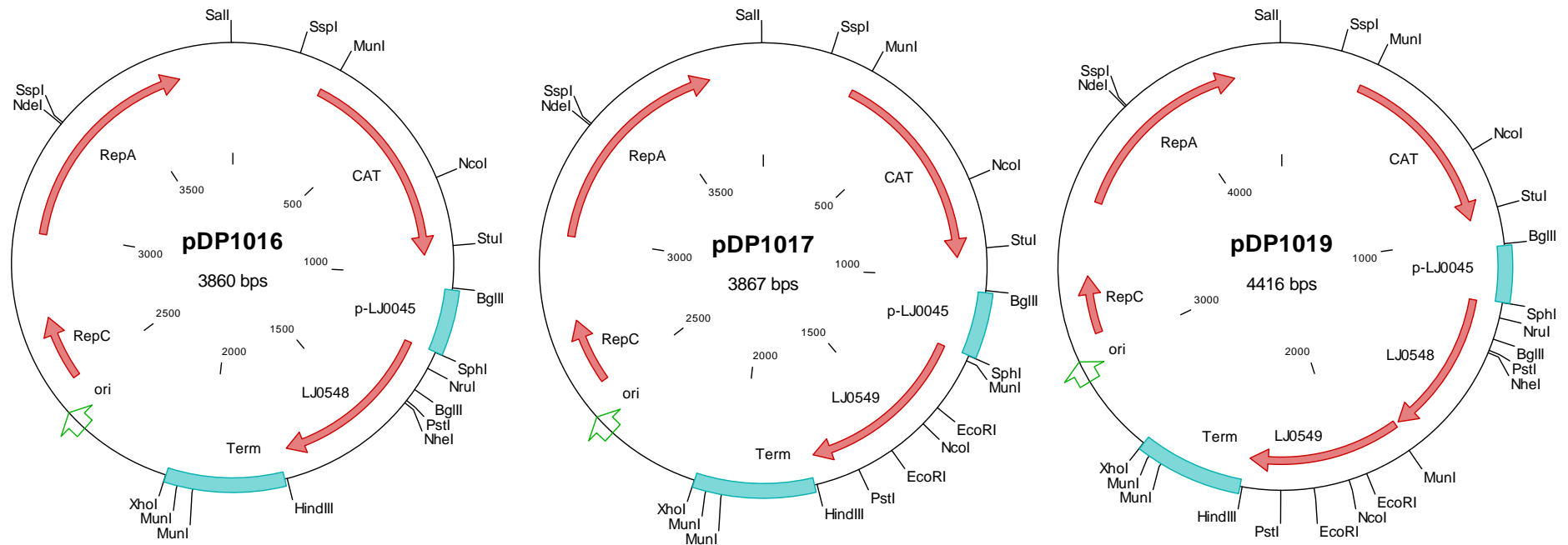


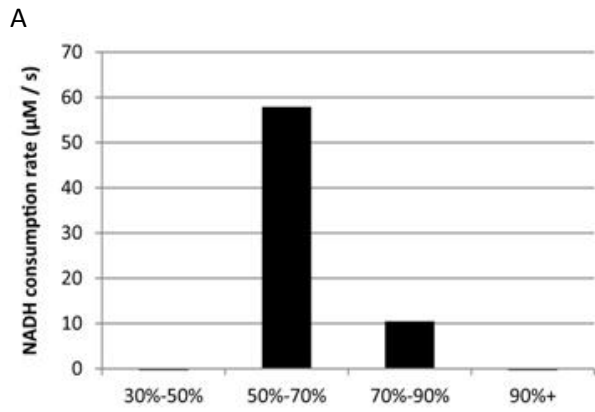
1 Supplementary material

2 **Table S1: Primers used in construction of *L. johnsonii* mutant strains.**

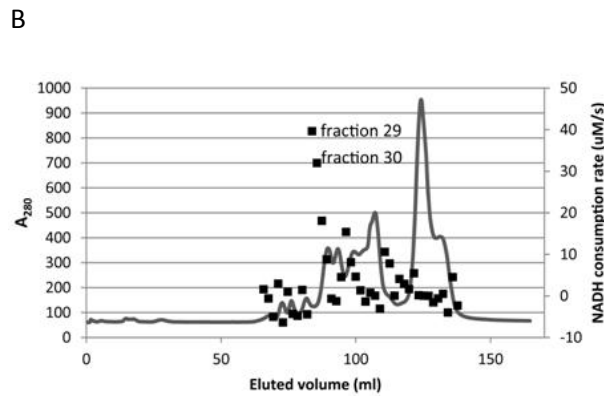
Region	ID	Sequence
<i>LJ_1826</i> 5' homology 5' primer	A	CTACTCCAGAAGAAGTCG
<i>LJ_1826</i> 5' homology 3' primer	B	ATATATGGATCCAAGGGTGAAGGACAAAGC
<i>LJ_1853</i> 3' homology 5' primer	C	GGGTTTTGCATTCCAGTC
<i>LJ_1853</i> 3' homology 3' primer	D	ATATATGGTACCTTGCCAAAAGTTGGAGC
<i>LJ_1254- LJ_1255</i> 5' homology 5'	E	ATATATGAGCTCAATACTCAATGTAAGCGC
<i>LJ_1254- LJ_1255</i> 5' homology 3'	F	ATATATGGATCCTGATTGCAGGTCCACCTG
<i>LJ_1254- LJ_1255</i> 3' homology 5'	G	ATATATGAATTCTGATTTAGTAGCTGCTGG
<i>LJ_1254- LJ_1255</i> 3' homology 3'	H	ATATATGGTACCCAGAACTATGAAGGCTC
<i>LJ_0548- LJ_0549</i> 5' homology 5'	I	ATATATGAGCTCGCTGCAAATGAAGGGCTAGA
