Finding a niche: The habits and habitats of purple non-sulfur bacteria

Rachel S. Poretsky

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Abstract

Purple non-sulfur (PNS) isolates obtained by members of the 2003 Microbial Divesity course were characterized in terms of their salt and sulfide tolerances and preferences. I investigated growth as well as cell pigment and protein content and found a variety of different behaviors by the same isolates from Great Sippewissett Marsh when grown in different conditions and a diversity of behaviors between the isolates I characterized. PNS bacteria from three points along a salinity gradient (0, 10, and 25 ppt) were also characterized by direct plating and by DGGE. Taken together, the results of my investigations indicate that microorganisms in a particular environment may not always be best suited to the conditions there. It is speculated that this is an ecology strategy by the PNS bacteria that may arise from interactions with the other organisms there and the changing conditions of the environment.

Rachel S. Poretsky
The University of Georgia
Department of Marine Sciences
Athens, GA 30602

poretsky@uga.edu

Introduction

The purple non-sulfur bacteria (PNS) are the most diverse and versatile of the phototrophic purple bacteria. Phylogenetically, many branch within the α-proteobacteria, however they are found within the β- and γ- Proteobacteria as well (3). Although they live primarily as photoheterotrophs, they have a wide range of growth modes and are able to grow photoheterotrophically under anaerobic conditions in the light and with a variety of organic substrates such as TCA-cycle intermediates. All probably use H_2 as electron donor and some PNS bacteria can obtain energy by fermentation or anaerobic respiration. Other electron donors may be S^-, $S_2O_3^-$, S^o. Electron acceptors include nitrate, DMS, and TMA. Many PNS bacteria can also grow as photoautotrophs or chemoheterotrophs under microaerobic or aerobic conditions in the dark and single species are known to switch from one mode of metabolism to another depending on the conditions available (11).

The purple bacteria are ecologically significant due to their involvement in a variety of biogeochemical cycles. In addition to fixing carbon, which can be used by chemotrophic organisms, they are capable of fixing nitrogen. Their use of reduced sulfur compounds renders them players in the global cycle.

PNS bacteria have been isolated from almost every environment, including freshwater, marine systems, plants, soils, and activated sludge (13). Although they are widely distributed in the environment, their phylogenetic, genotypic, and functional diversity is not well understood. A recent study by Oda, et al. examined PNS genotypic and phenotypic diversity in two different aquatic sediments (12). Many different genotypes were observed in the two systems, but these genotypes were found to mostly comprise a few major phylotypes. Using the sensitive method of REP-PCR, they found that the diversity instead existed within a few single, dominant bacterial species and was at the strain level. Results of this study suggested that certain strains of *Rhodopseudomans palustris*, for example, might be endemic to a particular site. Distinct genotypic and phenotypic differences existed between strains isolated from the two sites.

There have been many other studies of the seasonal and spatial distribution of microorganisms, and there seems to be great variation and evidence of adaptation of specific types of bacteria to specific niches (2). Niche theory has been a fruitful paradigm for studying adaptive evolution and speciation among phylogenetically related populations of macro-organisms, such as Darwin's finches, that share resources in an ecosystem, but has not been used systematically to study parallel processes among related bacteria that carry out similar functions within an ecosystem. The general goal of this study was to examine what conditions might drives that microbial niche separation and how the microbial populations within a group of bacteria- the PNS bacteria- interact to form a stable functional group within an ecosystem. More specifically, I aimed to characterize PNS isolates obtained by members of the 2003 Microbial Diversity summer course from the Great Sippewissett Marsh in terms of their tolerances to sulfide and salt. In addition, I attempted to analyze the PNS community along a salinity gradient in the Quashnet River Estuary to determine if and how the PNS community changes over the course of such a gradient and whether the PNS bacteria are uniquely adapted to a specific niche.

Materials and Methods

Characterization of isolates

PNS enrichment

Members of the course took cores from the Great Sippewissett Salt Marsh with the back end of a glass Pasteur pipette on June 17, 2003. Enrichments were carried out the following day by placing a small (~0.5 g) portion of the top 0.5 cm of the core sample into a sterile 15 ml tube. We added 5 ml of basal salts medium with no yeast extract or carbon source (1 g/l NaCl, 0.4 g/l $MgCl_2$ $6H_2O$, 0.15 g/l $CaCl_2$ $2H_20$, 0.2 g/l KH_2PO_4, 0.25 g/l KCl, and 0.25 NH_4Cl). Approximately 1 g of glass beads were added and the tube was vortexed for 15 sec., followed by shaking at room temperature for about 30 min. After allowing the material to settle for 20-30 min., 100 µl of the top layer was plated undiluted and at a 1:10 dilution on PNS plates: (10 g/l NaCl, 0.4 g/l $MgCl_2$ $6H_2O$, 0.1 g/l $CaCl_2$ $2H_20$, 0.2 g/l KH_2PO_4, 0.5 g/l KCl, 2.38 g/l HEPES, 0.1 g/l yeast extract, 10 mM carbon source- either ethanol or succinate. 1x trace elements SL12 (as described in (9)) and 1x vitamin mix (0.1 mg/ml biotin, 0.35 mg/ml niacin, 0.3 mg/ml thiamine dichloride, 0.2 mg/ml *p*-aminobenzoic acid, 0.1 mg/ml pyridoxolium HCl, 0.1 mg/ml Ca-panthothenate, 0.05 mg/ml vitamin B_{12}) were added after autoclaving and adjusting pH to 6.8). An N source was omitted from the plates in order to bias towards organisms that fix nitrogen and against heterotrophs. The plates were incubated in anaerobic BBL gas pack jars near 40 W lightbulbs. After one week of incubation, the plates were examined for red or brown colonies, which were then restreaked onto new PNS plates containing NH_4Cl (0.25 g/l). I continued to restreak and maintain several isolates from course members for my analyses.

Growth measurements

I prepared PNS liquid media without a carbon or nitrogen source and made it anaerobic by bubbling for 45 min with N_2. I then aliquoted 10 ml of media into Hungate tubes in the anaerobic glovebox. Tubes were sealed with mushroom stoppers, crimped and then autoclaved. After autoclaving, I added 1X vitamins, 1x trace elements SL12, and succinate to 10 mM. I inoculated ten tubes with ten different isolates. The isolates were grown for one week in the light until enough were sufficiently turbid to start the growth curves. Each of eight isolates was inoculated into media with three different sulfide (using Na_2S) concentrations: 100µM, 500 µM, and 2 mM and three different NaCl concentrations: 1 g/l, 10 g/l, and 30 g/l to a starting OD_{660} of 0.05. The sulfide treatments all had 10 g/l NaCl, whereas the salt treatments had no added sulfide. OD_{660} measurements were taken approximately every three hours during the day for seven days.

