

Appendix

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Appendix

A Mono- vs co-culture

These tables were used for figures 2 – 15.

Rep.	Pos.	NS/S	t0	t11	t22	t33	t44
1.1	703340	NS	0	0.998	1	1	0.997
3.5	703340	NS	0	0	0	0.411	0.732

Table S1: **All SNPs in the same position as a fixed SNP.** The fixed SNP 1 is in replicate 1.1. Rep.: microcosm and replicate; Pos.: position on the chromosome; NS: non-synonymous, S: synonymous; t0–t44: sequencing time points.

Rep.	Pos.	NS/S	t0	t11	t22	t33	t44
1.1	83654	NS	0	0.402	0.912	0.675	0.584
1.2	83654	NS	0	0.237	1	1	1
3.1	83654	NS	0	0	0.241	0	0
3.2	83654	NS	0	0	0.182	0	0
3.3	83654	NS	0	0	0.161	0	0
3.4	83654	NS	0	0	0.064	0	0
3.5	83654	NS	0	0.286	0	0.452	0.946
4.2	83654	NS	0	0	0.437	0	0
4.3	83654	NS	0	0	0	0	0.462

Table S2: **All SNPs in the same position as a fixed SNP.** The fixed SNP 3 is in replicate 1.2. Rep.: microcosm and replicate; Pos.: position on the chromosome; NS: non-synonymous, S: synonymous; t0–t44: sequencing time points.

Rep.	Pos.	NS/S	t0	t11	t22	t33	t44
3.2	677536	NS	0	0	0.245	0.813	0.988

Table S3: **All SNPs in the same position as a fixed SNP.** The fixed SNP 4 is in replicate 3.2. Rep.: microcosm and replicate; Pos.: position on the chromosome; NS: non-synonymous, S: synonymous; t0–t44: sequencing time points.

Rep.	Pos.	NS/S	t0	t11	t22	t33	t44
3.4	2863371	NS	0	0	0.769	0.977	0.999

Table S4: **All SNPs in the same position as a fixed SNP.** The fixed SNP 5 is in replicate 3.4. Rep.: microcosm and replicate; Pos.: position on the chromosome; NS: non-synonymous, S: synonymous; t0–t44: sequencing time points.

Rep.	Pos.	NS/S	t0	t11	t22	t33	t44
4.5	1372439	S	0	0	0	0	0.983

Table S5: **All SNPs in the same position as a fixed SNP.** The fixed SNP 6 is in replicate 4.5. Rep.: microcosm and replicate; Pos.: position on the chromosome; NS: non-synonymous, S: synonymous; t0–t44: sequencing time points.

Rep.	Pos.	NS/S	t0	t11	t22	t33	t44
4.1	555455	NS	0	0.995	0.997	0.999	0.983

Table S6: **All SNPs in the same position as a fixed SNP.** The fixed SNP 7 is in replicate 4.1. Rep.: microcosm and replicate; Pos.: position on the chromosome; NS: non-synonymous, S: synonymous; t0–t44: sequencing time points

Rep.	Pos.	NS/S	t0	t11	t22	t33	t44
4.4	181352	NS	0	0	0.996	0.997	0.999

Table S7: **All SNPs in the same position as a fixed SNP.** The fixed SNP 8 is in replicate 4.4. Rep.: microcosm and replicate; Pos.: position on the chromosome; NS: non-synonymous, S: synonymous; t0–t44: sequencing time points

Rep.	Pos.	NS/S	t0	t11	t22	t33	t44
2.1	5466695	NS	0	0	0	0.998	0.992
2.4	5466695	NS	0	0	0.071	0	0
3.5	5466695	NS	0	0	0	0.11	0
4.3	5466695	NS	0	0.332	0.244	0.256	0.481
4.4	5466695	NS	0	0.149	0	0	0
4.5	5466695	NS	0	0	0	0.16	0
4.5	5466695	S	0	0.346	0.149	0	0.232

Table S8: **All SNPs in the same position as a fixed SNP.** The fixed SNP 11 is in replicate 2.1. Rep.: microcosm and replicate; Pos.: position on the chromosome; NS: non-synonymous, S: synonymous; t0–t44: sequencing time points

