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Factors affecting the isolation and diversity of marine sponge associated bacteria

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Table S1 Percentage similarity of 16S rRNA partial gene sequence with nearest type strains.

Isolate	Highest Match	% Sequence Similarity	RFLP groups	Genbank accession number
RB37	<u><i>Bacillus algicola</i> KMM 3737T</u>	99.46	7	MN310361
YA6	<u><i>Bacillus algicola</i> KMM 3737T</u>	99.68	7	MN310362
LC2	<u><i>Bacillus flexus</i> NBRC 15715^T</u>	98.97	7	MN310326
YA36	<u><i>Bacillus haynesii</i> NRRL B-41327T</u>	99.47	13	MN310336
YAI	<u><i>Bacillus hwajinpoensis</i> SW-72T</u>	99.89	1	MN310359
RB73	<u><i>Bacillus hwajinpoensis</i> SW-72T</u>	99.46	5	MN310357
GB48	<u><i>Bacillus hwajinpoensis</i> SW-72T</u>	100	7	MN315519
LC16	<u><i>Bacillus hwajinpoensis</i> SW-72T</u>	99.68	10	MN310328
GB3	<u><i>Bacillus hwajinpoensis</i> SW-72T</u>	100	36	MN315520
RB138	<u><i>Bacillus infantis</i> NRRL B-14911</u>	99.46	7	MN310331
D5	<u><i>Bacillus licheniformis</i> ATCC 14580T</u>	99.15	8	MN310334
LC35	<u><i>Bacillus pumilus</i> ATCC 7061^T</u>	98.44	35	MN310333
GB21	<u><i>Bacillus qingshengii</i> G19^T</u>	98.25	1	MN310332
RB62	<u><i>Bacillus siamensis</i> KCTC 13613T</u>	99.14	22	MN310330
LC30	<u><i>Bacillus simplex</i> NBRC 15720T</u>	99.48	5	MN310327
RB99	<u><i>Bacillus simplex</i> NBRC 15720T</u>	99.87	7	MN310354
RB148	<u><i>Bacillus simplex</i> NBRC 15720T</u>	99.3	7	MN310352
RB14	<u><i>Bacillus sonorensis</i> NBRC 101234T</u>	99.36	13	MN310358
LC15	<u><i>Falsibacillus pallidus</i> CW 7T</u>	99.12	10	MN310329
LC7	<u><i>Fictibacillus rigui</i> WPCB074T</u>	99.48	9	MN310335
GB17	<u><i>Gordonia otitidis</i> NBRC 100426^T</u>	99.1	15	MN310343
GB17 ^a	<u><i>Gordonia otitidis</i> NBRC 100426^T</u>	98.89	15	MN310343
L40	<u><i>Isoptericola rhizophila</i> BKS 3-46^T</u>	97.87	19	MN310363
L40 ^a	<u><i>Isoptericola rhizophila</i> BKS 3-46^T</u>	98.3	19	MN315548
L3	<u><i>Isoptericola rhizophila</i> BKS 3-46^T</u>	97.87	19	MN315523
F12	<u><i>Isoptericola rhizophila</i> BKS 3-46^T</u>	97.87	19	MN315524
GB23	<u><i>Janibacter indicus</i> CGMCC 1.12511T</u>	99.79	1	MN310348
RB40	<u><i>Kocuria kristinae</i> NBRC 15354T</u>	99.45	21	MN310355
RB107	<u><i>Kocuria kristinae</i> NBRC 15354T</u>	99.45	34	MN310353
RB86	<u><i>Leisingera methylohalidivorans</i> DSM 14336^T</u>	99.10	16	MN310320

