CHAPTER VI

Characterization of *Synechococcus* swimming motility mutants

Abstract

Two components of the motility machinery involved in non-flagellar swimming in marine *Synechococcus*, SwmA and SwmB, have been localized to the cell surface. The nine motility mutants currently in culture, were characterized to determine the cellular localization of these two proteins as well as to identify other components of the outer membrane that may be involved in swimming motility. While SwmA and SwmB are not dependent on one another for proper localization on the cell surface, all motility mutants in culture have a defect in the localization of either SwmA or SwmB and in some instances both of these proteins. Additionally, two outer membrane polypeptides of 70 kDa and 80 kDa are absent in some of these mutants, suggesting a role in motility. This characterization implicates a multicomponent ABC transporter in the export of SwmA and reveals the importance of several glycosyltransferases for the proper localization of components of the motility apparatus.

Introduction

The mechanism of non-flagellar swimming motility in marine *Synechococcus* remains mysterious following several advances anticipated to shed light on this problem. Genome sequencing failed to identify components of the motility apparatus (12). Not only were no motility genes identified, completion of the genome sequence exposed the absence of chemotaxis genes, making the novel motility of these cells even more intriguing. Development of a transposon mutagenesis technique for use in marine *Synechococcus* identified three chromosomal regions involved in swimming

motility (8). While this technique has begun to delineate the genes required for swimming motility, the genes identified in these regions do not point to an obvious mechanism for motility.

Due to the absence of extracellular appendages and having ruled out several other models, Ehlers *et al.* argue that the cell surface itself must be the thrust-generating structure in these cells (5). Two cell surface proteins involved in non-flagellar motility in *Synechococcus* have been identified. One cell-surface component of the motility apparatus is SwmA, a glycosylated protein that is required for motility (1). This protein forms a para-crystalline S-layer on the cell surface (9). Inactivation of *swmA* abolishes motility although cells retain the ability to generate torque. Whether the S-layer plays a direct role in motility or a more indirect role, such as being required for the proper placement and functioning of other components of the motility apparatus, remains unclear. Another cell surface protein required for motility is SwmB. An extremely large protein, SwmB is found in the soluble fraction following removal of the outer membrane by EDTA treatment (1). How this megadalton sized protein functions in motility is still being investigated.

In addition to these two cell-surface proteins, transposon mutagenesis identified three chromosomal regions encoding genes required for motility (8). These genes include a number of multi-component ABC transporters, several putative glycosyltransferases, as well as conserved and hypothetical genes of unknown function (8). The requirement of particular transporters for swimming motility is not surprising as the few known components of the motility apparatus are located on or near the cell surface and these proteins must be exported from the cytoplasm to the

cell surface. Perhaps these transporters, which are non-essential for growth, are dedicated to exporting components of the motility apparatus. Similarly, the identification of glycosyltransferases required for motility was not unexpected. SwmA is a glycosylated protein (1) and glycosyltransferases are frequently involved in the biogenesis of other components of the cell envelope (3, 10). As the cell surface is of critical importance for non-flagellar swimming motility in *Synechococcus,* correct assembly of the cell envelope must be essential for the proper functioning of the motility apparatus.

For each motility gene identified by transposon mutagenesis, a directed mutation was constructed to confirm the non-motile phenotype of the original mutant (8). Several additional mutations were constructed to inactivate genes downstream of those directly affected by transposon insertion in an effort to rule out polar effects of insertion. In order to better understand swimming in *Synechococcus*, each motility mutant was then assayed for the production and cellular localization of the two known structural components of the motility apparatus: SwmA and SwmB. Outer membrane fractions of each strain were also analyzed to determine other differences in protein content between these mutants and wild-type swimming cells.