Rep.	Pos.	NS/S	t0	t11	t22	t33	t44
2.1	5466897	S	0	0	0.987	0	0
2.2	5466897	S	0	0.991	0.99	1	1
2.3	5466897	S	0	0.9	0.991	1	1
2.4	5466897	S	0	0.98	0.934	1	0.998
3.2	5466897	S	0	0.099	0.058	0	0
4.4	5466897	S	0	0	0.784	0.979	0.969

Table S9: **All SNPs in the same position as a fixed SNP.** The fixed SNPs 12 and 15 are in replicate 2.2 and 2.3, respectively. Rep.: microcosm and replicate; Pos.: position on the chromosome; NS: non-synonymous, S: synonymous; t0–t44: sequencing time points

Rep.	Pos.	NS/S	t0	t11	t22	t33	t44
2.3	5643748	NS	0	0	0.808	0.968	0.997

Table S10: **All SNPs in the same position as a fixed SNP.** The fixed SNP 16 is in replicate 2.3. Rep.: microcosm and replicate; Pos.: position on the chromosome; NS: non-synonymous, S: synonymous; t0–t44: sequencing time points

Rep.	Pos.	NS/S	t0	t11	t22	t33	t44
2.5	4436504	NS	0	0.966	1	1	0.991

Table S11: **All SNPs in the same position as a fixed SNP.** The fixed SNP 18 is in replicate 2.5. Rep.: microcosm and replicate; Pos.: position on the chromosome; NS: non-synonymous, S: synonymous; t0–t44: sequencing time points

Rep.	Pos.	NS/S	t0	t11	t22	t33	t44
2.5	5466537	NS	0	0.95	1	1	1
4.2	5466537	NS	0	0	0.074	0	0.092

Table S12: **All SNPs in the same position as a fixed SNP.** The fixed SNP 19 is in replicate 2.5. Rep.: microcosm and replicate; Pos.: position on the chromosome; NS: non-synonymous, S: synonymous; t0–t44: sequencing time points

Rep.	Pos.	NS/S	t0	t11	t22	t33	t44
3.1	5466905	NS	0	0	0	0.129	0
3.3	5466905	NS	0	0.289	0.58	0.932	1
4.5	5466905	NS	0	0	0.765	0.266	0.502

Table S13: **All SNPs in the same position as a fixed SNP.** The fixed SNP 24 is in replicate 3.3. Rep.: microcosm and replicate; Pos.: position on the chromosome; NS: non-synonymous, S: synonymous; t0–t44: sequencing time points

Rep.	Pos.	NS/S	t0	t11	t22	t33	t44
3.4	5466552	NS	0	0.264	0.682	0.982	1
4.3	5466552	NS	0	0.208	0.374	0.48	0.235
4.5	5466552	NS	0	0	0	0.455	0

Table S14: **All SNPs in the same position as a fixed SNP.** The fixed SNP 27 is in replicate 3.4. Rep.: microcosm and replicate; Pos.: position on the chromosome; NS: non-synonymous, S: synonymous; t0–t44: sequencing time points

B SNPs under selective pressure

These tables were used to calculate the dN/dS ratio in table 5.

Rep.	Pos.	NS/S
1	703340	NS
3	703340	NS

Table S15: **All SNPs of a gene.** SNP 1 belongs to gene AGTU.c01_006770/ropA_2. It is found in the first chromosome of *A. tumefaciens*. Rep.: microcosm; Pos.: position on the chromosome; NS: non-synonymous, S: synonymous.

Rep.	Pos.	NS/S
1	83654	NS
1	83654	NS
3	83654	NS
3	83654	NS
3	83654	NS
3	83654	NS
3	83654	NS
3	83654	NS
4	83654	NS
4	83654	NS

Table S16: **All SNPs of a gene.** SNP 3 belongs to gene AGTU.c01_000790/fadD. It is found in the first chromosome of *A. tumefaciens*. Rep.: microcosm; Pos.: position on the chromosome; NS: non-synonymous, S: synonymous.

