

Table S1. Nitrogenase distribution among genome-sequenced Methanosarcinales.

Species	Mo-nitrogenase (<i>nif</i>)	V-nitrogenase (<i>vnf</i>)	Fe-nitrogenase (<i>anf</i>)
<i>Methanococcoides burtonii</i> NC 007955			
<i>Methanococcoides methylutens</i> MM1 NZ CP009518			
<i>Methanohalobium evestigatum</i> Z-7303 NC 014253			
<i>Methanohalophilus halophilus</i> NZ CP017921			
<i>Methanohalophilus mahii</i> NC 014002			
<i>Methanolobus psychrophilus</i> R15 NC 018876	•		
<i>Methanolobus zinderi</i> NZ CP058215	•		
<i>Methanomethylovorans hollandica</i> NC 019977			
<i>Methanosaeta harundinacea</i> 6Ac NC 017527			
<i>Methanosalsum zhilinae</i> NC 015676			
<i>Methanosarcina acetivorans</i> C2A NC 003552	•	•	•
<i>Methanosarcina barkeri</i> 227 NZ CP009530	•	•	
<i>Methanosarcina barkeri</i> 3 NZ CP009517	•	•	•
<i>Methanosarcina barkeri</i> CM1 NZ CP008746	•	•	
<i>Methanosarcina barkeri</i> MS NZ CP009528	•	•	
<i>Methanosarcina barkeri</i> str. Fusaro NC 007355	•	•	— ^a
<i>Methanosarcina barkeri</i> str. Wiesmoor NZ CP009526	•	•	— ^a
<i>Methanosarcina flavescens</i> NZ CP032683			
<i>Methanosarcina horonobensis</i> HB-1 NZ CP009516	•		
<i>Methanosarcina lacustris</i> Z-7289 NZ CP009515			
<i>Methanosarcina mazei</i> zm-15 NZ CP042908	•		
<i>Methanosarcina mazei</i> C16 NZ CP009514	•		
<i>Methanosarcina mazei</i> Gö1 NC 003901	•		
<i>Methanosarcina mazei</i> LYC NZ CP009513	•		
<i>Methanosarcina mazei</i> S-6 NZ CP009512	•		
<i>Methanosarcina mazei</i> SarPi NZ CP009511	•		
<i>Methanosarcina mazei</i> Tuc01 NC 020389	•		
<i>Methanosarcina mazei</i> WWM610 NZ CP009505	•		
<i>Methanosarcina siciliae</i> C2J NZ CP009508	•	•	•
<i>Methanosarcina siciliae</i> HI350 NZ CP009507	•	•	
<i>Methanosarcina siciliae</i> T4/M NZ CP009506	•	•	•
<i>Methanosarcina</i> sp. Kolksee NZ CP009524	•	•	
<i>Methanosarcina</i> sp. MTP4 NZ CP009505			
<i>Methanosarcina</i> sp. WH1 NZ CP009504	•		
<i>Methanosarcina</i> sp. WWM596 NZ CP009503	•		
<i>Methanosarcina thermophila</i> MT-1 NZ CP009501	— ^b		
<i>Methanosarcina thermophila</i> CHTI-55 NZ CP009502			
<i>Methanosarcina thermophila</i> TM-1 NZ CP009501			
<i>Methanosarcina vacuolata</i> Z-761 NZ CP009520	•	•	
<i>Methanotherix soehngenii</i> GP6 NC 015416	•		
<i>Methanotherix thermoacetophila</i> PT NC 008553			

^aAnfH is truncated and likely non-functional.

^bNif-like genes present but not in an operon.