Pigment and protein analysis

Pigment compositions were determined spectrophotometrically by harvesting a 1 ml sample, extracting the pellet with acetone-methanol (7:2), as described by Cohen-Bazire et al. (8), and observing the absorbance between 400 and 1000 nm. A millimolar extinction coefficient of $76mM^{-1}cm^{-1}$ (770 nm) and a molecular weight of 911.5 were used (6) to calculate bacteriochlorophyll concentrations.

Proteins were solubilized by adding 10µl of 10N NaOH to 1 ml of cells and boiling for 5 min. After brief centrifugation, the total protein concentration was determined by the dye binding method described by Bradford (4) using BSA to generate a standard curve.

Sequencing

To sequence the isolates, 1 ml of the liquid culture was centrifuged and the pellet was resuspended in 50 µl milli-Q water and five freeze-thaw cycles were carried out in -80°C and 65°C. Prior to PCR amplification with general eubacterial primers 8F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'), the cells were incubated at 97°C for 7 min. PCR reactions were cycled in an Eppendorf thermocycler with a temperature profile as follows: 95°C for 5 min, followed by 30 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 1:30 min, with a final extension step at 72°C for 8:30 min. PCR products were viewed on a 1% agarose gel in TBE and cleaned up using Millipore Microcon centrifugal filter devices (Millipore, Bedford, MA). Sequencing was done by the sequencing facility at Michigan State University. I analyzed the sequences using the Ribosomal Database Project II (http://rdp.cme.msu.edu/html/) Sequence Match and Phylip Interface tools.

Characterization of communities along a salinity gradient

Sample collection and direct plating

The Waquoit Bay National Estuarine Research Reserve is located on the south shore of Cape Cod, MA (Fig. 1). The Quashnet River Estuary is the largest source of freshwater to Waquoit Bay. Water samples were collected on July 10, 2003 in 10 L acid-washed carboys from water at the shore of the Quashnet River and Estuary at three different salinities: 0 ppt freshwater (FW), ~10 ppt mid-salinity (BW), and ~25 ppt high salinity (SW). The salinity at each site was determined using a portable refractometer. Samples were then transported back to the lab and filtered onto 0.2 µm GTTP Isopore membrane filters (Millipore, Bedford, MA) with or without a pre-filtration through an 8 µm pore size filter. Approximately 300 ml of water passed through the filters before the filter completely clogged.

Filters were placed on the surface of PNS plates with 10 mM succinate as a carbon source and with different salt contents: 1 g/l NaCL and 0.4 g/l $MgCl_2$ $6H_2O$ (FW), 8 g/l NaCl and 1.2 g/l $MgCl_2$ $6H_2O$ (BW), or 20 g/l NaCl and 3.0 g/l $MgCl_2$ $6H_2O$ (SW). One filter from each environment was placed on each type of plate. Plates were incubated anaerobically in the dark for one week as described above and monitored for the appearance of brown or purple colonies. After approximately one week, select colonies from each filter were plated onto new PNS plates in order to obtain pure colonies. DNA was then extracted from these colonies (as described above for the extraction of DNA from isolates) and PCR amplified with purple non-sulfur bacteria primers as described below.

DNA extraction and PCR

Using the MoBio UltraClean Soil DNA Isolation Kit (Mo Bio Laboratories, Inc., Solana Beach, CA), I extracted DNA from FW, BW and SW filters onto which about 300 ml of sample water had been filtered. Following amplification, DNA was amplified with purple non-sulfur specific primers encoding the M subunit of the photosynthetic reaction center (1). The primers were: pufM.557F (5'-CGCACCTGGACTGGAC-3') and pufM.750R (5'-CCCATGGTCCAGCGCCAGAA-3'). The primer set was used in 50 µl total volume PCR amplification mixtures containing 45 µl Platinum PCR SuperMix (Invitrogen Life Technologies, Carlsbad, CA), 1.0 µl of each primer at 10 µM, 1-3 µl DNA template, and milli-Q water. Reactions were cycled in an Eppendorf thermocycler with a temperature profile as follows: 94°C for 1 min, followed by 30 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min, with a

final extension step at 72°C for 10 min. Products were electrophoresed on a 1% agarose gel in 1X TBE.

DGGE

Denaturing gradient gel electrophoresis was used to separate DNA fragments of the same size that differed in base composition. DGGE gels were prepared using a gradient former with the Econo Pump (Bio-Rad, Hercules, CA). A DCode Universal Mutation Detection System (Bio-Rad, Hercules, CA) was used to electrophorese the gel. All pufM PCR products (fragment size of 229 bp) were electrophoresed on a 10% acrylamide-bisacrylamide gel prepared from 30% acrylamide-bisacrylamide (Bio-Rad, Hercules, CA). The parameters for the pufM DGGE had been optimized previously (1) at a 20-80% gradient. The gel was run for 6 hours at 130V and a 60°C running temperature. Although GC clamped primers are typically used to PCR amplify products for DGGE, high resolution of DGGE bands was known to be obtained without GC clamps (1) and so I did not use GC-clamped primers in my PCR reactions. The DGGE gel was stained with Sybr Gold nucleic acid gel stain (Molecular Probes, Eugene, OR) and photographed.

Results

Sippewissett Isolate Growth

Of ten purple non-sulfur isolates selected from the Sippewissett enrichments, only eight (PNS1, 3, 4, 6, 7, 8, 9, and 10) grew rapidly enough to be included in a growth curve that could be carried out in the time of the course. These isolates exhibited slightly different colony morphologies from one another (Fig. 2). Most isolates were rod or cocci (Fig. 3) and there was no apparent difference between the isolates obtained using succinate or ethanol as a carbon source. Sequencing results were obtained only for two of the organisms, PNS3 and PNS4, both of whom are α-proteobacteria, closely related to *Rhodovulum sulfidophilum*.

The eight isolates exhibited different responses when grown in the different sulfide or salt concentrations (Fig. 4). Three isolates, PNS1, PNS6, and PNS10, grew poorest in the lowest salt concentration of 1 g/l. Isolates 1 with doubling times about twice as long as when grown in the higher salt concentrations (Table 1). PNS9 also could not in 1 g/l NaCl, but was unable to grow in 10 g/l NaCl as well and grew at the highest salinity with a long doubling time of 28 h. In contrast, two of the isolates, PNS7 and PNS8, experienced the slowest growth in the highest salinity and doubled most rapidly in the lowest salinity. PNS3 and PNS4 did not appear to be inhibited at any of the three salt concentrations.

