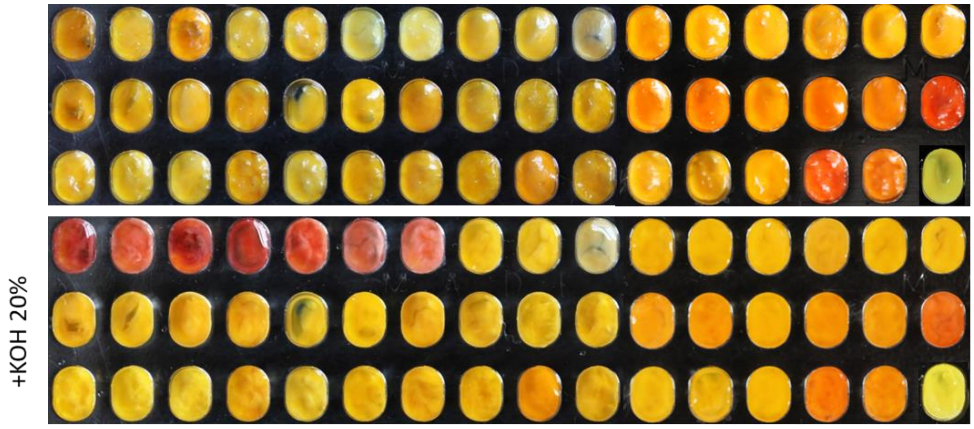
Supplementary Information

Light stimulates anoxic and oligotrophic growth of glacial *Flavobacterium* strains that produce zeaxanthin

This file contains Supplementary Figures (page 2) and Tables (page 9), which has provided additional information in the zeaxanthin-associated light stimulation of the glacial *Flavobacterium* strains in lower oxygen availability or poor nutrients.

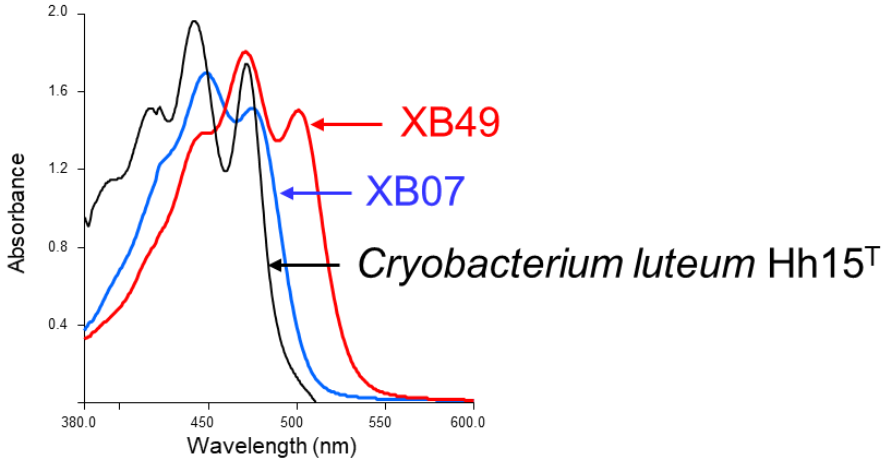
Supplementary figure titles and legends:

Supplementary Figure 1. Assay of flexirubin produced by some *Flavobacterium* strains using 20% KOH described in Methods. Beneath are the glacial strain names, and Hh15T is a strain of *Cryobacterium luteum* isolated from the same glacier.