Materials and Methods

Bacterial strains and growth conditions. The bacterial strains and plasmids used in this study are listed in Table 1**.** *E. coli* strains MC1061 (pRK24, pRL528) and DH5α were grown in Luria-Bertani (LB) medium (14). Ampicillin (100 μg/ml), kanamycin (50 μg/ml), and chloramphenicol (10 μg/ml) were used, when appropriate,

for the selection and maintenance of plasmids in *E.coli*. Cyanobacterial strains were grown in either SN medium (16) made with seawater obtained from the Scripps Pier (Scripps Institution of Oceanography, La Jolla, CA), or in SN medium prepared with synthetic ocean water (SOW) (13). Cyanobacterial cultures were incubated at 25°C with a constant illumination of 25 $\mu E \cdot m^{-2} \cdot sec^{-1}$ and were maintained as either 4-ml cultures in 17 mm × 100 mm polystyrene tubes (Becton Dickinson, Franklin Lakes, NJ) or as 50-ml cultures in 125-ml glass flasks without shaking. Kanamycin was added to a final concentration of 25 μg/ml for pour plates and 20 μg/ml for liquid cultures, where appropriate, to maintain selection for insertions.

Directed Mutagenesis. Directed inactivations were accomplished by cloning a completely internal fragment of a gene into the suicide vector pMUT100 as previously described (2). 20-mer oligonucleotide primers (Integrated DNA Technologies, Inc. Coralville, IA) were used to amplify DNA fragments (details given in Table 1) for cloning into pMUT100. These constructions were introduced into *Synechococcus sp.* WH8102 by conjugation with *E. coli* followed by subsequent selection of exconjugants on solidified medium containing kanamycin. Clonal isolates were grown in liquid medium to confirm the mutant phenotype of the original transposon mutant. Complete segregation of mutant chromosomes was confirmed by Southern blotting and by PCR. To confirm complete segregation of mutant chromosomes, DNA from each cyanobacterial mutant strain was isolated using the QIAGEN (Valencia, CA) DNeasy Tissue Kit with the following modification. Prior to proteinase K treatment, cells were incubated in 20 mM Tris-Cl (pH8.0), 2 mM Na_2EDTA, 1.2% Triton X-100, 50 mg/ml lysozyme for 30 minutes at 37°C. This

DNA was then used as a template for PCR analysis utilizing primers flanking the fragment used for inactivation. Primer pairs used in these tests were confirmed to amplify a fragment of the expected size from wild-type DNA. Failure to amplify a fragment of the wild-type size from a mutant strain's DNA confirms the absence of the intact gene among the clonal population of mutant cells. This same DNA sample was used as template in another PCR reaction utilizing primers directed to another ORF as a positive control to confirm that the DNA was of sufficient quality for PCR amplification.

Cell fractionation. Outer membrane (OM) proteins were isolated from all strains as described by Brahamsha (1). Briefly, exponentially growing cultures were centrifuged, washed once with 30 ml sterile SN medium, and resuspended at ~125× concentration in ice-cold stripping buffer (50 mM Tris HCl + 50 mM Na_2EDTA + 15% sucrose, pH 8.0) to strip off the outer membranes. After a 30 minute incubation on ice, cells were removed by centrifugation for 10 minutes at 6277 × *g*. The resulting outer membrane fraction was subjected to a high-speed spin of 100,466 × *g* for 90 minutes at 4°C to pellet the insoluble membrane fraction (high-speed pellet: HSP) and yield a supernatant containing the soluble OM proteins (high-speed supernatant: HSS). HSS proteins were concentrated using Amicon ultra 30,000 molecular weight cut-off (MWCO) centrifugal filters (Millipore, Bedford, MA) as directed by the manufacturer. Following removal of cells from spent media by centrifugation, proteins were recovered and concentrated using an ultra-filtration cell (Amicon) with 30,000 MWCO filters and further concentrated with 30,000 MWCO centrifugal filters. Cell density was determined prior to fractionating each sample using a Petroff-Hauser

counting chamber (Hauser Scientific Co., Horsham, PA). Gels were loaded normalizing cell number with a corresponding sample from wild-type strain WH8102 for comparison. Gel electrophoresis was conducted using Nu-PAGE Novex Tris-Acetate 3-8% and Novex Tricine 10-20% gradient gels as recommended by the manufacturer (Invitrogen, Carlsbad, CA). SYPRO Ruby staining (Sigma, St. Louis, MO) of gels was conducted as recommended by manufacturers.