Different growth rates were observed when the isolates were grown with three different sulfide concentrations (Fig. 5). All the sulfide treatments were grown with 10 g/l NaCl as well. PNS7 and PNS9 were the only isolates for which the growth in 500 μM S^- did not parallel the growth in 100 μM S^-. In the course of 7 days, PNS 9 never grew in the lowest sulfide concentration. PNS7 grew faster in the middle sulfide concentration of 500 μM, but it reached log phase in this sample about two days after it reached log phase in the 100 μM treatment. This pattern was observed with several other isolates at the highest sulfide concentrations. PNS1, PNS3, PNS4, PNS6, PNS9, and PNS10 all remained in lag phase longer in the 2 mM S^- treatment than in the treatments with lower sulfide concentrations. A few of these, PNS3, PNS6, PNS9, and PNS, in fact had doubling times the same or faster in the high sulfide sample once

they began growing in log phase (Table 1). PNS7 and PNS8 both appear to begin growing early, but then die after reaching a peak OD_{660} of approximately 0.2.

Pigment and protein analyses

All of the eight isolates exhibited color changes when grown under different conditions. The most vibrant red color was seen in the low sulfide treatments (Fig. 6) and varied both within and among isolates. In order to elucidate the potential reasons for these differences, I measured pigment spectra as well as protein concentrations in two of the eight isolates. These isolates, PNS1 and PNS10, had similar color patterns (Fig. 6) but slightly different growth patterns (Table 1, Figs 4 and 5). Both PNS1 and PNS10 had a bacteriochlorophyll *a* peak around 771 nm and a carotenoid peak at either 456 nm or 483 nm (data not shown). Sequencing shows PNS1 is an α-proteobacteria in the group *Rhodobacteraceae.* The PNS10 sequence most closely matched members of the A*eromonas* species. In both 30 g/l salt samples, a double peak was observed in the carotenoid region. The peak intensities varied between organisms and between treatments, and the carotenoid:chlorophyll ratios are displayed in Table 2. Protein compositions were then looked at in order to determine if differences in pigments were simply a consequence of protein differences or if there was a true pigment. The results show that the organisms grown with sulfide additions produced more protein than those grown without (Fig. 7). Chlorophyll concentrations were also higher in these treatments, however the chlorophyll:protein ratios did not follow the same pattern. In the NaCl treatments, it appears that a larger percentage of the proteins in the cells is chlorophyll. This was more dramatic with PNS10 than with PNS1.

Direct isolations

I obtained a large number of morphologically diverse colonies on 0.2 μm filters from different salinities (freshwater= FW, mid-salinity= BW, and salt water= SW) in the Quashnet River Estuary. It appears that filters from the BW site are most dense, and those from the FW site are least dense. In fact, no isolates were obtained from the FW sample when the filter was placed on a SW plate. Filters from all three sites were densest on the freshwater plates and least dense on the SW plates, regardless of the source of the inoculum. The colors of the colonies appeared to be slightly different on filters from different environments (Fig. 8).

DGGE

Amplification products were obtained from the environmental samples as well as the isolates using the pufM primer set. When run on a DGGE gel, the purple non-sulfur community looks fairly different in the different salinity environments, however there are several bands that appear to be present in two or all three samples (Fig. 9). The BW and the SW sample are more similar to each other than either is to the FW sample. This seems true for the isolates as well. A bright band at the same point in the gel is seen in both the SW and BW isolates that were obtained from filters inoculated on SW plates. There were also common bands in all of the BW samples and some bands in common between the SW samples.

Discussion

Characterization of the purple non-sulfur isolates from Sippewissett Marsh revealed a diversity of salt and sulfide tolerances among the isolates. Some organisms, such as PNS9, coul not grow in the lowest salt concentration of 1g/l while others, such as PNS did not grow well under the highest salt concentration of 30 g/l. Although tidal cycles and freshwater inflows into Sippewissett influence the salinity on a daily basis, the salinity does not change all that much, ranging from about 28-32 ppt (14). This might lead one to speculate that the organisms found there would all have high salt tolerances and would grow best in the 30 g/l salt treatment. Several isolates did, in fact, have the most rapid doubling times when grown in the presence of high salt concentrations, however the fact that some appeared to be inhibited by high levels of salt was somewhat surprising. Perhaps these organisms are situated in the marsh near a source of freshwater input such as an area that accumulates rainwater or receives substantial groundwater flow. The highly conductive aquifers of glacial till deposits in Sippewissett, which are composed of low organic coastal sands, tend to have large groundwater discharge (14). Unfortunately, there is no record of the types of areas where samples were taken from the marsh for the PNS enrichments. It would be interesting to determine whether the organisms that prefer low or no salt are found near fresher water in the marsh, or if they are inactive but present in the high salt regions.

Different sulfide tolerances were also observed among the isolates. Early biogeochemical studies in Sippewissett found soluble sulfide concentrations in the pore waters to vary on an annual basis, ranging from near zero up to 2 mM sulfide (10) (7). Most of the isolates grew well in high (2 mM) sulfide only after a long lag phase, but eventually reached the same OD_{660} at stationary phase as when grown in lower sulfide concentrations. The range of sulfide tolerances between the isolates might be explained by the variable sulfide concentrations in the marsh pore waters, however a better correlation would involve measuring sulfide concentrations at the location from which samples were enriched.

The different colors exhibited by the same isolate when grown under different conditions seems to be attributable to a change in pigments, both chlorophyll and carotenoids. Because the pigment and protein analyses were done after the isolates had reached stationary phase in which both isolates PNS1 and PNS10 had an OD_{660} of approximately 1.0 in the NaCl treatments and 1.8 in the sulfide treatments, it seems possible that the more vibrant red color in the sulfide treatments had to do with cell density. Previous studies have shown that some bacteria have different adaptations in pigment content and the pigment content can be a good indicator of the physiological status of the cell *in situ* (5). Cohen-Bazire et al. also demonstrated that pigment contents can be adjusted in PNS to light intensity and bacteriochlorophyll synthesis can be regulated by oxygen (8). These data suggest that the isolates I studied may have distinct physiological responses as a result of different conditions.