<i>LJ_0548- LJ_0549</i> 5' homology 3'	J	ATATATGGATCCGCGTTGCTACCTACAATGGC
<i>LJ_0548- LJ_0549</i> 3' homology 5'	K	ATATATGAATTCTTTAATTGGTCATGCTGCAG
<i>LJ_0548- LJ_0549</i> 3' homology 3'	L	ATATATGGTACCAGCTCGCCTTCACTACGGAG
<i>LJ_1125</i> term 5'	O	ATATATAAGCTTTGCCAATGGATAACCAGG
<i>LJ_1125</i> term 3'	P	ATATATCTCGAGAATCTCTCTTGGACTTGC
<i>LJ_0045</i> promoter 5'	R	ATATATAGATCTCATTATCATAAGGCACCC
<i>LJ_0045</i> promoter 3'	S	ATATATGAGCTCGCTAGCGCATGCATTAAACCTCC GTC
SphI site before <i>LJ_0548</i>	T	ATATATGCATGCAACTCTTGCCATTGTAGG
SphI site before <i>LJ_0549</i>	U	ATATATGCATGCAATTACTAGCAATTGTTGG
HindIII site after <i>LJ_0548</i>	V	ATATATAAGCTTAATTTTCATGGGTCGTTCTC
HindIII site after <i>LJ_0549</i>	W	ATATATAAGCTTGGTTTTAACTTATTTTTGAGCTTG



3 **Figure S1: Genetic maps of *LJ_0548* and/or *LJ_0549* overexpression plasmids.**