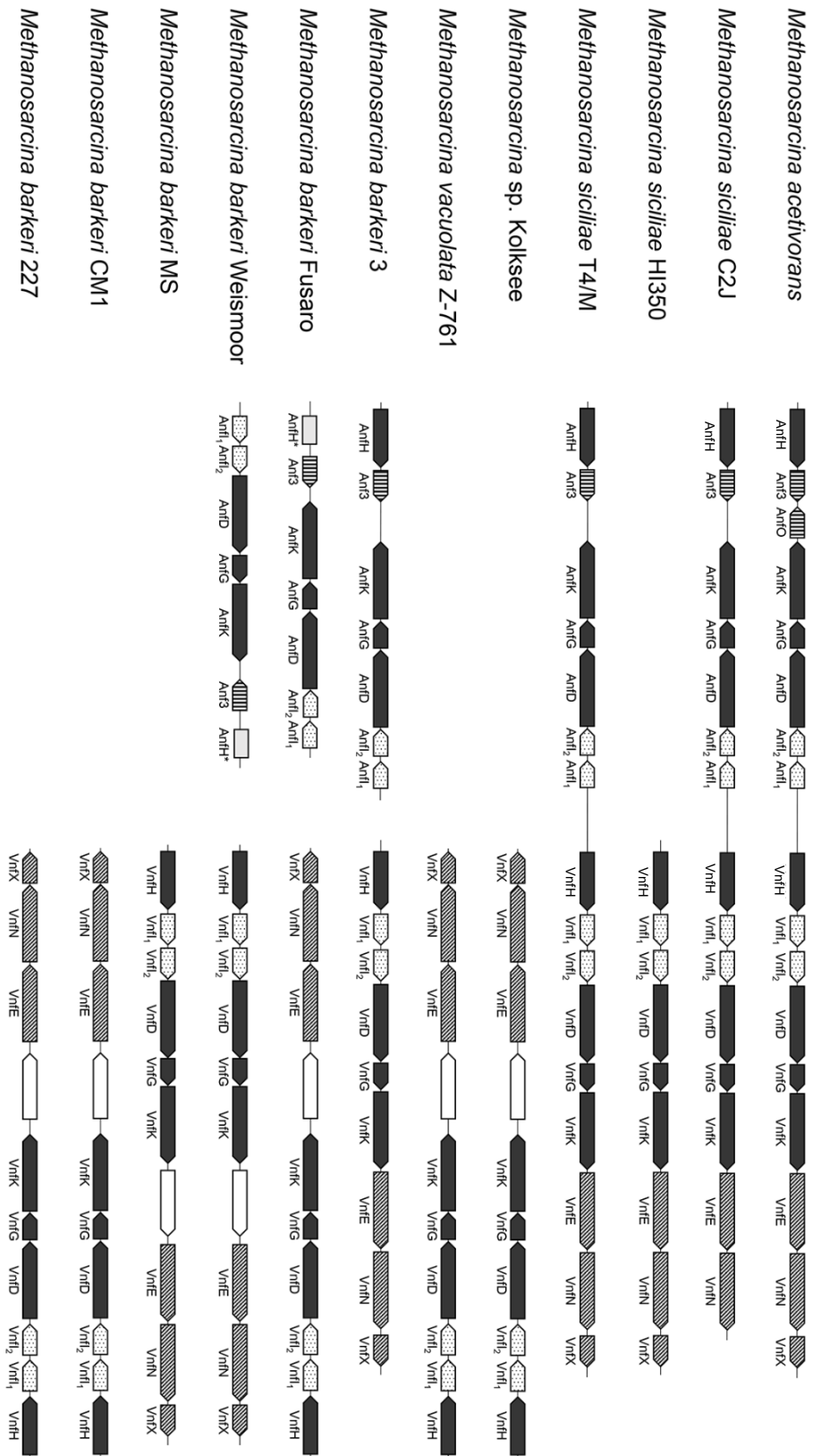


Fig. S1. Arrangement of the *vnf* and *anf* gene clusters in *Methanosarcinales* species that contain both alternative nitrogenases.