The Quashnet River direct isolations and DGGE analysis supported the observation that there are organisms that can be isolated from an environment that are not necessarily most well suited to the conditions there. The system is dynamic there, so it is possible that there is a constant influx of bacteria that are simply "passing through." Perhaps, however, there are all types of organisms present in a particular environment and they are simply inactive or slow growing until conditions become more suitable for them. The PNS bacteria tend to be extremely versatile organisms, however my results indicate that they do have specific salinity and sulfide preferences. It would be interesting to observe competition between these organisms to

determine how they respond when they are surrounded by organisms seem to prefer a given condition. It would also be useful to quantify the organisms that appear in all points across the gradient.

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References

1. Achenbach, L. A., J. Carey, and M. T. Madigan. 2001. Photosynthetic and phylogenetic primers for detection of anoxygenic phototrophs in natural environments. Applied and Environmental Microbiology **67:**2922-2926.
2. Amann, R. I., W. Ludwig, and K. H. Schleifer. 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol Rev **59:**143-69.
3. Blankenship, R. E., M. T. Madigan, and C. E. Bauer. 1995. Anoxygenic photosynthetic bacteria. Kluwer Academic Publishers, Dordrecht ; Boston.
4. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:**248-254.
5. Casamayor, E. O., J. Mas, and C. Pedros-Alio. 2001. In situ assessment on the physiological state of purple and green sulfur bacteria through the analyses of pigment and 5S rRNA content. Microbial Ecology **42:**427-437.
6. Clayton, R. K. 1966. Spectroscopic analysis of bacteriochlorophylls in vitro and in vivo. Photochem. Photobiol. **5:**669-677.
7. Cohen, Y., R. W. Castenholz, H. O. Halvorson, and Marine Biological Laboratory (Woods Hole Mass.). Ecosystems Center. 1984. Microbial mats, stromatolites : based on the proceedings of the Integrated Approach to the Study of Microbial Mats, July 26-31, 1982, sponsored by microbial ecology and marine ecology courses, and the Ecosystems Center, Marine Biological Laboratory, Woods Hole, Massachusetts. A.R. Liss, New York.
8. Cohen-Bazire, G., W.R. Sistrom, R.Y. Stanier. 1957. Kinetic studies of pigment synthesis by non-sulfur purple bacteria. J. Cell. Comp. Physiol. **49:**25-78.
9. Eichler, B., and N. Pfenning. 1988. A new purple sulfur bacterium from stratified fresh water lakes, Amoebobacter purpureus sp. nov. Arch. Microbiol. **155**.
10. **Howarth, R. W., A. Giblin, J. Gale, B.J. Peterson, and G.W. Luther.** 1983. Reduced sulfur compounds in the pore waters of a New England salt marsh, p. 135-152. *In* R. Hallberg (ed.), Environmental biogeochemistry : proceedings of the 5th International Symposium on Environmental Biogeochemistry (ISEB) arranged in Stockholm 1-5 June, 1981, vol. 35. Ecological Bulletins Pub. House, Stockholm.

11. **Imhoff, J. F.** 1995. Taxonomy and Physiology of Phototrophic Purple Bacteria and Green Sulfur Bacteria, p. 1-15. *In* R. E. Blankenship, M.T. Madigan, C.E. Bauer (ed.), Anoxygenic Photosynthetic Bacteria. Kluwer Academic Publishers, The Netherlands.
12. Oda, Y., W. Wanders, L. A. Huisman, W. G. Meijer, J. C. Gottschal, and L. J. Forney. 2002. Genotypic and phenotypic diversity within species of purple nonsulfur bacteria isolated from aquatic sediments. Applied and Environmental Microbiology **68:**3467-3477.
13. Overmann, J., and F. Garcia-Pichel July 25, 2000 July 25, 2000, posting date. The Phototrophic Way of Life. Springer-Verlag 3.http://link.springer-ny.com/link/service/books/10125/
14. Valiela, I., J.M. Teal, S. Volkmann, D.Shafer, E.J. Carpenter. 1978. Nutrient and Particulate Fluxes in a Salt Marsh Ecosystem: Tidal Exchanges and Inputs by Precipitation and Groundwater. Limnology and Oceanography **23:**798-812.

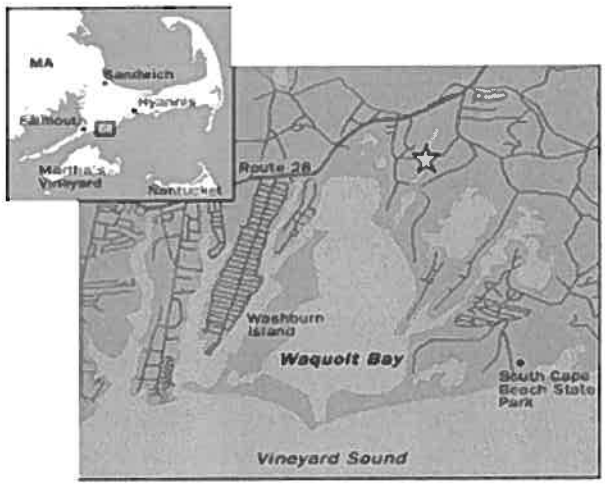

Figure 1: The Waquoit Bay, Cape Cod, MA. Sampling was carried out in The Quashnet River Estuary, located at the star.