For Western analysis, proteins were transferred to Invitrolon PVDF (Invitrogen) membranes in NuPAGE transfer buffer (Invitrogen) + 10% MeOH with 110V constant voltage for 2 hours at 4°C. Following overnight blocking at 4°C in BLOTTO (7), membranes were incubated for 1.5 hours at room temperature with primary antibodies diluted (production and purification described in Chapter V) in BLOTTO (1:500,000 and 1:50,000 for anti-SwmA and anti-SwmB respectively). Membranes were then washed 4 × 15 minutes in PBS + 0.05% Tween 20 (Fisher Scientific, Fair Lawn, New Jersey). Following washes, membranes were incubated 1.5 hours at room temperature with a peroxidase-conjugated anti-rabbit IgG (Sigma, St. Louis, MO) diluted 1:40000 in BLOTTO. Secondary antibody incubation was followed with another 4 × 15 minute washes in PBS + 0.05% Tween 20 and detected with Super Signal West Dura (Pierce, Rockford, IL) as recommended by the manufacturer. Densitometry analysis of these Westerns was performed using AlphaEase FC version 3.2.2 software (Alpha Innotech, San Leandro, CA). Bands from mutant strains were compared to the corresponding band in wild-type strain WH8102 to quantify protein abundance in relation to wild-type cells. Periodic acid-Schiff (PAS) staining was performed as described (15) to detect glycosylation.

Results

Mutagenesis. Directed mutagenesis of each motility gene identified by transposon mutagenesis confirmed the non-motile phenotype of the transposon mutant (8). Due to their close downstream proximity, three additional genes (SYNW0089, SYNW0194, and SYNW0195) were inactivated by insertional mutagenesis (Fig. 1). Inactivation of SYNW0089 did not affect motility. Directed inactivation of both SYNW0194 and SYNW0195 resulted in a loss of motility.

Cellular fractionation. Whole cells, OM preparations separated into insoluble and soluble fractions by high-speed centrifugation (HSP and HSS, respectively), and spent medium were analyzed by gel electrophoresis and SYPRO staining as well as by western analysis using antibodies to both SwmA and SwmB for each mutant strain. Protein profiles of each fraction were analyzed by comparison to the corresponding wild-type fraction to detect differences in the protein content of each mutant strain (Figs. 2-10). Aside from changes in SwmA and SwmB, two major differences were detected. The abundant outer membrane protein of 70 kDa (1) appears to be absent in two mutant strains (SYNW0087$^-$ and SYNW0195$^-$) (Figs. 4 and 9). Additionally, four mutant strains (S1A, SYNW0192$^-$, SYNW0193$^-$, and SYNW0194$^-$) lack an 80 kDa protein typically present in the spent medium of wild-type cells (Figs. 3, and 6-8).

SwmA Western analysis. Wild-type cellular localization of SwmA was disrupted for every motility mutant except for the SwmB mutant strain Swm-2 (Figs 2-10). Inactivation of *swmB* had no apparent effect on SwmA localization. Excluding

Swm-2 cells, SwmA was not detected in either whole cells or the HSP for all motility mutants. SwmA was detected in the HSS of SYNW0195$^-$ cells at approximately 22% (by densitometry) of the level of wild-type (Fig. 9). While SwmA was, for the most part, not detected on cells or in cellular fractions for all of these mutants, several strains still produced SwmA (SYNW0079$^-$, SYNW0087$^-$, SYNW0088$^-$, and SYNW0195$^-$), which accumulated in the media in concentrations similar to wild-type (Figs. 2, 4, 5 and 9). Western analysis of these media fractions show a band of wild-type size but two smaller bands (approximately 108 kDa and 94 kDa) are also detected with the polyclonal anti-SwmA antibody (Figs. 2, 4, 5 and 9). These smaller bands are never seen in western analysis of the wild-type strain.