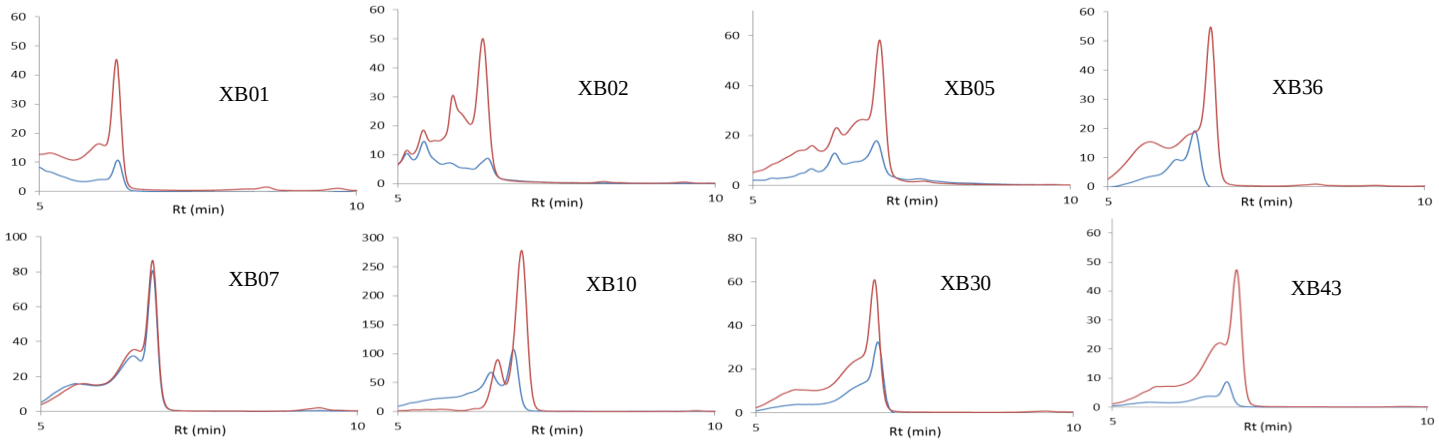


XB01	XB02	XB03	XB45	XB36	XB41	XB47	XB31	XB05	XB43	XB13	XB37	XB30	XB21	XB48	XB25
XB51	XB33	XB08	XB44	XB32	XB35	XB09	XB26	XB39	XB17	XB15	XB24	XB12	XB19	XB23	XB49
XB06	XB20	XB28	XB04	XB22	XB50	XB11	XB07	XB10	XB14	XB18	XB16	XB27	XB40	XB38	Hh15^{T}

Supplementary Figure 2. The spectra of carotenoids extracted from the glacial *Cryobacterium luteum* Hh15^{T} and *Flavobacterium* XB07 and XB49.

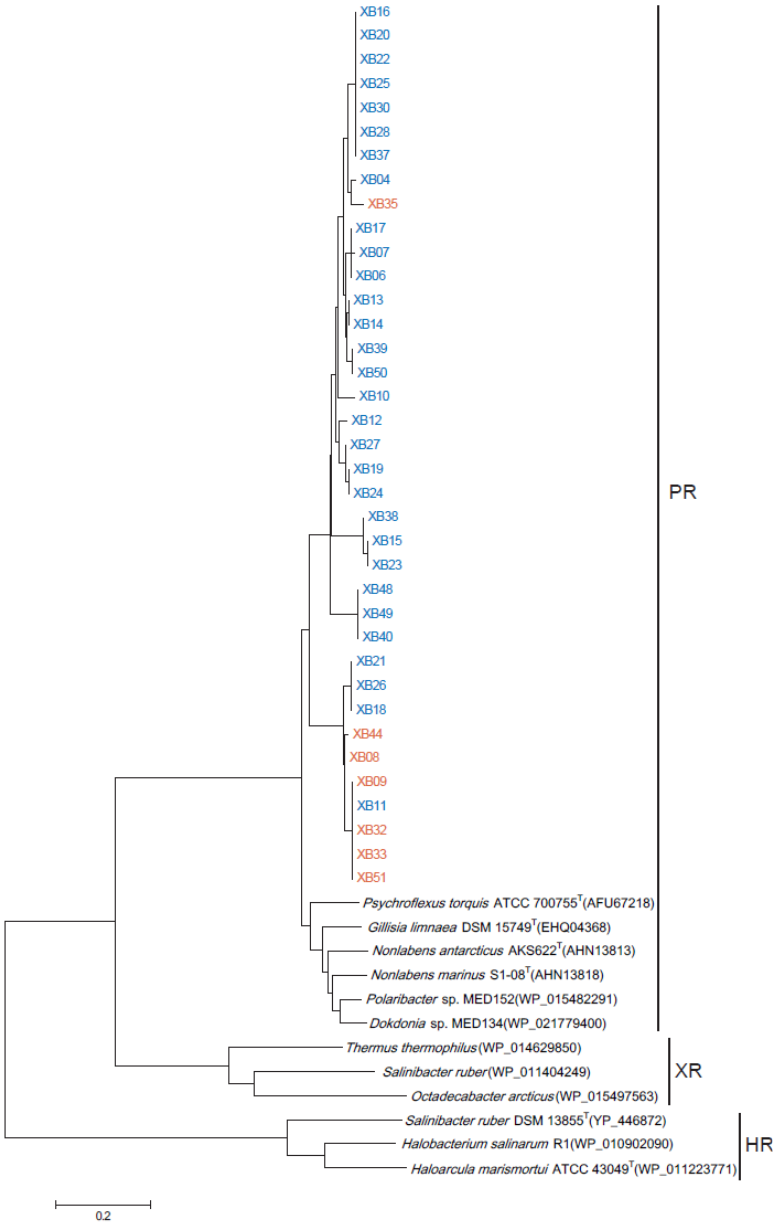


Supplementary Figure 3. **Light induced zeaxanthin production of the glacial *Flavobacterium* strains**. HPLC determined the carotenoid yields in the cultures of light exposure (red) and in dark (blue) from each four strains that carry the proteorhodopsin gene (lower) or not (upper). The same assay procedure was used as that in Figure 4c. Extraction of zeaxanthin was described in the methods section. Rt: retention time.