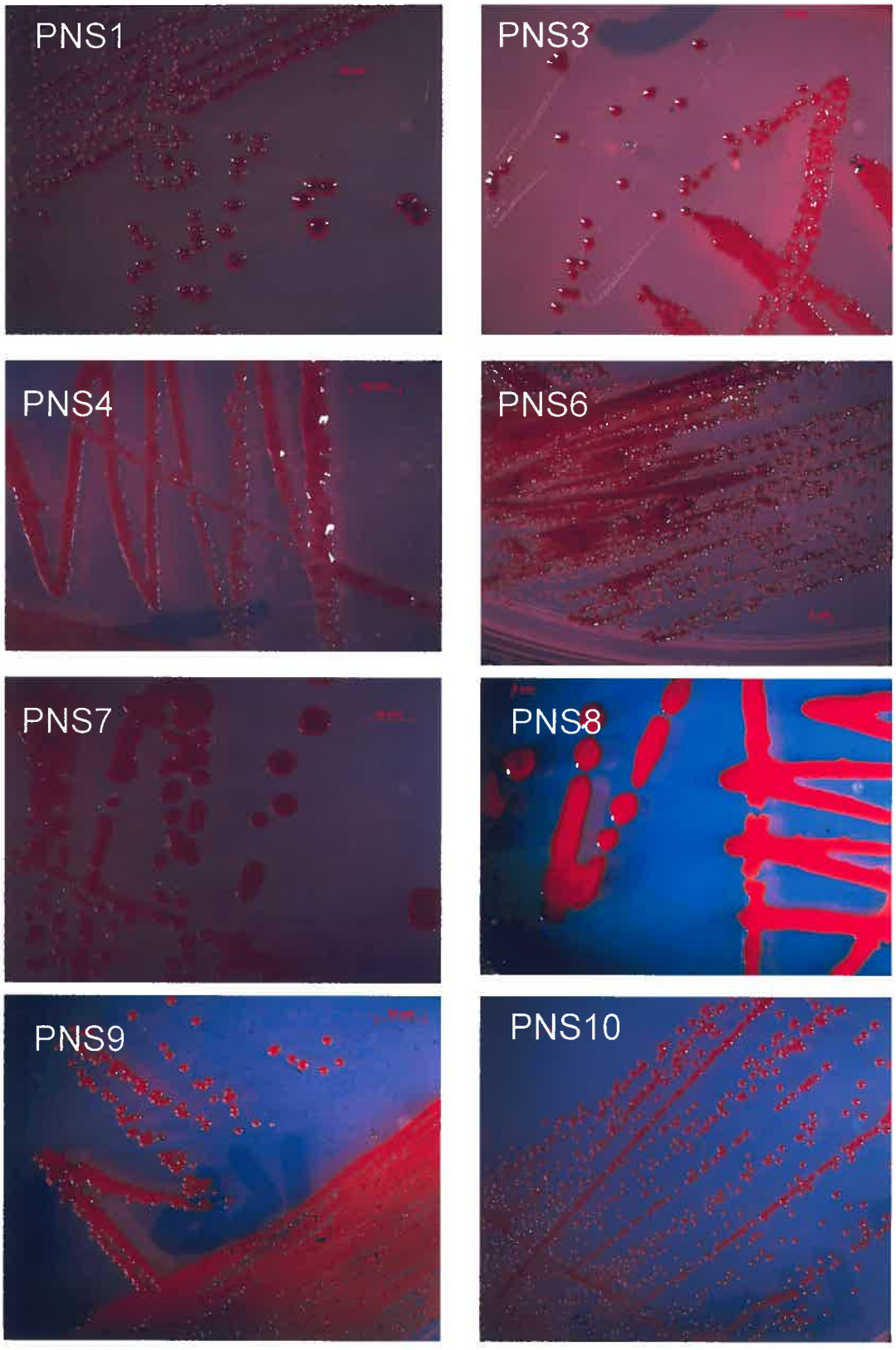

Fig 2. Colonies of varying sizes and colors used for growth measurements plated on PNS plates with succinate or ethanol and NH_4Cl (see Methods).

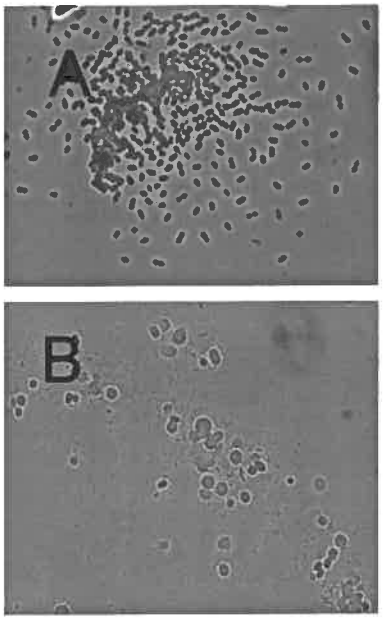

Fig 3. Examples of some of the purple non-sulfur cell types isolated from Sippewissett and growth with succinate (A) and ethanol (B) viewed with phase contrast under 100X magnification.

Isolate #	1	3	4	6	7	8	9	10
1g/L NaCl	16	12	17		23	18		25
10g/l NaCl	12	19	17	17	36	28		22
30 g/l NaC	8	12	15	21	34	21	28	10
100 uM S	7	5	15	15	19	16		15
500 uM S	7	6	16	11	13	18	17	14
2 mM S	13	4	29	10	19	23	15	14

Table 1: Doubling times in hours of Sippewissett isolates grown under different conditions.