SwmB Western analysis. In contrast to SwmA, SwmB was detected among total cellular proteins in all strains (Figs. 2-10). Three strains (SYNW0079$^-$, SYNW0088$^-$, and SYNW0193$^-$) lack SwmB in the HSP fraction and although it could be detected in the HSS fraction it was only at a fraction of the wild-type level (29%, 26%, and 23% respectively, by densitometry) (Figs. 2, 5 and 7). In all strains however SwmB is present in spent media in amounts similar to wild-type. Much like what was observed in anti-SwmA Westerns, the spent medium of one strain (SYNW0087$^-$) contains full-length SwmB as well as three smaller bands (approximately 98 kDa, 64 kDa, and 57 kDa) that are detected using the polyclonal antibody to SwmB (Fig. 4). Again, these smaller bands that react with the SwmB antibody are never seen in the wild-type strain. A summary of all cellular fractionation results is presented in Table 2.

PAS staining. For each strain that still produces SwmA, samples of spent medium concentrate were assayed for SwmA glycosylation by PAS. All strains revealed a band of 130 kDa, corresponding to SwmA, that tested positive for glycosylation by this test.

Discussion

SwmA and SwmB represent the only two characterized proteins required for non-flagellar swimming in marine *Synechococcus*. Presumably the production and proper localization of each of these components of the motility apparatus is crucial for locomotion. Inactivation of *swmB* has no apparent effect on SwmA production and localization on the cell surface. The reverse is also true. Mutation of *swmA* results in cells that still produce SwmB at wild-type levels and with wild-type localization. There does not appear to be any interdependence of one protein for the other in regards to the production and surface localization of these two proteins.

The same is not true for a number of the other mutants screened. Every mutant, other than *swmB* mutant strain Swm-2, had some defect in SwmA physiology. Three mutants (four including the *swmA* mutant strain S1A) produce no detectable SwmA at all. The corresponding genes inactivated in these mutants are all sequentially encoded in cluster two (Fig. 1) and are predicted to encode the pieces of a multi-component ATP-binding cassette (ABC) transporter. None of these mutations affect SwmB transport across the cell envelope however. While mutation to SYNW0193 results in SwmB being found primarily in spent media, mutations to SYNW0192 and SYNW0194 do not affect SwmB localization. Clearly SwmB can be

exported and attached on the cell surface in the absence of this transporter. Perhaps SYNW0192, SYNW0193, and SYNW0194 comprise a transporter dedicated to exporting SwmA. The observation that no SwmA is found in the medium supports this assertion. It appears that without this transporter, SwmA is not produced at all. How *swmA* transcription and/or translation is controlled remains to be determined. The four strains that lack SwmA all appear to lack an 80 kDa protein that is typically present in the spent media. Discussed in greater detail below, this polypeptide is unlikely to be merely a breakdown product of SwmA as Western analysis of media from wild-type cells does not detect any polypeptides other than intact SwmA. Perhaps this is a component of the motility apparatus that is dependent on the presence of SwmA for production.

Four mutations result in SwmA being found almost exclusively in the media. While SwmA transport out of the cytoplasm is not a problem for these motility mutants, correct localization is. Three of the four predicted gene products are glycosyltransferases, and the fourth is a conserved hypothetical. It is tempting to speculate that these proteins function in the modification of SwmA, which is known to be glycosylated (1). Although the predicted glycosyltransferases can be assigned to glycosyltransferase protein families based on sequence homology (3) (http://afmb.cnrs-mrs.fr/CAZY/), these families are general classifications including many enzymes involved in cell envelope biogenesis. Thus determining whether any would act directly on SwmA is not possible based solely on sequence information. Moreover, SwmA from these strains has the same electrophoretic mobility as wild-type and is positive for glycosylation by PAS staining. Thus it seems that the products

encoded by these motility genes do not act to directly modify SwmA, or do not act independently. They are clearly important for motility however, suggesting that certain components of the cell envelope must be modified for proper interaction with SwmA.