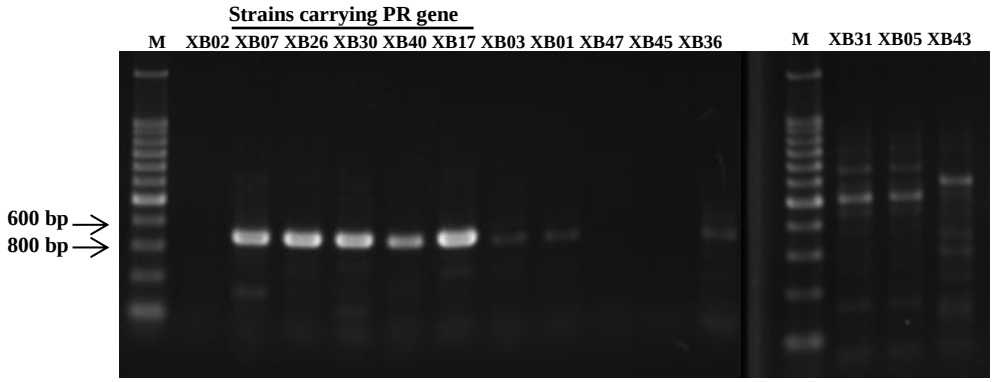


Supplementary Figure 4. **A Bayesian phylogenetic tree of the rhodopsin proteins from 37 glacial *Flavobacterium* strains and other types of rhodopsin proteins.**

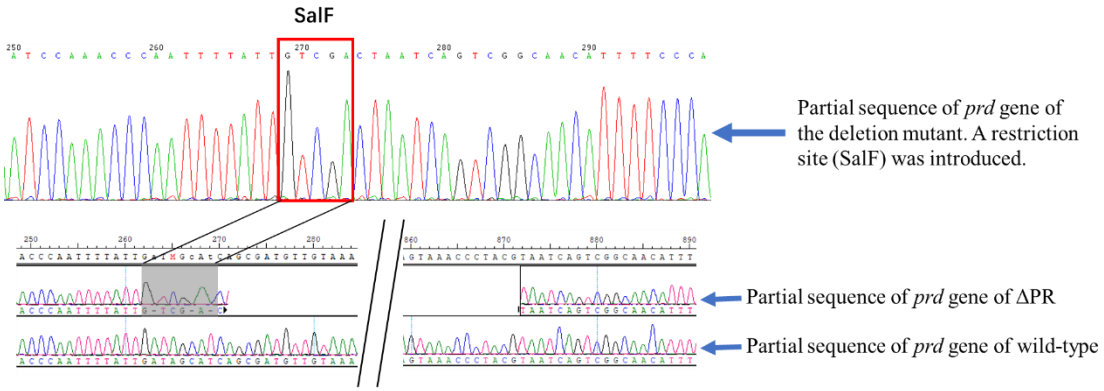
Strain numbers in blue and orange indicate those isolated from northern and southern glaciers, respectively. PR, proteorhodopsin; XR, xanthorhodopsin; HR, halorhodopsin. Bar, 0.2 amino acid substitution/site.