GB11	<u>Limimarinicola hongkongensis</u> DSM 17492 ^T	98.77	4	MN315530
GB46	<u>Limimarinicola hongkongensis</u> DSM 17492 ^T	98.95	6	MN310319
GB33	<u>Limimarinicola hongkongensis</u> DSM 17492 ^T	98.56	6	MN315531
A4	<u>Microbacterium aquimaris</u> JS54-2 ^T	99.35	20	MN315525
YA34	<u>Microbacterium aquimaris</u> JS54-2 ^T	98.74	26	MN310337
RB5	<u>Micrococcus aloeverae</u> AE-6 ^T	99.60	23	MN310360
LC12	<u>Micrococcus aloeverae</u> AE-6 ^T	99.60	24	MN310338
RB166	<u>Micrococcus aloeverae</u> AE-6 ^T	99.43	29	MN315526
GB37	<u>Muricauda aquimarina</u> SW-63 ^T	97.67	3	MN310365
GB37 ^a	<u>Muricauda aquimarina</u> SW-63 ^T	98.13	3	MK367393
LC1	<u>Mycolicibacterium iranikum</u> DSM 45541 ^T	98.68	17	MN310364
LC1 ^a	<u>Mycolicibacterium iranikum</u> DSM 45541 ^T	98	17	MK358953
RB122	<u>Pseudoalteromonas marina</u> Mano4 ^T	99.28	25	MN310325
LC17	<u>Pseudomonas oleovorans subsp. oleovorans</u> DSM 1045 ^T	100	14	MN315527
YA11	<u>Pseudonocardia carboxydivorans</u> Y8 ^T	99.74	30	MN310349
LC21	<u>Rhodococcus corynebacterioides</u> DSM 20151 ^T	99.06	12	MN310339
GB15	<u>Rhodovulum iodosum</u> N1 ^T	98.09	11	MN310321
RB71	<u>Rothia kristinae</u> NBRC 15354 ^T	99.86	33	MN310345
L11	<u>Staphylococcus hominis subsp. novobiosepticus</u> GTC 1228 ^T	100	18	MN315522
RB38	<u>Streptomyces ambofaciens</u> ATCC 23877 ^T	98.60	32	MN310342
YA19	<u>Streptomyces anthocyanicus</u> NBRC 14892 ^T	98.55	31	MN310340
H8	<u>Streptomyces antimycoticus</u> NBRC 12839 ^T	100	38	MN310344
YA20	<u>Streptomyces bacillaris</u> NBRC 13487 ^T	98.87	31	MN315528
YA27	<u>Streptomyces badius</u> NRRL B-2567 ^T	99.84	31	MN315521
RB128	<u>Streptomyces hyderabadensis</u> OU-40 ^T	99.44	31	MN310350
RB27	<u>Streptomyces lienomycini</u> LMG 20091 ^T	99.83	32	MN315546
RB27 ^a	<u>Streptomyces lienomycini</u> LMG 20091 ^T	99.92	32	MN315546
YA22	<u>Streptomyces prasinusporus</u> NRRL B-12431 ^T	98.40	31	MN310366
YA22 ^a	<u>Streptomyces prasinusporus</u> NRRL B-12431 ^T	98.94	31	MN315547
LC14	<u>Streptomyces setonii</u> NRRL ISP-5322 ^T	99.85	37	MN310347
RB154	<u>Streptomyces sundarbansensis</u> MS1/7 ^T	98.30	31	MN310351
YA2	<u>Streptomyces tendae</u> ATCC 19812 ^T	99.82	31	MN310341

RB56	<u><i>Streptomyces violaceorubidus</i> LMG 20319T</u>	99.65	31	MN310346
RB58	<u><i>Sulfitobacter delicatus</i> DSM 16477T</u>	99.89	2	MN310356
GB12	<u><i>Sulfitobacter faviae</i> S5-53T</u>	99.30	1	MN315529
GB44	<u><i>Sulfitobacter faviae</i> S5-53T</u>	99.27	2	MN310324
GB39	<u><i>Sulfitobacter indolifex</i> HEL-45T</u>	98.87	27	MN310322
GB6	<u><i>Sulfitobacter indolifex</i> HEL-45T</u>	99.20	28	MN310323