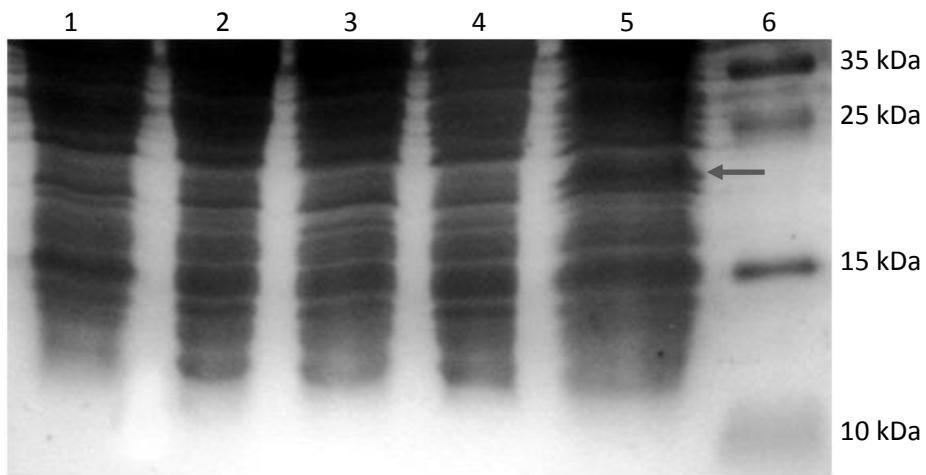


4

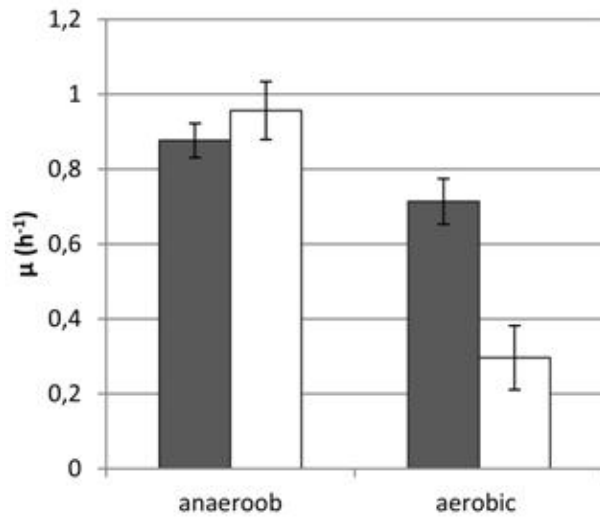


5

6 **Figure S2: Purification of NADH flavin reductase activity in cell free extract of wild type *L. johnsonii*. NADH**
 7 **consumption rate (in the presence of 250 µM FAD) is determined in fractions obtained during ammonium**
 8 **sulphate precipitation (panel A). The fraction with highest activity is subsequently used for anion exchange**
 9 **chromatography. All fractions eluting from the Q column (panel B) are tested for NADH flavin reductase**
 10 **activity. On the left axis, absorption at 280 nm is shown which is an indicator of protein concentration. On**
 11 **the right axis, NADH consumption rate is shown in the eluting fractions.**



13 **Figure S3: SDS gel of cell free extracts of *L. johnsonii* NCC 533 (wt, lane 1), NCC 9359 + pDP 1016 (Δnfr +**
14 ***LJ_0548*, lane 2), NCC 9359 + pDP 1017 (Δnfr + *LJ_0549*, lane 3), NCC 9359 (Δnfr , lane 4), NCC 9359 + pDP**
15 **1019 (Δnfr + *LJ_0548-LJ_0549*, lane 5), PageRuler™ marker (lane 6).**



16

17 **Figure S4: Growth rate of *L. johnsonii* NCC 533 (grey bars) and its *nfr*-deletion derivative NCC 9359 (white**
18 **bars) in MRS-medium in stirred pH controlled sparged with 750 ml/min of N₂ + 5% CO₂ (anaerobic) or N₂+**
19 **20% O₂+ 5% CO₂ (aerobic). Growth rates were determined as explained in Materials & Methods. Data are**
20 **average of triplicate experiments ± standard deviation.**

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L. johnsonii LJ_0549      ----MKLLAIVGTNADFSYNRFLDQFMAKRYKDQAEIEVY-EIADLPRFK 45
L. johnsonii LJ_0548      ----MKLFAIVGSNADHSYNRDLLNFIKKHFTDRYDIELG-EVKDLPMFK 45
E. coli K12 ChrR          MSEKLQVVTLLGSLRKGSEFNGMVARTLPKIAPASMEVNALPSIADIPLYD 50
                          :::::.*: . *:* : . : *      :::  .. *:* ..

L. johnsonii LJ_0549      KEAQP----DSKVEEFKNKIREADGVIFATPEYDHGIPSALKSAMWTGS 91
L. johnsonii LJ_0548      EGVKE----PAAVASFAKKVADADAVLISTPEQQHSVPSSLKSALEWLSS 91
E. coli K12 ChrR          ADVQEEGFPATVEALAEQIRQADGVVIVTPEYNYSVPGGLKNAIDWLSR 100
                          .:      : * : ::: :**.*:: *** ::.:*..**.*:* .

L. johnsonii LJ_0549      HAQGNADVMMKMPAMVLGTSYGIQGASRAQEEMREIILSPDQSANVLPGN 141
L. johnsonii LJ_0548      AEHP----FKDKPVVIVGTSVLPQGSARGQSHLKLVLSSPGFGAKVFNGD 137
E. coli K12 ChrR          LPDQP---LAGKPVLIQTSSMGVIGGARCQYHLRQILVFLD--AMVMNKP 145
                          .      : **.: : *  *.* * .: : * . * *:

L. johnsonii LJ_0549      EVLIGHAADKFDKNTGDLDDQETIHAIDLAFNNFVKFVEQAQK 184
L. johnsonii LJ_0548      EFMMGTAPEQFDENGN--LPAGTVKFLDHHFFDEFDSFYAEVSK 178
E. coli K12 ChrR          EFMGGVIQNKVDPQTGEVIDQGTLDHLTGQLTAFGEFIQRVKI 188
                          *.: *  :.:* : . : *.: : : * .* ...

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21

22 **Figure S5: ClustalW2 comparison of LJ_0548 / LJ_0549 to chromate reductase (ChrR) from E. coli.**

23 **Highlighted in grey are the residues that constitute the flavin binding site, highlighted in yellow are the**

24 **similarities in these residues in the L. johnsonii genes.**