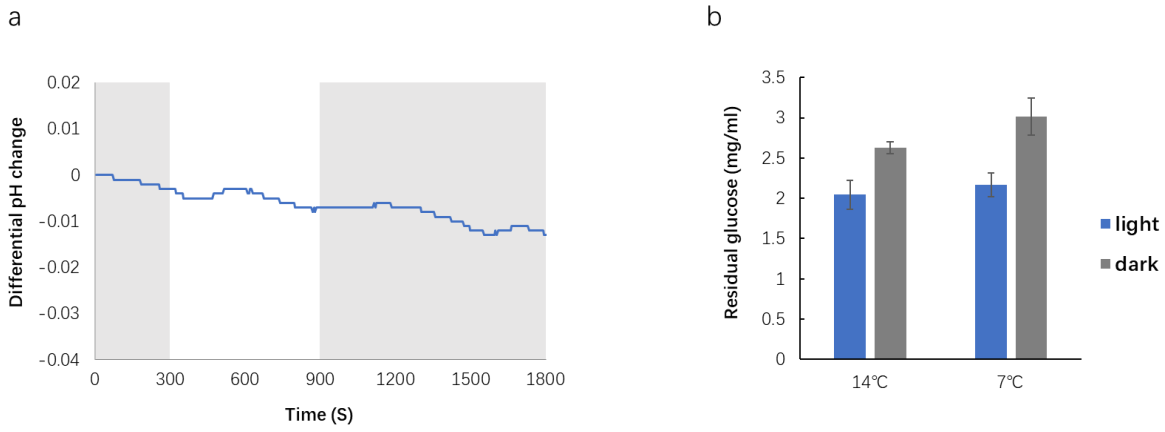
Supplementary Figure 5. PCR verification of the absence of the not sequenced *prd* gene in some glacial *Flavobacterium* genomes. A pair of primers listed in Supplementary Table S2 that specifically hybrid to the PR gene was used. Strains that have the *prd* gene sequenced were included as the references. M, DNA ladder.



Supplementary Figure 6. Verification of the *prd* gene deletion in strain XB07 via DNA sequencing. The genomic DNAs of strain XB07 wild type and the *prd* gene deletion mutant were sanger sequenced using primer PR_delR (Supplementary Table 2) on ABI 3730. A sequencing chart presents an insertion of cleavage motif of the restriction DNase SalF replaced the *prd* encoding region comparing with that of the wild type.



Supplementary Figure 7. Assay of light-driven proton pump activity (a) and enhanced glucose consumption (b) in the glacial *Flavobacterium* XB07. a. Using the protocol of Feng et al. (2013), light-driven proton pump activity was determined via measuring the pH changes in the cell suspension (initial pH 7.0) at 14° C under photo flux of 50 photon $m^{-2} \cdot s^{-1}$. Shaded areas represent dark conditions. **b**. The stationary spent PGY cultures that were grown in light and dark, and at 7 ºC and 14° C were assayed for the residual glucose contents using 3,5-dinitrosalicylic acid (DNS) reagent [Miller, 1959]. Triplicated cultures were assayed, and the averages and standard derivations are shown.