Rep.	Pos.	NS/S
3	677536	NS
4	677412	NS
4	677452	NS

Table S17: **All SNPs of a gene.** SNP 4 belongs to gene AGTU.c01_006500/AGTU_00650. It is found in the first chromosome of *A. tumefaciens*. Rep.: microcosm; Pos.: position on the chromosome; NS: non-synonymous, S: synonymous.

Rep.	Pos.	NS/S
1	2863324	S
3	2863324	S
3	2863324	S
3	2863324	S
3	2863371	NS
3	2863324	S
4	2863324	S
4	2863324	S
4	2863324	S
4	2863324	S
4	2863324	S

Table S18: **All SNPs of a gene.** SNP 5 belongs to gene AGTU.c01_027410/yjgN. It is found in the first chromosome of *A. tumefaciens*. Rep.: microcosm; Pos.: position on the chromosome; NS: non-synonymous, S: synonymous.

Rep.	Pos.	NS/S
4	1372426	NS
4	1372426	NS
4	1372427	S
4	1372438	NS
4	1372439	S
4	1372444	NS
4	1372445	NS

Table S19: **All SNPs of a gene.** SNP 6 belongs to gene AGTU.c01_013260/AGTU_01326. It is found in the first chromosome of *A. tumefaciens*. Rep.: microcosm; Pos.: position on the chromosome; NS: non-synonymous, S: synonymous.

Rep.	Pos.	NS/S
4	555455	NS

Table S20: **All SNPs of a gene.** SNP 7 belongs to gene AGTU.c02_004900/secA. It is found in the second chromosome of *A. tumefaciens*. Rep.: microcosm; Pos.: position on the chromosome; NS: non-synonymous, S: synonymous.

Rep.	Pos.	NS/S
1	181433	S
1	181288	S
1	181412	S
3	181477	S
3	181433	S
4	181417	NS
4	181427	S
4	181337	NS
4	181426	NS
4	181352	NS
4	181297	S

Table S21: **All SNPs of a gene.** SNP 8 belongs to gene AGTU.p02_001750/frmR. It is found in the second plasmid of *A. tumefaciens*. Rep.: microcosm; Pos.: position on the chromosome; NS: non-synonymous, S: synonymous.

Rep.	Pos.	NS/S
2	5466695	NS
2	5466897	S
2	5466897	S
2	5466843	NS
2	5466897	S
2	5466695	NS
2	5466897	S
2	5466537	NS
3	5466516	NS
3	5466573	NS
3	5466855	NS
3	5466905	NS
3	5466500	NS
3	5466516	NS
3	5466534	NS
3	5466565	S
3	5466897	S
3	5466500	NS
3	5466558	S
3	5466905	NS
3	5466516	NS
3	5466538	S
3	5466552	NS
3	5466534	NS
3	5466695	NS
4	5466495	NS
4	5466500	NS
4	5466534	NS
4	5466537	NS
4	5466564	S
4	5466543	NS
4	5466552	NS
4	5466564	S
4	5466695	NS
4	5466516	NS
4	5466543	NS
4	5466695	NS
4	5466855	NS
4	5466897	S
4	5466534	NS
4	5466543	NS
4	5466552	NS
4	5466695	NS
4	5466695	S
4	5466905	NS
4	5466960	NS

Table S22: **All SNPs of a gene.** SNPs11, 12, 15, 19, 24, and 27 belong to gene COTE.c01_050720/nasR. It is found in the first chromosome of *C. testosteroni*. Rep.: microcosm; Pos.: position on the chromosome; NS: non-synonymous, S: synonymous.

Rep.	Pos.	NS/S
2	5643680	S
2	5643748	NS
4	5644709	NS
4	5643683	NS

Table S23: **All SNPs of a gene.** SNP 16 belongs to gene COTE.c01_052100/trkH. It is found in the first chromosome of *C. testosteroni*. Rep.: microcosm; Pos.: position on the chromosome; NS: non-synonymous, S: synonymous.