Table S2 Genus diversity with respect to isolation temperature

Genera	15°C	27°C	Genera	15°C	27°C
<i>Gordonia</i>	2	5	<i>Bacillus</i>	30	61
<i>Isoptericola</i>	1	4	<i>Fictibacillus</i>	0	7
<i>Janibacter</i>	0	1	<i>Flasibacillus</i>	0	6
<i>Kocuria</i>	11	20	<i>Staphylococcus</i>	0	5
<i>Microbacterium</i>	0	17	<i>Leisingera</i>	2	3
<i>Micrococcus</i>	7	8	<i>Limimarinicola</i>	8	9
<i>Mycobacterium</i>	0	3	<i>Pseudoalteromonas</i>	0	1
<i>Pseudonocardia</i>	0	7	<i>Pseudomonas</i>	4	2
<i>Rhodococcus</i>	2	6	<i>Rhodovulum</i>	2	3
<i>Streptomyces</i>	25	86	<i>Sulfitobacter</i>	13	19
<i>Muricauda</i>	0	3			

Table S3 List of Phyla identified by Next Generation Sequencing from sponges *Aplysilla sulfurea* and *Carteriospongia foliascens*.

<i>Aplysilla sulfurea</i> (n=12)	<i>Carteriospongia foliascens</i> (n=13)
<i>Actinobacteria</i>	<i>Actinobacteria</i>
<i>Bacteroidetes</i>	<i>Bacteroidetes</i>
<i>Chlamydiae</i>	<i>Chlamydiae</i>
<i>Chloroflexi</i>	<i>Crenarchaeota</i>
<i>Crenarchaeota</i>	<i>Cyanobacteria</i>
<i>Cyanobacteria</i>	<i>Euryarchaeota</i>
<i>Firmicutes</i>	<i>Firmicutes</i>
<i>Fusobacteria</i>	<i>Fusobacteria</i>
<i>Planctomycetes</i>	<i>Planctomycetes</i>
<i>Proteobacteria</i>	<i>Proteobacteria</i>
<i>Spirochaetes</i>	<i>Spirochaetes</i>
<i>Verrucomicrobia</i>	<i>Tenericutes</i>
	<i>Verrucomicrobia</i>

Table S4 Known and candidate genera identified by Next Generation Sequencing from sponge *Aplysilla sulfurea*.

137 known genera identified by NGS			
<i>Acidaminobacter</i>	<i>Desulfovibrio</i>	<i>Parachlamydia</i>	<i>Thermoanaerobacter</i>
<i>Acinetobacter</i>	<i>Dialister</i>	<i>Paracoccus</i>	<i>Treponema</i>
<i>Actinobacillus</i>	<i>Dinoroseobacter</i>	<i>Persicirhabdus</i>	<i>Turicibacter</i>
<i>Actinomyces</i>	<i>Dolichospermum</i>	<i>Persicobacter</i>	<i>Ulvibacter</i>
<i>Aggregatibacter</i>	<i>Elizabethkingia</i>	<i>Phaeobacter</i>	<i>Veillonella</i>
<i>Agrobacterium</i>	<i>Erwinia</i>	<i>Photobacterium</i>	<i>Verrucomicrobium</i>
<i>Alkaliphilus</i>	<i>Erythrobacter</i>	<i>Planctomyces</i>	<i>Vibrio</i>
<i>Alteromonas</i>	<i>Ferrimonas</i>	<i>Pleomorphomonas</i>	<i>Waddlia</i>
<i>Amaricoccus</i>	<i>Flavobacterium</i>	<i>Plesiomonas</i>	<i>Winogradskyella</i>
<i>Aminobacter</i>	<i>Fluviicola</i>	<i>Polaribacter</i>	<i>Wolbachia</i>
<i>Amycolatopsis</i>	<i>Fusibacter</i>	<i>Porphyromonas</i>	<i>Zoogloea</i>
<i>Antarctobacter</i>	<i>Fusobacterium</i>	<i>Prevotella</i>	<i>Candidatus</i> <i>Xiphinematobacter</i>
<i>Aquicella</i>	<i>Gemella</i>	<i>Prochlorococcus</i>	<i>Capnocytophaga</i>
<i>Aquimarina</i>	<i>Glaciecola</i>	<i>Propionibacterium</i>	<i>Chryseobacterium</i>
<i>Arcobacter</i>	<i>Gramella</i>	<i>Pseudaminobacter</i>	<i>Clostridium</i>
<i>Atopobium</i>	<i>Granulicatella</i>	<i>Pseudidiomarina</i>	<i>Cobetia</i>
<i>Bacteroides</i>	<i>Haemophilus</i>	<i>Pseudoalteromonas</i>	<i>Cohaesibacter</i>
<i>Bdellovibrio</i>	<i>Inquilinus</i>	<i>Pseudomonas</i>	<i>Collinsella</i>
<i>Bifidobacterium</i>	<i>Jannaschia</i>	<i>Pseudonocardia</i>	<i>Congregibacter</i>
<i>Burkholderia</i>	<i>Kaistia</i>	<i>Psychrilyobacter</i>	<i>Coprococcus</i>
<i>Campylobacter</i>	<i>Kingella</i>	<i>Reinekea</i>	<i>Coralimargarita</i>
<i>Candidatus Arthromitus</i>	<i>Klebsiella</i>	<i>Rhodobacter</i>	<i>Corynebacterium</i>
<i>Candidatus Portiera</i>	<i>Lactobacillus</i>	<i>Robiginitalea</i>	<i>Coxiella</i>
<i>Candidatus</i> <i>Protochlamydia</i>	<i>Leptotrichia</i>	<i>Roseivirga</i>	<i>Crenothrix</i>