Supplementary tables

Supplementary Table 1. Strains used in this study*

Isolates	Strain No. in CGMCC	16S rRNA gene Acc. #	Most related species (16S rRNA similarity %)	Isolation location	Opt. T (℃)	Genome acc No of NCBI
XB01	1.10046	JX290502	*F. frigidimaris* KUC-1 (98.90)	Hailuogou glacier	14	SRR5506633
XB02	1.9982	JX290495	*F. hibernum* ATCC 51468 (99.03)	Hailuogou glacier	14	SRR5506632
XB03	1.10006	JX290498	*F. frigidimaris* KUC-1 (98.89)	Hailuogou glacier	14	SRR5506631
XB04	1.9356	JX290459	*F. limicola* ST-82 (98.34)	Toumingmengke glacier	14	SRR5506630
XB05	1.9759	JX290489	*F. sinopsychrotolerans* 0533 (98.91)	Midui glacier	14	SRR5506629
XB06	1.9686	JX290486	*F. sinopsychrotolerans* 0533 (99.85)	Toumingmengke glacier	14	SRR5506628
XB07	1.9225	JX949246	*F. psychrolimnae* LMG 22018 (99.35)	Xinjiang No.1 glacier	14	SRR5506627
XB08	1.10002	JX290496	*F. xinjiangense* JCM 11314 (99.78)	Hailuogou glacier	14	SRR5506626
XB09	1.9905	JX290493	*F. xinjiangense* JCM 11314 (99.71)	Midui glacier	14	SRR5506625
XB10	**1.2747^{T}**	AF433174	***F. omnivorum* JCM 11313^{T,1}**	Xinjiang No.1 glacier	<20	SRR5506624
XB11	**1.2749^{T}**	AF433173	***F. xinjiangense* JCM 11314^{T,1}**	Xinjiang No.1 glacier	<20	SRR5506623
XB12	**1.6847^{T}**	EU036219	***F. tiangeerense* 0563^{T,2}**	Xinjiang No.1 glacier	26	SRR5506622
XB13	1.6461	JX290448	*F. sinopsychrotolerans* 0533 (99.93)	Xinjiang No.1 glacier	14	SRR5506621
XB14	**1.8704^{T}**	FJ654474	***F. sinopsychrotolerans* 0533^{T,3}**	Xinjiang No.1 glacier	25	SRR5506620
XB15	1.9255	JX290454	*F. sinopsychrotolerans* 0533 (98.11)	Xinjiang No.1 glacier	14	SRR5506619
XB16	1.9360	JX290460	*F. tiangeerense* 563 (98.70)	Toumingmengke glacier	14	SRR5506618
XB17	1.9455	JX290469	*F. sinopsychrotolerans* 0533 (99.85)	Toumingmengke glacier	14	SRR5506617
XB18	1.6442	JX290447	*F. urumqiense* Sr25 (99.93)	Xinjiang No.1 glacier	14	SRR5506616
XB19	1.9224	JX290451	*F. granuli* Kw05 (98.48)	Xinjiang No.1 glacier	14	SRR5506615
XB20	1.9381	JX290467	*F. psychrolimnae* LMG 22018 (98.69)	Toumingmengke glacier	14	SRR5506614
XB21	1.6440	JX290446	*F. urumqiense* Sr25 (99.92)	Xinjiang No.1 glacier	14	SRR5506613
XB22	1.9365	JX290463	*F. xueshanense* Sr22 (99.78)	Toumingmengke glacier	14	SRR5506612
XB23	1.9215	JX290450	*F.* sinopsychrotolerans 0533 (98.18)	Xinjiang No.1 glacier	14	SRR5506611
XB24	1.9246	JX290452	*F. granuli* Kw05 (98.47)	Xinjiang No.1 glacier	14	SRR5506610
XB25	1.9361	JX290461	*F. xueshanense* Sr22 (100)	Toumingmengke glacier	14	SRR5506609
XB26	1.9340	JX290457	*F. urumqiense* Sr25 (99.20)	Toumingmengke glacier	14	SRR5506608
XB27	1.9175	JX290449	*F. pectinovorum* DSM 6368 (98.07)	Xinjiang No.1 glacier	14	SRR5506607
XB28	1.9376	JX290465	*F. psychrolimnae* LMG 22018 (98.63)	Toumingmengke glacier	14	SRR5506606
XB30	1.9364	JX290462	*F. psychrolimnae* LMG 22018 (98.47)	Toumingmengke glacier	14	SRR5506605
XB31	1.11100	JX290506	*F. sinopsychrotolerans* 0533 (98.90)	Midui glacier	14	SRR5506604
XB32	1.11107	JX290507	*F. xinjiangense* JCM 11314 (99.71)	Midui glacier	14	SRR5506603
XB33	1.10050	JX290503	*F. xinjiangense* JCM 11314 (100)	Hailuogou glacier	14	SRR5506602
XB35	1.11109	JX290508	*F. glaciei* 0499(98.41)	Midui glacier	14	SRR5506601
XB36	1.9830	JX290491	*F. xinjiangense* JCM 11314 (99.26)	Midui glacier	14	SRR5506600
XB37	**1.9227^{T}**	HQ436466	***F. xueshanense* Sr22^{T,4}**	Xinjiang No.1 glacier	18	SRR5506599
XB38	1.9621	JX290480	*F. sinopsychrotolerans* 0533 (98.47)	Toumingmengke glacier	14	SRR5506598
XB39	1.9469	JX290470	*F. sinopsychrotolerans* 0533 (98.98)	Toumingmengke glacier	14	SRR5506597
XB40	1.9333	JX290456	*F. tiangeerense* 563 (97.93)	Xinjiang No.1 glacier	14	SRR5506596

XB41	1.9683	JX290485	*F. limicola* ST-82 (98.40)	Toumingmengke glacier	14	SRR5506595
XB43	1.9569	JX290476	*F. sinopsychrotolerans* 0533 (98.98)	Toumingmengke glacier	14	SRR5506594
XB44	1.9784	JX290490	*F. xinjiangense* JCM 11314 (99.64)	Midui glacier	14	SRR5506593
XB45	**1.10076^{T}**	JQ002654	***F. noncentrifugens* R-HLS-17^{T,5}**	Hailuogou glacier	29	SRR5506592
XB47	1.9680	JX290484	*F. limicola* ST-82 (98.20)	Toumingmengke glacier	14	SRR5506591
XB48	1.9672	JX290483	*F. xueshanense* Sr22 (97.76)	Toumingmengke glacier	14	SRR5506590
XB49	1.9702	JX290488	*F. sinopsychrotolerans* 0533 (96.15)	Toumingmengke glacier	14	SRR5506589
XB50	1.9696	JX290487	*F.* sinopsychrotolerans 0533 (98.91)	Toumingmengke glacier	14	SRR5506588
XB51	1.10018	JX290499	*F. xinjiangense* JCM 11314 (100)	Hailuogou glacier	14	SRR5506587
Hh15^{T}	**1.11210^{T}**		***Cryobacterium luteum*** [6]		14	