Rep.	Pos.	NS/S
2	4436504	NS
4	4436637	S

Table S24: **All SNPs of a gene.** SNP 18 belongs to gene COTE.c01_041260/COTE_04126. It is found in the first chromosome of *C. testosteroni*. Rep.: microcosm; Pos.: position on the chromosome; NS: non-synonymous, S: synonymous.

Rep.	Pos.	NS/S
3	1628511	NS

Table S25: **All SNPs of a gene.** SNP 30 belongs to gene MISA.c01_015490/MISA_01549. It is found in the first chromosome of *M. saperdae*. Rep.: microcosm; Pos.: position on the chromosome; NS: non-synonymous, S: synonymous.

Rep.	Pos.	NS/S
3	2587910	NS
3	2587947	S
3	2587957	S
3	2587968	NS
3	2587969	NS
3	2587971	S
3	2587974	NS
3	2587957	NS
3	2587957	S
3	2587959	S
3	2587975	NS
3	2587947	S
3	2587957	NS
3	2587957	S
3	2587265	S
3	2587322	NS
3	2588195	NS
3	2588369	NS
4	2588096	NS
4	2588348	NS

Table S26: **All SNPs of a gene.** SNP 31 belongs to gene MISA.c01_024740/puuP_3. It is found in the first chromosome of *M. saperdae*. Rep.: microcosm; Pos.: position on the chromosome; NS: non-synonymous, S: synonymous.

Rep.	Pos.	NS/S
3	699404	NS
3	699272	NS
4	699272	NS
4	698447	NS
4	699259	NS
4	699272	NS

Table S27: **All SNPs of a gene.** SNP 32 belongs to gene MISA.c01_006580/ftsH_1 It is found in the first chromosome of *M. saperdae*. Rep.: microcosm; Pos.: position on the chromosome; NS: non-synonymous, S: synonymous.

Rep.	Pos.	NS/S
3	1281919	S
3	1281919	S
4	1282387	NS
4	1281695	NS
4	1281972	NS

Table S28: **All SNPs of a gene.** SNP 33 belongs to gene MISA.c01_012280/dedA_1 It is found in the first chromosome of *M. saperdae*. Rep.: microcosm; Pos.: position on the chromosome; NS: non-synonymous, S: synonymous.

Rep.	Pos.	NS/S
3	3643922	S
3	3643435	NS
4	3643534	NS
4	3644008	NS

Table S29: **All SNPs of a gene.** SNP 34 belongs to gene MISA.c01_035320/srlR. It is found in the first chromosome of *M. saperdae*. Rep.: microcosm; Pos.: position on the chromosome; NS: non-synonymous, S: synonymous.

Rep.	Pos.	NS/S
4	2116572	NS
4	2116572	NS
4	2116572	NS
4	2116572	NS
4	2116572	NS

Table S30: **All SNPs of a gene.** SNPs 38, 39, 40, and 41 belong to gene OCAN.c02_020540/yjiA. It is found in the first chromosome of *M. saperdae*. Rep.: microcosm; Pos.: position on the chromosome; NS: non-synonymous, S: synonymous.

C Discussion

Here all remaining genes are discussed in detail in terms of how a mutation affects evolution in MWF.

