## Cultivation strategies for permafrost microorganisms Reference: DOI: 10.1002/ppp.1987

Organism's ecophysiology	Media used for enrichment/cultivation	Special extra requirements, conditions or devices used for cultivation	Example of isolated organisms
Aerobic heterotrophs	Hickey-Tresner revised medium ( <u>Katayama et al.</u> 2007)	Direct plating	Tomitella biformata (Katayama et al. 2010)
	HM medium ( <u>Ventosa et al, 1982</u> )	1/4 HM medium, incubated with shaking for two weeks	Virgibacillus arcticus (Niederberger et al, 2009)
	PYGV (DSMZ medium 621)	Direct plating	Chryseobacterium xinjiangense (Zhao et al. 2011)
			Epilithonimonas psychrotolerans (Ge et al, 2015)
			Hymenobacter psychrotolerans (Zhang et al. 2008)
			Polymorphobacter fuscus ( <u>Jia et al, 2015</u> )
		Serial dilutions on modified PYGV, low temperature incubation conditions, modification with NaHCO3 (alkaliphilic organisms).	Paracoccus tibetensis (Zhu et al, 2013)
	R2A (Reasoner and Geldreich, 1985)	Direct plating	Chryseobacterium frigidisoli ( <u>Bajerski et al. 2013</u> )

			Demequina lutea (Finster et al, 2009)
			Psychrobacter muriicola (Shcherbakova et al. 2009)
			Spirosoma luteum and Spirosoma spitsbergense (Finster et al, 2009, Hansen et al 2007)
			Tumebacillus permanentifrigoris (Steven et al, 2008)
			Undibacterium terreum (Liu et al, 2013)
		1/10 strength R2A as enrichment medium	Glaciimonas frigoris (Margesin et al, 2016).
		Supplementation with sodium acetate	Planococcus halocryophilus (Mykytczuk et al. 2012)
	Nutrient broth	Permafrost suspended in saline solution, supplemented with sodium pyruvate before plating	Cohnella kolymensis (Kudryashova et al. 2018)
	Luria broth	Plates supplemented with minimal medium plus 0.5% glucose	Glaciibacter superstes (Katayama et al, 2009)
	Tryptic soy broth (TSB)		Carnobacterium inhibens (Jöborn et al, 1999)

		TSB at different strengths (full, ½ and 1/10). Sucrose and NaCl as osmoprotectants.	Psychrobacter cryhalolentis and P. arcticus (Bakermans et al 2006, Vishnivetskaya et al 2000)
			Exiguobacterium sibiricum ( <u>Frigi</u> Rodrigues, 2005)
	Gause 1 ( <u>Wang et al.</u> 2018).		Massilia violaceinigra (Wang et al. 2018).
Anaerobes	GPM (Shcherbakova et al, 2013)	Trace element solution of DSMZ medium 320. Serial dilution in Hungate tubes. Cultivation on solid media with a 100% nitrogen atmosphere	Celerinatantimonas yamalensis (Shcherbakova et al. 2013)
	Marine broth (BD biosciences)		Celerinatantimonas yamalensis
	Basal medium (Rivkina et al, 2007)	Enrichment with vitamin solution and trace elements (Balch et al, 1979) . H2 and CO2 used as carbon sources. Medium supplemented with glucose and peptone for Sphaerochaeta associata.	Methanobacterium arcticum (Shcherbakova et al. 2011)
			Methanobacterium veterum (Krivushin et al., 2010)

Basal medium (Wagner et al, 2013)		Sphaerochaeta associata (Troshina et al, 2015)  Methanosarcina soligelidi (Wagner et al, 2013)
Medium according to Trubitsyn et al (2023)		Methanobacterium spitsbergense ( <u>Trubitsyn</u> et al. 2023)
Mineral mixture with sodium ascorbate, glucose and peptone as carbon sources (Shcherbakova et al. 2005)	Incubation for three months in dark conditions	Clostridium algoriphilum (Shcherbakova et al., 2005)
		Clostridium tagluense (Suetin et al, 2009)
Mineral solution (Wolin et al, 1963)	Trace minerals (Whitman et al, 1982), dilution method in Hungate tubes	Carnobacterium pleistocenium ( <u>Pikuta et al., 2005</u> )

Anaerobes, sulfate-reducing bacteria		Trace element solution of DSMZ medium 320, sodium lactate, Na2S and resazurin	Desulfovibrio gilichinskyi (Ryzhmanova et al. 2019)
		Enrichment using NaHCO3, HCl and sodium lactate	Desulfovibrio arcticus (Pecheritsyna et al., 2012)
	Postgate B (in Jain, 1995)	Medium previously reduced using sodium dithionite	Desulfosporosinus hippei (Vatsurina et al, 2008)