* Strains have been deposited in public culture collection centers as indicated. CGMCC, China General Microorganism Culture Collection Center. Bold letters refer to the validate published species in the references as indicated by superscripted numbers as following,

1. Zhu F, Wang S, Zhou P. (2003) *Flavobacterium xinjiangense* sp. nov. and *Flavobacterium omnivorum* sp. nov., novel psychrophiles from China No1 glacier. IJSEM 53: 853-857.

2, Xin, Y.H., Liang, Z.H., Zhang, D.C., Liu, H.C., Zhang, J.L., Yu, Y., Xu, M.S., Zhou, P.J., and Zhou, Y.G. (2009). *Flavobacterium tiangeerense* sp. nov., a cold-living bacterium isolated from a glacier. Int J Syst Evol Microbiol 59, 2773-2777.

3, Xu, M., Xin, Y., Tian, J., Dong, K., Yu, Y., Zhang, J., Liu, H., and Zhou, Y. (2011). *Flavobacterium sinopsychrotolerans* sp. nov., isolated from a glacier. Int J Syst Evol Microbiol 61, 20-24

4, Dong, K., Liu, H., Zhang, J., Zhou, Y., and Xin, Y. (2012). *Flavobacterium xueshanense* sp. nov. and *Flavobacterium urumqiense* sp. nov., two psychrophilic bacteria isolated from glacier ice. Int J Syst Evol Microbiol 62, 1151-1157.

5, Zhu, L., Liu, Q., Liu, H., Zhang, J., Dong, X., Zhou, Y., and Xin, Y. (2013). *Flavobacterium noncentrifugens* sp. nov., a psychrotolerant bacterium isolated from glacier meltwater. Int J Syst Evol Microbiol 63, 2032-2037.

6, Liu Q, Liu HC, Wen Y, Zhou YG, Xin YH. (2012). *Cryobacterium flavum* sp. nov. and *Cryobacterium luteum* sp. nov., isolated from glacier ice. Int J Syst Evol Microbiol 62, 1296-1299.

Supplementary Table 2. Primers used in this study

Primer	Sequence (5'→3')	Purpose
PRf	ATGTTATTHCAGGATTAGTYGSDA	Amplification of PR gene
PRr	CAAANCCDATTTTRTTRATNGCATC	
XB32PR379F	GGAGAAGCGGTAGACAGAC	
XB32PR554R	GACCATCCAACTAATACGAACC	
XB26PR419f	TAATCTCAGGTATCGCTTAC	
XB26PR590r	CCGTCAGTTCCTAACATAT	
XB07PR237f	GTCACCAACTTTCTTTCG	Quantification of the PR transcript copies
XB07PR399r	AACTTCACCAATGTAACCT	
XB10PR40f	ATGTTACCTACTGATTATGTTG	
XB10PR200r	TAATGTACCGCAGCAATA	
XB30PR244f	ACTTTCTTTCGTTATGTTGAC	
XB30PR416r	GCACTATCCGTGAATACC	
PRup-SalF	ACGCGTCGACTAATCAGTCGGCAACATTTTCC	
PRup-SphR	ACATGCATGCGTCAGACCAATAAAGCCAAA	
PRdn-KpnF	CGGGGTACCTGCCAAAAAATCCGATG	Deletion of the PR gene
PRdn-SalR	ACGCGTCGACAATAAAATTGGGTTTGGATTA	
PR_delF	ACATTAAGAAGTTGCTCT	
PR_delR	ACCACTACATAATACCCT	

Supplementary Table 3. Growth rates (μ/d) and final cell yields (OD600) of light (L)- vs. dark(D)-cultures of the 47 glacial *Flavobacterium* strains[&]

[&], triplicate cultures of each strain were assayed; growth rates were calculated based on the regression of growth rate of the triplicate culture, and the final OD600 averages and standard deviates were shown. Hh15^T, *Cryobacterium luteum*.

*, only the final OD600 values are recorded due to floculent cultures;

#, light-dark growths of the strains were also tested in pre-illuminated medium.

†, light-dark growths of strain XB07 at oligotrophic condition.