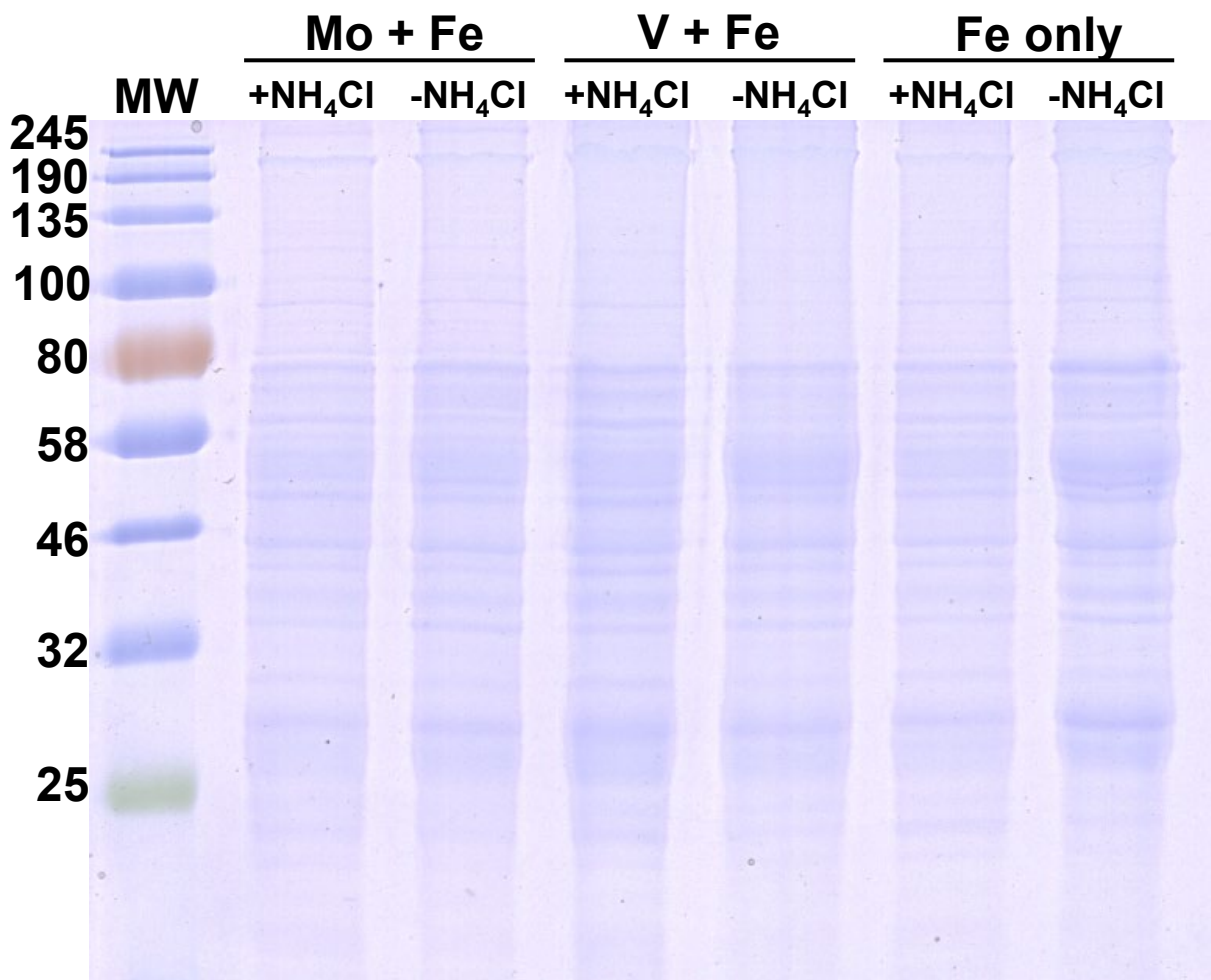


Fig. S2. SDS-PAGE of cell lysates used for Western blot in Fig. 5. MW, molecular weight standard