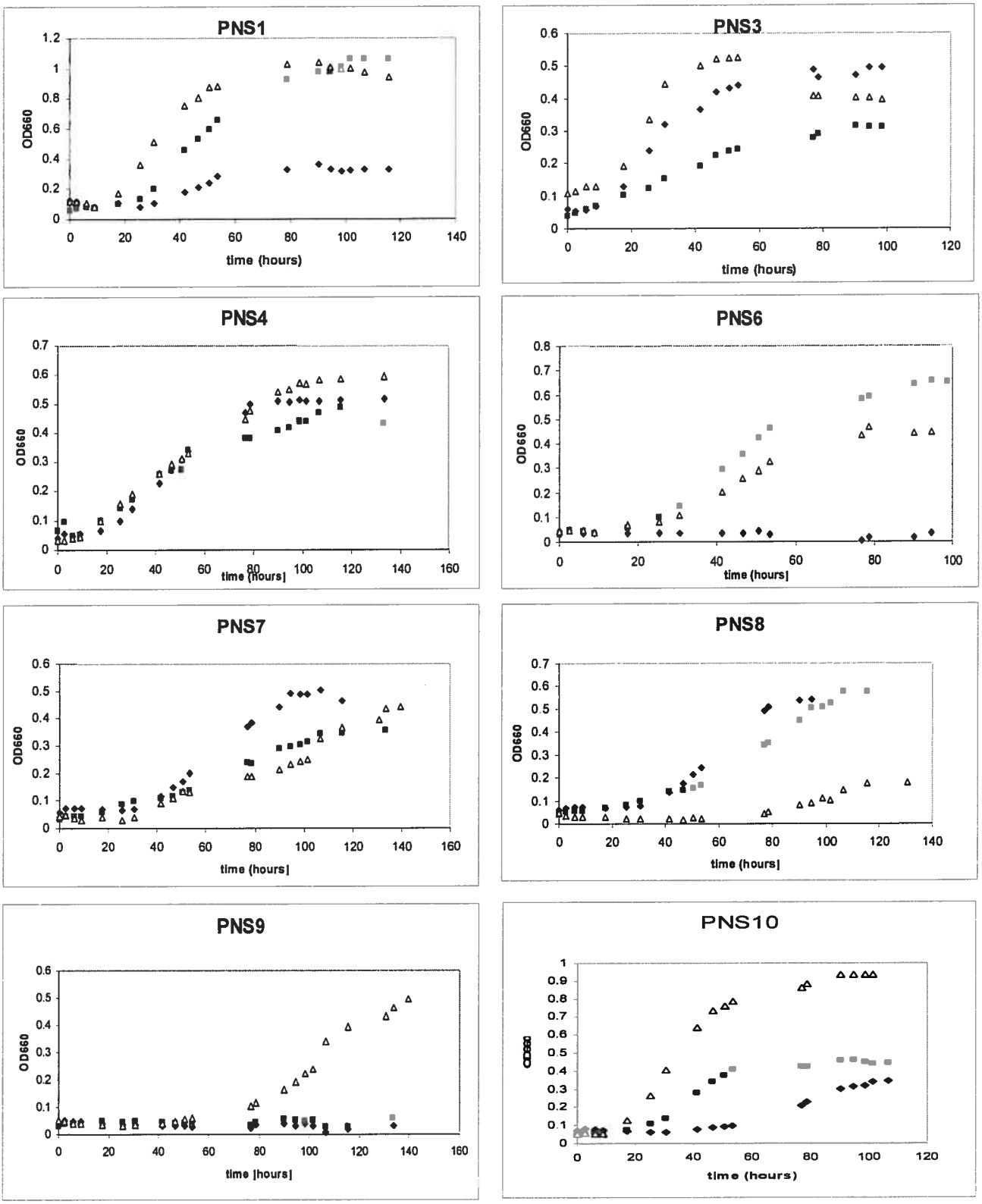

Fig 4. Growth curves of eight different PNS isolates under different salt concentrations. ♦= 1g/l, ■=10 g/l, and △=30 g/l.

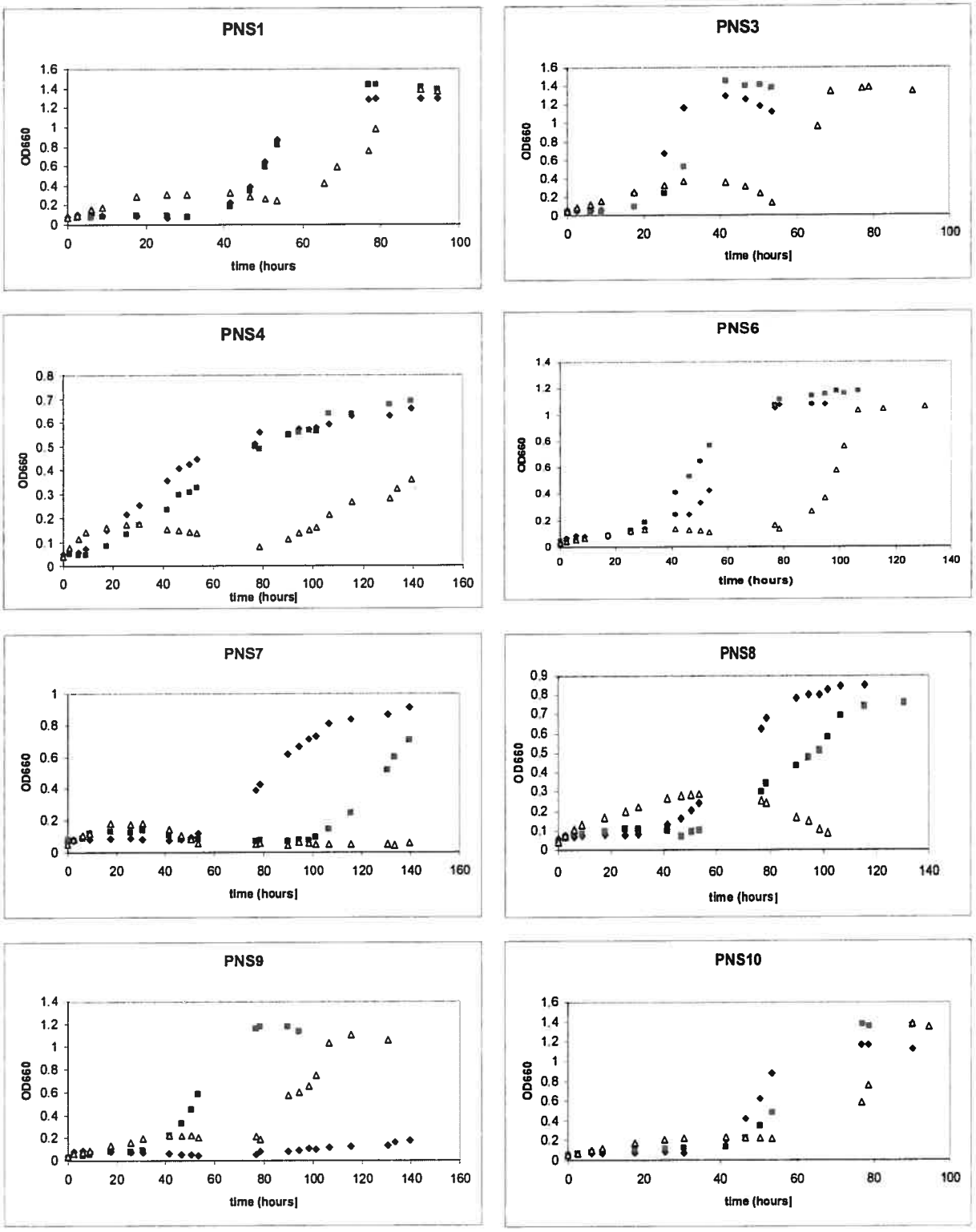

Fig 5. Growth curves of eight different PNS isolates under different sulfide concentrations. ♦= 100μM, ■=500 μM, and △=2mM Na_2S.