Stains	μ/dark	μ/light	μ fold of L/D	Final OD600 /dark	Final OD600 /light	Final OD600 fold of L/D
XB01	0.11	0.13	1.2	0.52±0.026	0.65±0.03	1.3
XB02	0.17	0.22	1.3	0.84±0.02	1.12±0.02	1.3
XB03	0.16	0.28	1.8	0.61±0.01	0.99±0.02	1.6
XB04	0.06	0.09	1.5	0.51±0.01	0.85±0.015	1.8
XB05[#]	0.03	0.08	2.7	0.43±0.012	0.49±0.08	1.1
XB06	0.09	0.13	1.4	1.14±0.02	1.72±0.05	1.5
XB07[#]	0.04	0.09	2.2	0.42±0.016	0.92±0.031	2.2
XB07†	0.01	0.05	5.00	0.15±0.02	0.39±0.00	2.5
XB08	0.15	0.25	1.7	1.23±0.07	1.51±0.08	1.2
XB09	0.08	0.13	1.6	0.87±0.01	1.29±0.02	1.5
XB10	0.06	0.11	1.8	0.58±0.023	0.97±0.016	1.7
XB11	0.06	0.13	2.2	1.4±0.04	1.52±0.07	1.1
XB12	0.05	0.09	1.8	0.87±0.02	1.38±0.05	1.6
XB13	0.12	0.19	1.6	1.43±0.04	1.9±0.05	1.3
XB14	0.12	0.19	1.6	1.16±0.05	1.5±0.07	1.3
XB15	0.04	0.11	2.8	0.81±0.02	1.33±0.04	1.6
XB16	0.03	0.1	3.3	0.72±0.01	1.33±0.03	1.9
XB17	0.08	0.12	1.5	1.04±0.02	1.12±0.02	1.1
XB18	0.07	0.13	1.9	0.93±0.04	1.13±0.03	1.2
XB19	0.03	0.08	2.7	0.99±0.02	1.29±0.025	1.3
XB20	0.03	0.05	1.7	0.88±0.01	1.06±0.02	1.2
XB21	0.05	0.08	1.6	0.87±0.015	1.38±0.03	1.6
XB22	0.03	0.05	1.7	0.68±0.01	0.85±0.04	1.3
XB23	0.03	0.07	2.3	0.75±0.03	1.32±0.05	1.8
XB24	0.04	0.07	1.8	0.7±0.02	1.03±0.05	1.5
XB25	0.06	0.12	2.0	0.92±0.02	1.16±0.035	1.3
XB26	0.04	0.07	1.8	0.41±0.021	0.78±0.035	1.9
XB27	0.03	0.05	1.7	0.68±0.01	0.78±0.01	1.2
XB28	0.04	0.07	1.8	0.43±0.01	0.84±0.02	2.0
XB30	0.03	0.06	2.0	0.32±0.04	0.54±0.04	1.7
XB31	0.05	0.09	1.8	0.68±0.01	0.93±0.02	1.4
XB32	0.05	0.08	1.6	0.59±0.01	0.92±0.036	1.6
XB33	0.07	0.11	1.6	1.23±0.01	1.46±0.04	1.2
XB35	0.08	0.15	1.9	1.07±0.01	1.69±0.01	1.6

XB36	0.06	0.09	1.5	0.64±0.03	1.26±0.00	1.6
XB37	0.01	0.04	4.0	0.41±0.01	0.85±0.02	2.1
XB38	0.01	0.02	2.0	0.29±0.01	0.54±0.01	1.9
XB39*	NA	NA		0.39±0.005	0.52±0.02	
XB40	0.05	0.09	1.8	0.58±0.01	0.93±0.02	1.6
XB41	0.03	0		NA	0	
XB43	0.04	0.08	2.0	0.45±0.034	0.94±0.04	2.1
XB44	0.09	0.17	1.9	1.41±0.02	1.86±0.04	1.3
XB45	0.03	0.08	2.7	0.55±0.01	0.66±0.03	1.2
XB47	0.04	0		NA	0	
XB48	0.05	0.12	2.4	1.21±0.02	1.66±0.03	1.4
XB49	0.02	0.07	3.5	0.53±0.01	0.72±0.02	1.4
XB50	0.03	0.06	2.0	0.71±0.02	1.23±0.04	1.7
XB51	0.08	0.15	1.9	1.06±0.01	1.17±0.02	1.1
Hh15^T	0.06	0.06	1.0	0.56±0.07	0.56±0.08	1.0

Supplementary Table 4. Genome sizes and numbers of the over-representative genes in each of 47 glacier strains*

* *crtBIYZ*, the gene cluster encoding zeaxanthin; *dar*, the gene cluster encoding flexirubin; *prd*, the proteorhodopsin gene; *blh*, the gene for β-carotene dioxygenease; NF, not found.