<i>Alkaliphilus</i>	<i>Erythrobacter</i>	<i>Planctomyces</i>	<i>Vibrio</i>
<i>Candidatus</i> <i>Rhabdochlamydia</i>	<i>Leuconostoc</i>	<i>Roseobacter</i>	<i>Desulfobacter</i>
<i>Desulfocapsa</i>	<i>Loktanella</i>	<i>Roseovarius</i>	<i>Moritella</i>
<i>Desulfosarcina</i>	<i>Luteolibacter</i>	<i>Rothia</i>	<i>Nautella</i>
<i>Desulfosporosinus</i>	<i>Lutimonas</i>	<i>Rubritalea</i>	<i>Neptunomonas</i>
<i>Maribacter</i>	<i>Ruegeria</i>	<i>Microbulbifer</i>	<i>Nitrosopumilus</i>
<i>Marivita</i>	<i>Selenomonas</i>	<i>Micrococcus</i>	<i>Octadecabacter</i>
<i>Megasphaera</i>	<i>Shewanella</i>	<i>Moraxella</i>	<i>Oleibacter</i>
<i>Oribacterium</i>	<i>Staphylococcus</i>	<i>Streptococcus</i>	<i>Sutterella</i>
<i>Parabacteroides</i>	<i>Stenotrophomonas</i>	<i>Streptomyces</i>	<i>Synechococcus</i>
<i>Syntrophomonas</i>	<i>Tepidibacter</i>	<i>Thalassobius</i>	<i>Thalassomonas</i>
<i>Tenacibaculum</i>			
12 candidate genera identified by NGS			
<i>A1</i>	<i>HB2-32-21</i>	<i>[Prevotella]</i>	<i>WH1-8</i>
<i>BD2-13</i>	<i>HTCC</i>	<i>T78</i>	<i>ZD0117</i>
<i>Blvii28</i>	<i>MSBL3</i>	<i>Ucs1325</i>	<i>5-7N15</i>

Table S5 Known and candidate genera identified by Next Generation Sequencing from sponge *Carteriospongia foliascens*

