 0.1330	**	0.0019
Strain021 vs. Strain 075	0.08803	0.03002 to 0.1460	***	0.0001
Strain021 vs. Strain 079	0.1998	0.1418 to 0.2578	****	<0.0001
Strain021 vs. Strain 080	0.2414	0.1834 to 0.2994	****	<0.0001

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Summary	p value
Strain021 vs. Strain 101	0.1397	0.08169 to 0.1977	****	<0.0001
Strain021 vs. Strain 133	0.1048	0.04679 to 0.1628	****	<0.0001
Strain021 vs. Strain 162	-0.06117	-0.1192 to -0.003153	*	0.0288
Strain021 vs. Strain 215	0.2415	0.1835 to 0.2995	****	<0.0001
Strain021 vs. Strain 216	0.1917	0.1337 to 0.2497	****	<0.0001
Strain022 vs Strain063	0.1158	0.05779 to 0.1738	****	<0.0001
Strain022 vs. Strain064	0.07893	0.02092 to 0.1369	***	0.0009
Strain022 vs. Strain 070	0.09553	0.03752 to 0.1535	****	<0.0001
Strain022 vs. Strain 075	0.1288	0.07079 to 0.1868	****	<0.0001
Strain022 vs. Strain 079	0.2406	0.1826 to 0.2986	****	<0.0001
Strain022 vs. Strain 080	0.2822	0.2242 to 0.3402	****	<0.0001
Strain022 vs. Strain 101	0.1805	0.1225 to 0.2385	****	<0.0001
Strain022 vs. Strain 133	0.1456	0.08755 to 0.2036	****	<0.0001
Strain022 vs. Strain 215	0.2823	0.2243 to 0.3403	****	<0.0001
Strain022 vs. Strain 216	0.2325	0.1745 to 0.2905	****	<0.0001
Strain063 vs. Strain 079	0.1248	0.06679 to 0.1828	****	<0.0001
Strain063 vs. Strain 080	0.1664	0.1084 to 0.2244	****	<0.0001
Strain063 vs. Strain 101	0.06467	0.006653 to 0.1227	*	0.0151
Strain063 vs. Strain 162	-0.1362	-0.1942 to -0.07819	****	<0.0001
Strain063 vs. Strain 215	0.1665	0.1085 to 0.2245	****	<0.0001
Strain063 vs. Strain 216	0.1167	0.05865 to 0.1747	****	<0.0001
Strain064 vs. Strain 079	0.1617	0.1037 to 0.2197	****	<0.0001
Strain064 vs. Strain 080	0.2033	0.1453 to 0.2613	****	<0.0001
Strain064 vs. Strain 101	0.1015	0.04352 to 0.1595	****	<0.0001
Strain064 vs. Strain 133	0.06663	0.008620 to 0.1246	*	0.0104
Strain064 vs. Strain 162	-0.09933	-0.1573 to -0.04132	****	<0.0001
Strain064 vs. Strain 215	0.2033	0.1453 to 0.2613	****	<0.0001
Strain064 vs. Strain 216	0.1535	0.09552 to 0.2115	****	<0.0001
Strain 070 vs. Strain 079	0.1451	0.08705 to 0.2031	****	<0.0001
Strain 070 vs. Strain 080	0.1867	0.1287 to 0.2447	****	<0.0001

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Summary	p value
Strain 070 vs. Strain 101	0.08493	0.02692 to 0.1429	***	0.0002
Strain 070 vs. Strain 162	-0.1159	-0.1739 to -0.05792	****	<0.0001
Strain 070 vs. Strain 215	0.1867	0.1287 to 0.2447	****	<0.0001
Strain 070 vs. Strain 216	0.1369	0.07892 to 0.1949	****	<0.0001
Strain075 vs. Strain 079	0.1118	0.05379 to 0.1698	****	<0.0001
Strain075 vs. Strain 080	0.1534	0.09539 to 0.2114	****	<0.0001
Strain075 vs. Strain 162	-0.1492	-0.2072 to -0.09119	****	<0.0001
Strain075 vs. Strain 215	0.1535	0.09545 to 0.2115	****	<0.0001
Strain075 vs. Strain 216	0.1037	0.04565 to 0.1617	****	<0.0001
Strain079 vs. Strain 101	-0.06013	-0.1181 to -0.002120	*	0.0346
Strain079 vs. Strain 133	-0.09503	-0.1530 to -0.03702	****	<0.0001
Strain079 vs. Strain 162	-0.261	-0.3190 to -0.2030	****	<0.0001
Strain080 vs. Strain 101	-0.1017	-0.1597 to -0.04372	****	<0.0001
Strain080 vs. Strain 133	-0.1366	-0.1946 to -0.07862	****	<0.0001
Strain080 vs. Strain 162	-0.3026	-0.3606 to -0.2446	****	<0.0001
Strain101 vs. Strain 162	-0.2009	-0.2589 to -0.1429	****	<0.0001
Strain101 vs. Strain 215	0.1018	0.04379 to 0.1598	****	<0.0001
Strain133 vs. Strain 162	-0.166	-0.2240 to -0.1080	****	<0.0001
Strain133 vs. Strain 215	0.1367	0.07869 to 0.1947	****	<0.0001
Strain133 vs. Strain 216	0.0869	0.02889 to 0.1449	***	0.0002
Strain162 vs. Strain 215	0.3027	0.2447 to 0.3607	****	<0.0001
Strain162 vs. Strain 216	0.2529	0.1949 to 0.3109	****	<0.0001