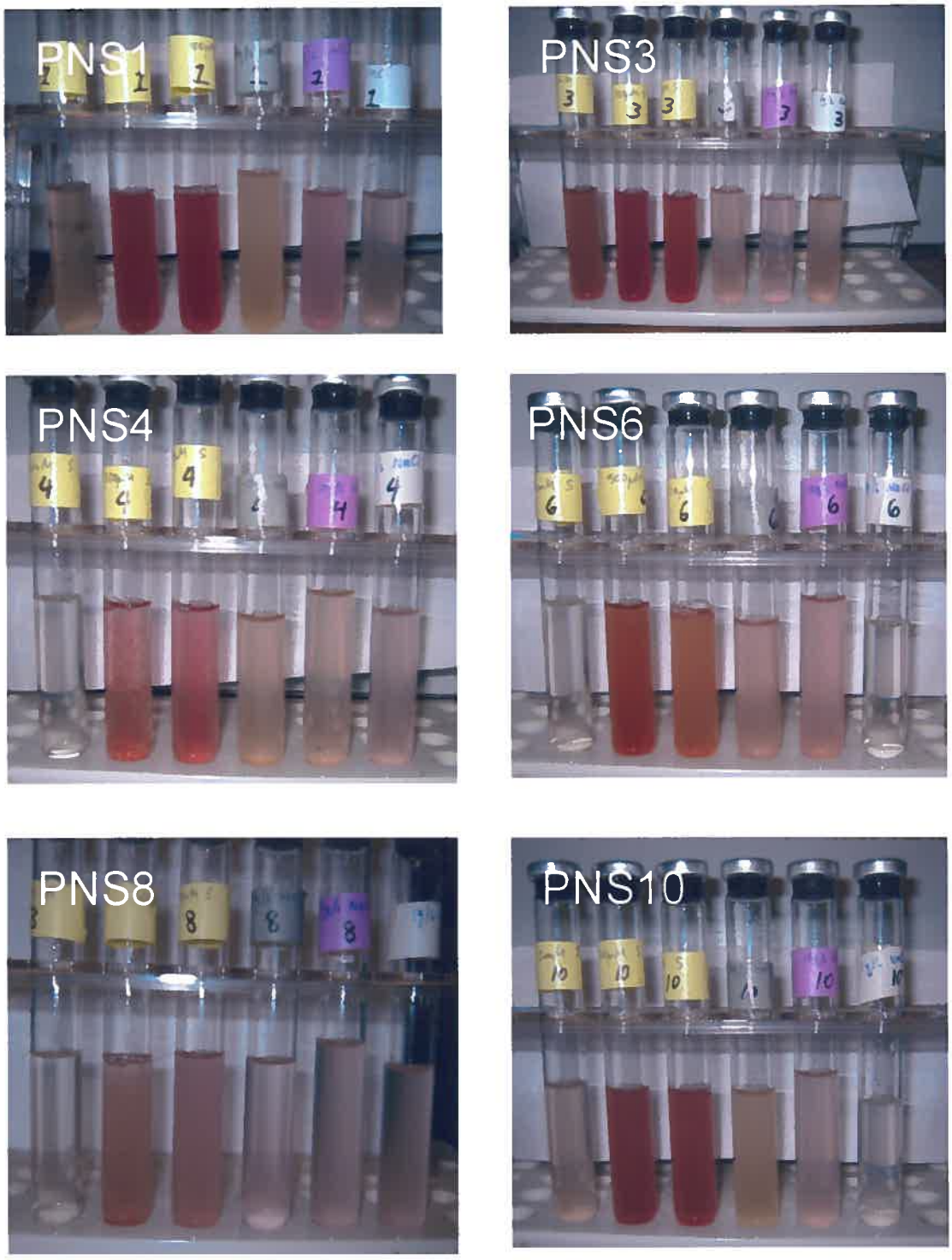

Fig 6. PNS isolates from Sippewissett Marsh grown under (from left to right): 2 mM S^-, 500 µM S^-, 100 µM S^-, 30 g/l NaCl, 10 g/l NaCl, and 1 g/L NaCl.

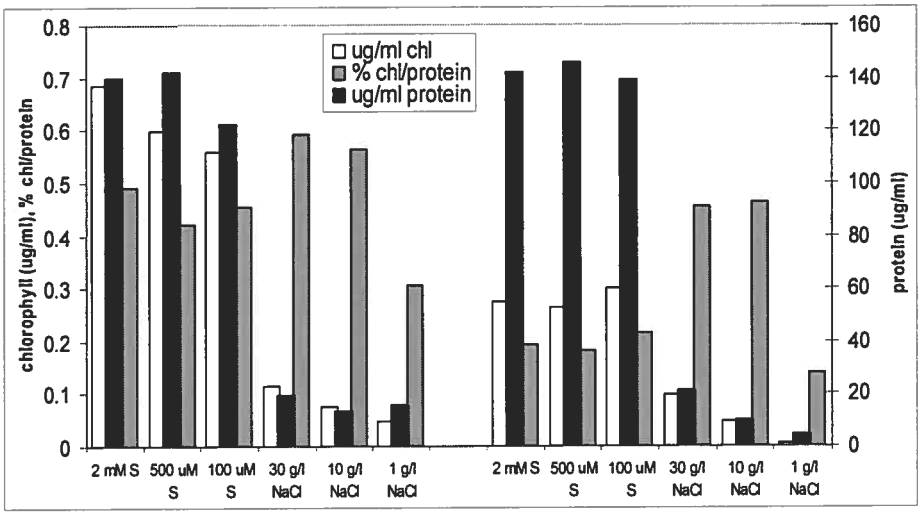

Fig. 7: Protein and bacteriochlorophyll contents in Sippewissett isolates PNS1 (left) and PNS10 (right) grown under different sulfide concentrations and different NaCl concentrations.

		peak wavelength		peak height		
		chl	carotenoids	chl	carotenoids	**Ratio carotenoid:chl**
PNS1	2mM S	772	483	0.57	0.625	**1.10**
	500 uM S	772	483	0.498	0.618	**1.24**
	100 uM S	770	483	0.466	0.652	**1.40**
	30 g/l NaCl	770	456	0.097	0.194	**2.00**
	10 g/l NaCl	772	485	0.063	0.142	**2.25**
	1 g/l NaCl	770	485	0.04	0.103	**2.58**
PNS10	2mM S	771	483	0.23	0.442	**1.92**
	500 uM S	772	482	0.221	0.385	**1.74**
	100 uM S	770	483.9	0.25	0.493	**1.97**
	30 g/l NaCl	771	456	0.082	0.197	**2.40**
	10 g/l NaCl	772.9	480	0.04	0.104	**2.60**
	1 g/l NaCl	893	480	0.005	0.047	**9.40**