183 known genera identified by NGS				
<i>Acaryochloris</i>	<i>Cetobacterium</i>	<i>Haemophilus</i>	<i>Odoribacter</i>	<i>Roseivivax</i>
<i>Acholeplasma</i>	<i>Clostridiisalibacter</i>	<i>Haloplanus</i>	<i>Oleibacter</i>	<i>Roseobacter</i>
<i>Achromobacter</i>	<i>Clostridium</i>	<i>Halorhabdus</i>	<i>Oscillospira</i>	<i>Roseovarius</i>
<i>Actinobacillus</i>	<i>Cohaesibacter</i>	<i>Hyphomicrobium</i>	<i>Paenibacillus</i>	<i>Rothia</i>
<i>Actinomyces</i>	<i>Collinsella</i>	<i>Hyphomonas</i>	<i>Parabacteroides</i>	<i>Rubritalea</i>
<i>Adlercreutzia</i>	<i>Congregibacter</i>	<i>Idiomarina</i>	<i>Parachlamydia</i>	<i>Ruegeria</i>
<i>Akkermansia</i>	<i>Coprococcus</i>	<i>Inquilinus</i>	<i>Paracoccus</i>	<i>Ruminococcus</i>
<i>Alcanivorax</i>	<i>Coralimargarita</i>	<i>Janibacter</i>	<i>Paulinella</i>	<i>Rummeliibacillus</i>
<i>Aliivibrio</i>	<i>Corynebacterium</i>	<i>Jannaschia</i>	<i>Persicirhabdus</i>	<i>Saprospira</i>
<i>Alkaliphilus</i>	<i>Coxiella</i>	<i>Jeotgalicoccus</i>	<i>Persicobacter</i>	<i>Shewanella</i>
<i>Allobaculum</i>	<i>Dehalobacterium</i>	<i>Klebsiella</i>	<i>Phaeobacter</i>	<i>Spongiibacter</i>
<i>Alteromonas</i>	<i>Demequina</i>	<i>Lactobacillus</i>	<i>Photobacterium</i>	<i>Staphylococcus</i>
<i>Amaricoccus</i>	<i>Desulfarculus</i>	<i>Lactococcus</i>	<i>Piscirickettsia</i>	<i>Stenotrophomonas</i>
<i>Aminobacter</i>	<i>Desulfobacter</i>	<i>Legionella</i>	<i>Planctomyces</i>	<i>Streptococcus</i>
<i>Amphritea</i>	<i>Desulfococcus</i>	<i>Leptolyngbya</i>	<i>Pleomorphomonas</i>	<i>Streptomyces</i>
<i>Amycolatopsis</i>	<i>Desulfofaba</i>	<i>Leptotrichia</i>	<i>Pleomorphomonas</i>	<i>Psychrilyobacter</i>
<i>Anaerococcus</i>	<i>Desulfomonile</i>	<i>Leuconostoc</i>	<i>Polaribacter</i>	<i>Succinivibrio</i>
<i>Anaeroplasm</i>	<i>Desulforhopalus</i>	<i>Loktanella</i>	<i>Prevotella</i>	<i>Succinivibrio</i>
<i>Anaerospira</i>	<i>Desulfotalea</i>	<i>Luteolibacter</i>	<i>Prochlorococcus</i>	<i>Synechococcus</i>
<i>Aquicella</i>	<i>Desulfovibrio</i>	<i>Lutibacterium</i>	<i>Propionibacterium</i>	<i>Syntrophomonas</i>
<i>Arcobacter</i>	<i>Devosia</i>	<i>Lutimonas</i>	<i>Propionigenium</i>	<i>Tatlockia</i>
<i>Arthrobacter</i>	<i>Dinoroseobacter</i>	<i>Marinicella</i>	<i>Pseudaminobacter</i>	<i>Tepidibacter</i>
<i>Atopobium</i>	<i>Dolichospermum</i>	<i>Marinobacter</i>	<i>Pseudoalteromonas</i>	<i>Tepidimonas</i>
<i>Azospirillum</i>	<i>Dorea</i>	<i>Marinomonas</i>	<i>Pseudomonas</i>	<i>Thalassobius</i>
<i>Bacillus</i>	<i>Elizabethkingia</i>	<i>Marivita</i>	<i>Pseudonocardia</i>	<i>Thalassomonas</i>