#, the absence of the *prd* gene has been verified by no PCR product amplified using the gene generated primers listed in supplementary table 4.

Isolates	N50 (Kbp)	Genome size (Mbp)	*crtBIYZ*	*dar*	*prd*#	*blh*	TonB-dependent receptor numbers	ECF-type sigma factor numbers (total σ factor numbers)	Two-component system numbers		Cold shock protein numbers
									Histidine kinase	Response regulator	
Strains isolated from southern glaciers											
XB01	241,851	6.5	+	+	-	-	100	19(28)	53	79	5
XB02	460,503	5.6	+	+	-	-	89	23(36)	40	53	1
XB03	369,172	6.5	+	+	-	-	101	19(28)	54	79	5
XB05	761,497	4.1	+	-	-	-	51	8(15)	27	34	1
XB08	396,009	3.4	+	-	+	NF	29	5(8)	21	25	1
XB09	234,309	3.7	+	-	+	+	28	5(9)	32	37	1
XB31	761,345	4.1	+	-	-	-	52	10(16)	26	34	1
XB32	379,396	3.8	+	-	+	+	28	7(10)	28	37	1
XB33	155,378	4.0	+	-	+	+	32	7(10)	25	40	1
XB35	562,988	3.7	+	-	+	+	37	9(35)	23	31	1
XB36	417,640	5.5	+	+	-	-	77	20(32)	41	57	3
XB44	818,938	3.8	+	-	+	+	34	6(9)	21	35	1
XB45	1,059,330	4.0	+	+	-	-	26	9(10)	27	52	1
XB51	215,962	4.0	+	-	+	+	28	7(10)	25	35	1
Strains isolated from northern glaciers											
XB04	67,384	3.5	+	-	+	+	27	5(9)	19	26	1

XB06	269,129	3.6	+	-	+	+	31	9(13)	27	27	1
XB07	302,468	3.7	+	-	+	+	38	9(14)	28	28	1
XB10	175,403	3.7	+	-	+	+	33	8(15)	24	32	1
XB11	235,828	3.9	+	-	+	NF	29	5(9)	33	37	1
XB12	558,867	3.5	+	-	+	+	32	7(9)	19	25	2
XB13	300,785	3.5	+	-	+	+	32	10(15)	27	27	1
XB14	353,682	3.5	+	-	+	+	32	7(14)	25	28	1
XB15	215,725	3.4	+	-	+	+	28	5(9)	17	20	1
XB16	212,768	3.5	+	-	+	+	31	8(14)	25	25	1
XB17	298,385	3.6	+	-	+	+	31	9(13)	28	27	1
XB18	189,248	3.7	+	-	+	+	25	5(9)	23	30	1
XB19	216,389	3.7	+	-	+	+	30	9(12)	23	22	1
XB20	219,145	3.6	+	-	+	+	34	9(16)	26	25	1
XB21	462,002	3.4	+	-	+	NF	29	5(9)	22	28	1
XB22	345,953	3.5	+	-	+	+	25	6(10)	30	22	1
XB23	481,111	3.4	+	-	+	+	62	6(12)	62	19	1
XB24	216,315	3.7	+	-	+	+	29	9(12)	23	22	1
XB25	187,798	3.5	+	-	+	+	32	6(11)	22	21	1
XB26	283,155	3.6	+	-	+	+	28	6(10)	20	31	1
XB27	340,820	3.0	+	-	+	+	24	7(10)	20	18	1
XB28	193,754	3.5	+	-	+	+	35	9(16)	26	25	1
XB30	185,953	3.5	+	-	+	+	31	8(14)	25	25	1
XB37	159,605	3.5	+	-	+	+	29	8(12)	24	23	1
XB38	121,516	3.5	+	-	+	+	36	9(15)	18	23	1
XB39	137,582	3.6	+	-	+	+	30	5(8)	22	35	1
XB40	193,433	3.4	+	-	+	+	28	9(12)	23	19	1

XB41	116,021	4.1	-	+	-	-	50	10(15)	20	23	1
XB43	183,002	4.6	+	-	-	-	52	10(16)	27	41	1
XB47	116,001	4.1	-	+	-	-	50	10(15)	22	23	1
XB48	240,098	3.4	+	-	+	+	41	7(15)	12	21	1
XB49	233,761	3.4	+	-	+	+	41	7(15)	12	20	1
XB50	309,273	3.4	+	-	+	+	23	6(7)	20	30	1