Table 2: Pigment spectra from Sippewissett isolates PNS1 and PNS10

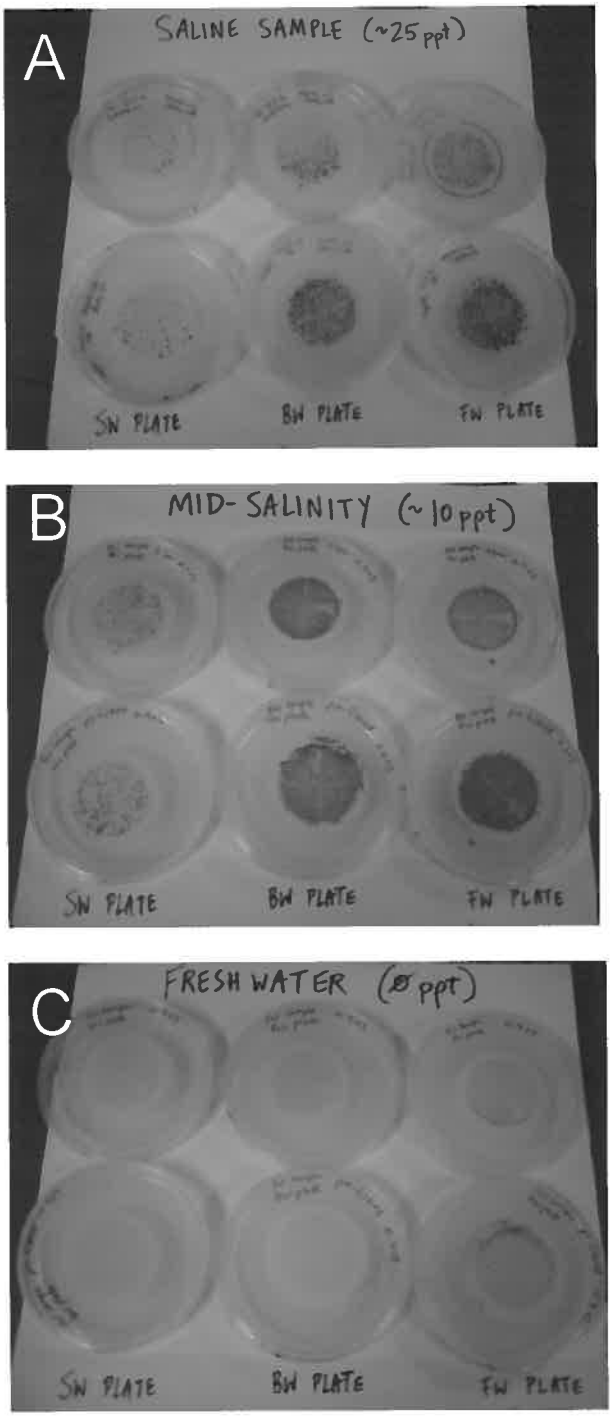

Fig. 8: Direct isolations on 0.2 µm filters from 300 ml of water of varying salinities. Filters from each site were placed on high, mid, and low salinity plates. Top and bottom rows of each photograph are duplicates.

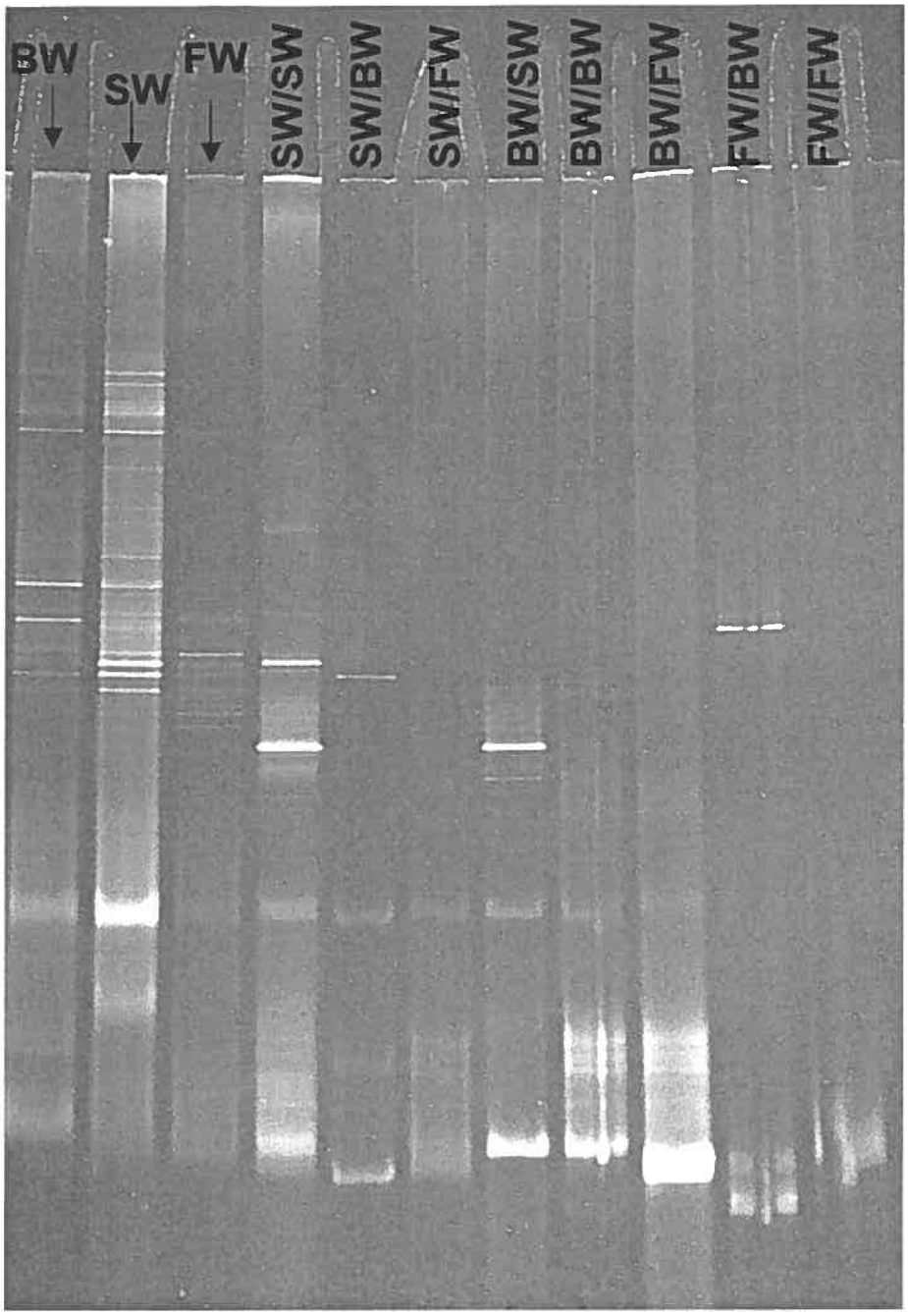

Fig 9. DGGE gel of *pufM* amplification products from environmental samples (lanes 1-3) as well as from isolates grown on filters. The isolates are labeled as the site from which they were obtained/the plate the filter was grown on.