<i>Bacteroides</i>	<i>Enterobacter</i>	<i>Methylobacterium</i>	<i>Pseudoruegeria</i>	<i>Thiohalorhabdus</i>
<i>Balneola</i>	<i>Enterococcus</i>	<i>Microbulbifer</i>	<i>Psychrilyobacter</i>	<i>Tolimonas</i>
<i>Bdellovibrio</i>	<i>Erwinia</i>	<i>Moraxella</i>	<i>Psychrobacter</i>	<i>Trichococcus</i>
<i>Bifidobacterium</i>	<i>Faecalibacterium</i>	<i>Moritella</i>	<i>Psychromonas</i>	<i>Trichodesmium</i>
<i>Blautia</i>	<i>Ferrimonas</i>	<i>MSBL3</i>	<i>Ralstonia</i>	<i>Turicibacter</i>
<i>Burkholderia</i>	<i>Flavobacterium</i>	<i>Nautella</i>	<i>Reinekea</i>	<i>Veillonella</i>
<i>Campylobacter</i>	<i>Fluviicola</i>	<i>Neisseria</i>	<i>Rhizobium</i>	<i>Verrucomicrobiu m</i>
<i>Candidatus Hepatoplasma</i>	<i>Gemella</i>	<i>Nitratireductor</i>	<i>Rhodovulum</i>	<i>Vibrio</i>
<i>Candidatus Portiera</i>	<i>Geobacter</i>	<i>Nitrosopumilus</i>	<i>Rickettsiella</i>	<i>Waddlia</i>
<i>Candidatus Protochlamydia</i>	<i>Gloeobacter</i>	<i>Nitrospina</i>	<i>Robiginitalea</i>	<i>Winogradskyella</i>
<i>Candidatus Rhabdochlamydia</i>	<i>Gluconacetobacter</i>	<i>Oceanospirillum</i>	<i>Roseivirga</i>	<i>Wolbachia</i>
<i>Candidatus Xiphinematobacter</i>	<i>Granulicatella</i>	<i>Octadecabacter</i>		
9 candidate genera identified by NGS				
5-7N15	A17	B46	heteroC45	HTCC
SJA-88	SJA-88	Ucs1325	Ucs1325	

Figure S1: Twelve sponge samples collected from two sites. RB 1, RB 2, RB 3, RB 11, RB 12, RB 16, RB 17, and RB 18 were collected from Rapid Bay; GB 1, GB 08, GB 21, and GB 23 were collected from Glenelg Blocks.



RB 1



RB 2



RB 3



RB 11



RB 12



RB 16



RB 17



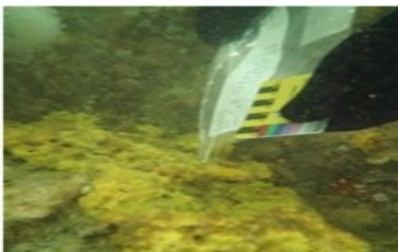
RB 18



GB 1



GB 08



GB 21



GB 23

Figure S2: Cumulative abundance of isolates collected over the 16 weeks of incubation.