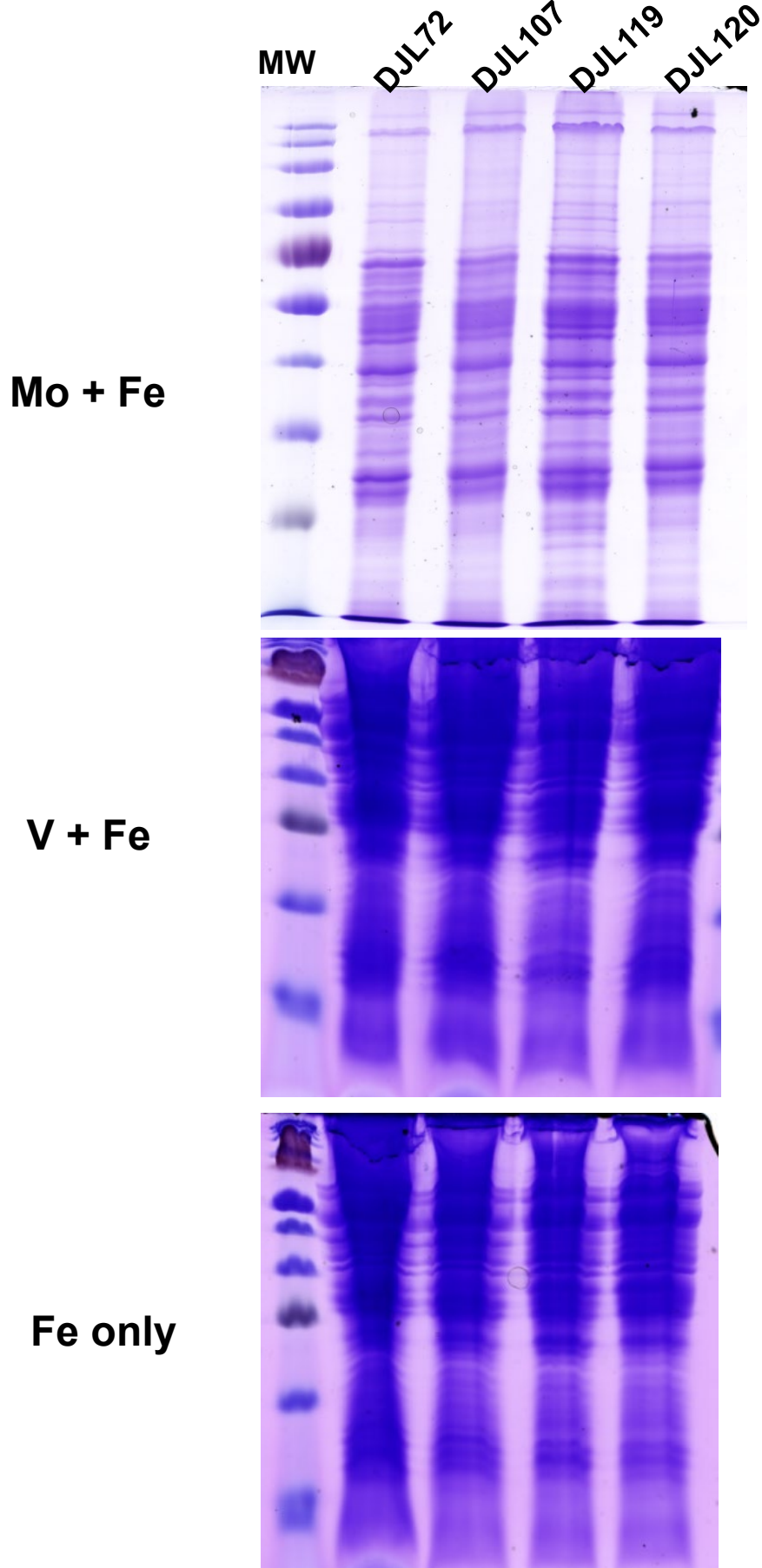


Fig. S4. Loading control SDS-PAGE of cell lysates used for Western blot in Fig. 7. MW, molecular weight standard

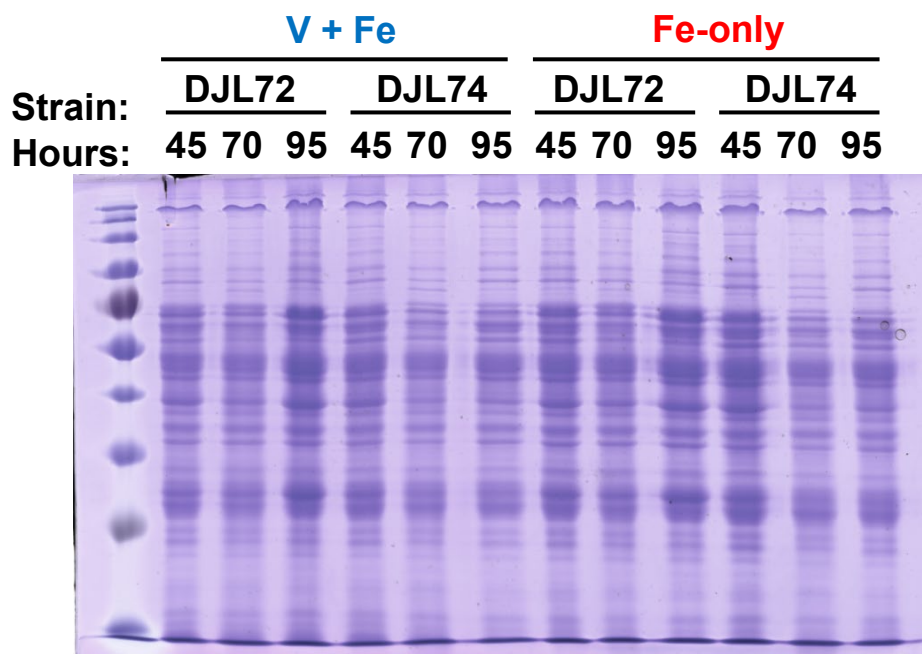


Fig. S5. Loading control SDS-PAGE of cell lysates used for western blot in Fig. 6E. MW, molecular weight standard