- **ropA__2/SNP 1.** I assume this gene is under positive or neutral selection because only non-synonymous (NS) SNPs occurred. The outer membrane protein IIIA has a porin activity. In the environment of the MWF, it might be necessary to adapt certain transporter proteins to let substances in or keep them out of the cell. Additionally, the fixed SNP occurs in the monoculture. Therefore, I assume that a mutation of this gene is an adaption to the environment.
- **AGTU__00650/SNP 4.** I assume this gene is under positive or neutral selection because only NS SNPs occurred. The function of the protein is uncertain, but it likely has a structural rather than an enzymatic function. Additionally, the fixed SNP occurs in a co-culture. However, I do not make an assumption about why there needs to be an adaptation of this protein.
- **yjgN/SNP 5.** I assume this gene is under negative selection because a dN/dS ratio of 0.25 in microcosm 3 was calculated, and only the fixed SNP is NS. All other SNPs are synonymous (S). Additionally, the fixed SNP occurs in a co-culture. The function of the protein is unknown. Therefore, I do not make an assumption about why this gene has a mutation.
- **AGTU__01326/SNP 6.** I assume this gene is under positive selection because a dN/dS ratio of 2.5 was calculated. However, the fixed SNP is S and occurs in a co-culture. The gene codes for an uncharacterised protein whose function we do not know. Therefore, I do not make an assumption about why there needs to be an adaptation of this protein.
- **secA/SNP 7.** I assume this gene is under positive or neutral selection because only the fixed SNP occurs, which is NS. The protein SecA, part of the Sec protein translocase complex, acts as an ATPase. Because of the other organisms and the environment of the MWF, it might be that ATP is limited. Additionally, the fixed SNP occurs in a co-culture. Therefore, I assume that a mutation of this gene is an adaption to the other microbes.
- **trkH/SNP 16.** I assume this gene is under positive or neutral selection because a dN/dS ratio of 1 in microcosm 2 was calculated, and all but one SNP was found to be NS. Additionally, the fixed SNP is NS. The gene encodes for a potassium-chloride-symporter. An adaptation of this transporter might be necessary because of the special composition of the MWF. This adaptation may be necessary because the protein is essential for survival. Additionally, the fixed SNP occurs in the monoculture. Therefore, I assume that a mutation of this gene is an adaption to the environment.
- **COTE__04126/SNP 18.** I assume this gene is under neutral selection because one NS SNP and one S SNP in two different microcosms were found. However, the fixed SNP is NS and occurs in the monoculture. This gene codes for an uncharacterised protein whose function we do not know. Therefore, I do not make an assumption about why this gene has a mutation.
- **MISA__01549/SNP 30.** I assume this gene is under positive or neutral selection because only the fixed SNP occurs, which is NS. The protein has ATP-binding activity and belongs to the pho regulon. In MWF, the production of ATP might be hard, and ATP might be limited. Therefore, an adaptation of this protein might be necessary. The pho regulon is involved in the transport and utilisation of various forms of combined phosphates and free phosphate. It is activated when phosphate is limited. Depending on the composition of MWF, this might be the case, and an adaptation of the regulon might be necessary. Additionally, the fixed SNP occurs in a co-culture. However, I assume that a mutation of this gene is an adaption to the environment.

- **puuP_3/SNP 31.** I assume this gene is under positive selection because, in microcosm 3, a dN/dS ratio of 1.25 was calculated and in microcosm 4, only NS SNPs were found. Additionally, the fixed SNP is NS. The protein helps in the uptake of an alternative energy source when glucose is not available. In the MWF, glucose might be limited, if even present. Depending on the competitive strength of the microbe, the other microbes already took all glucose. An adaptation to take up another energy source might be necessary. Additionally, the fixed SNP occurs in a co-culture. However, I assume that a mutation of this gene is an adaption to the environment.
- **ftsH_1/SNP 32.** I assume this gene is under positive or neutral selection because only NS SNPs occurred. The protein is ATP dependent. In MWF, the production of ATP might be hard, and energy sources to make ATP might be limited because of the other microbes. An adaptation of this protein might be necessary. Additionally, the fixed SNP occurs in a co-culture. Therefore, I assume that a mutation of this gene is an adaption to the other microbes.
- **dedA_1/SNP 33.** I assume this gene is under neutral selection because only S SNPs in microcosm 3 and only NS SNP in microcosm 4 were found. However, the fixed SNP is NS and occurs in a co-culture. The protein belongs to a family of uncharacterised proteins. Therefore, I do not make an assumption about why this gene has a mutation.
- **srlR/SNP 34.** I assume this gene is under positive or neutral selection because a dN/dS ratio of 1 in microcosm 3 was calculated, and in microcosm 4, only NS SNPs occurred. Additionally, the fixed SNP is NS. The protein is a repressor of a system responsible for the uptake and phosphorylation of specific carbohydrates. In the MWF, it might be necessary to adapt this system because the specific carbohydrates might not be present. Additionally, the fixed SNP occurs in a co-culture. However, I assume that a mutation of this gene is an adaption to the environment.