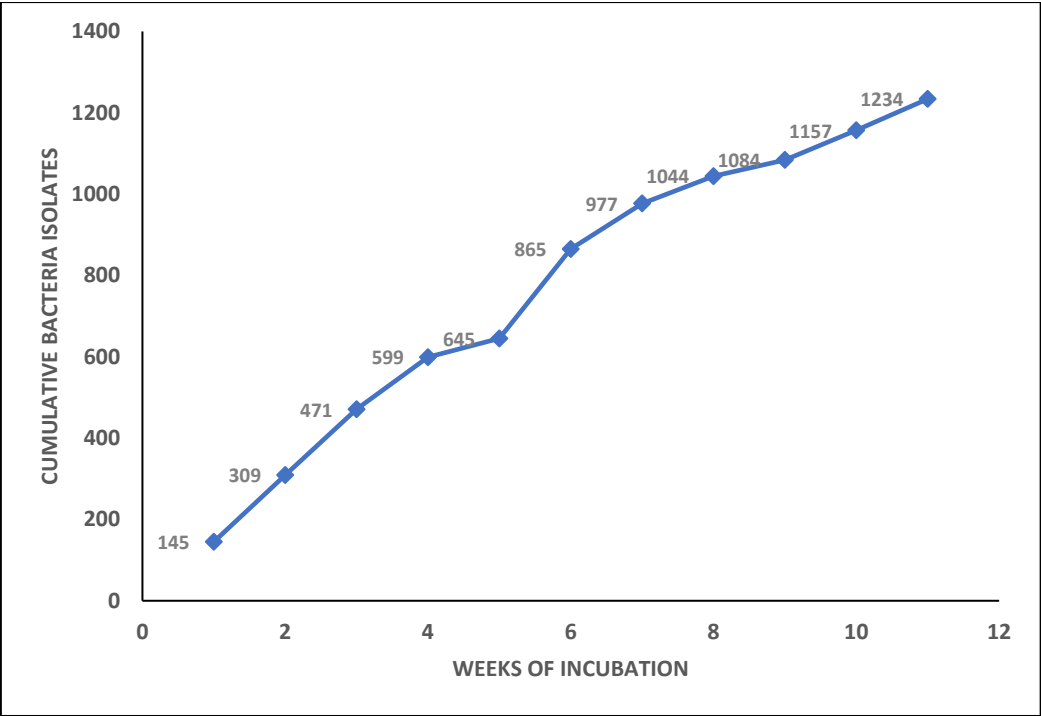


Figure S3 Abundance and diversity of bacteria isolated under different incubation conditions.

a) total colony forming bacteria and morphological forms; **b)** the diversity of genera isolated at different oxygen levels

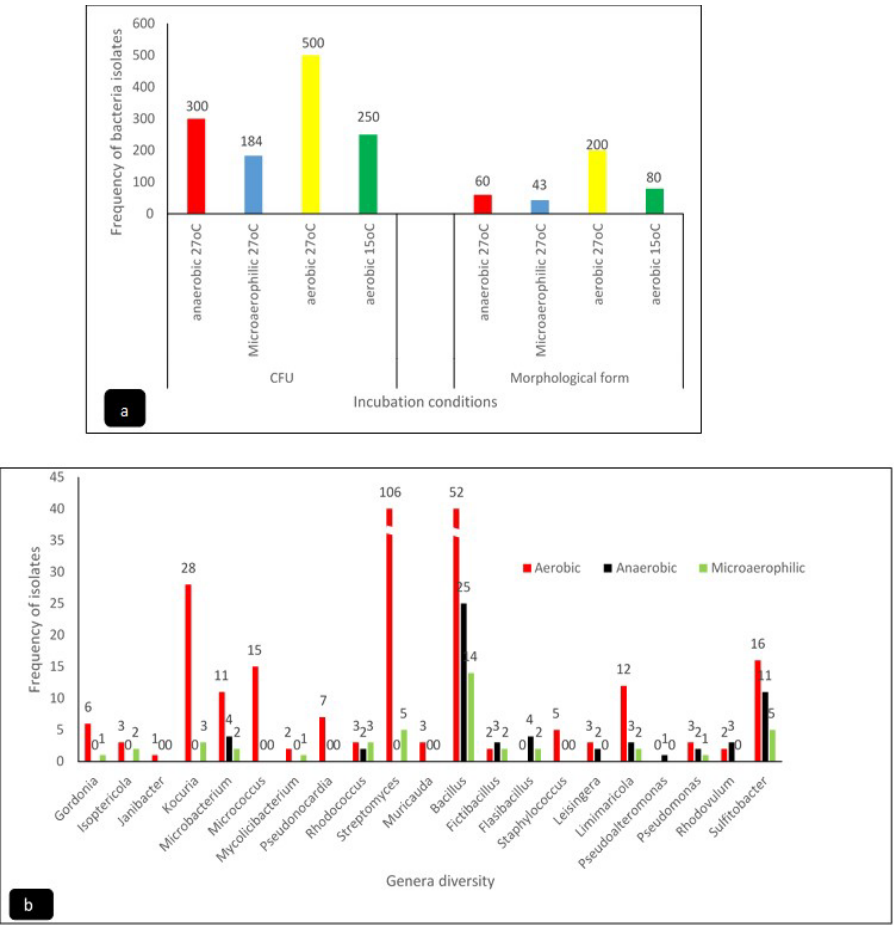


Figure S4: Bacterial growth at different NaCl concentrations presented as a percentage of total number.