Intriguingly, Western analysis of spent medium proteins from these same mutants show wild-type size SwmA as well as two bands of lesser molecular weight. These smaller molecular weight polypeptides (approximately 108 kDa and 94 kDa) are unlikely to be merely non-specific breakdown products as these bands are never seen in the wild-type. Perhaps SwmA is specifically degraded when not properly attached and these bands represent breakdown products. Another possibility is that these two polypeptides are SwmA in partially modified form. The predicted molecular weight of SwmA is 83.6 kDa although the mature post-translationally modified protein has an apparent molecular weight of 130 kDa (1). Both of the additional bands detected by Western analysis are larger than 83.6 kDa leaving open the possibility that they represent the full intact peptide sequence of SwmA that has not been modified to the full extent that wild-type SwmA is.

While all of the motility mutants obtained produce SwmB (with the exception of *swmB* mutant strain Swm-2), three of the mutants have aberrant SwmB localization. In each of these three strains SwmB is detectable in whole cells as well as in the HSS but at dramatically reduced levels compared to wild-type cells. SwmB accumulates in the spent medium at concentrations similar to wild-type. The disrupted genes resulting in this phenotype are present in chromosomal regions 1 and 2 (Fig 1.) and encode an MFP component of an ABC transporter, a predicted glycosyltransferase and

a conserved hypothetical. How the products of these genes with disparate functions result in the same abnormal localization of SwmB is not clear. These mutations seem similar in some respects to those previously described in that SwmB is being produced and traverses the cell envelope but appears to have a defect in proper attachment at the cell surface.

Western analysis of spent medium from SYNW0087$^-$ cells reveals three polypeptides, in addition to full length SwmB, reacting with antibodies to SwmB. As these bands are never observed in the wild-type strain they are unlikely to be non-specific breakdown products of SwmB. SwmB appears to have normal localization in this strain however and an explanation for the additional anti-SwmB reactive bands is not obvious.

One other major component of the outer membrane fraction appears greatly reduced or absent in two strains. The glycosylated 70 kDa protein (1) found in abundance in the HSP and spent media fractions of wild-type cultures, appears absent in strains SYNW0087$^-$ and SYNW0195$^-$. Perhaps the 70 kDa protein is either not present at all in these cellular fractions or is not glycosylated as it is in wild-type cells, resulting in a shift of electrophoretic mobility. ORFs SYNW0087 and SYNW0195 are both predicted to be glycosyltransferases. Again, it is tempting to speculate that these proteins function in the modification of the 70 kDa glycoprotein. Alternatively, the 70 kDa protein may require other components of the cell to be properly modified before it can be properly localized itself.

Analysis of the present collection of motility mutants reveals a variety of differences in the protein content of outer membrane and spent media fractions. Two

general trends regarding the two known motility components have been observed. One class of mutations affects proper localization of a given motility protein while a second class of mutation affects production of the protein. Another trend observed is the presence of multiple forms of these motility proteins in some mutants as detected by western analysis. It is not clear whether this is due to the same process affecting both SwmA and SwmB (*e.g.* specific degradation of components of the motility apparatus when it is improperly constructed), or if it is simply a similar result caused by disparate processes. Three basic SwmA phenotypes are observed: 1) wild-type abundance and distribution of SwmA, 2) SwmA present but only in the media, and 3) total lack of SwmA. Likewise the same three classes can be applied to mutations affecting SwmB. Additionally mutations affecting the 70 kDa OM protein are observed as well. Unfortunately no trends are observed in the combinations of these phenotypes. Mutations affecting one of these three proteins does not necessarily have an effect on another. The presence or location of one of the proteins cannot predict that of another. Perhaps with the isolation of additional motility mutants, commonalities in protein complements of mutant strains will become apparent. For the time being, several proteins of interest, specifically the 70 and 80 kDa proteins, appear to have a role in swimming motility and warrant further investigation.

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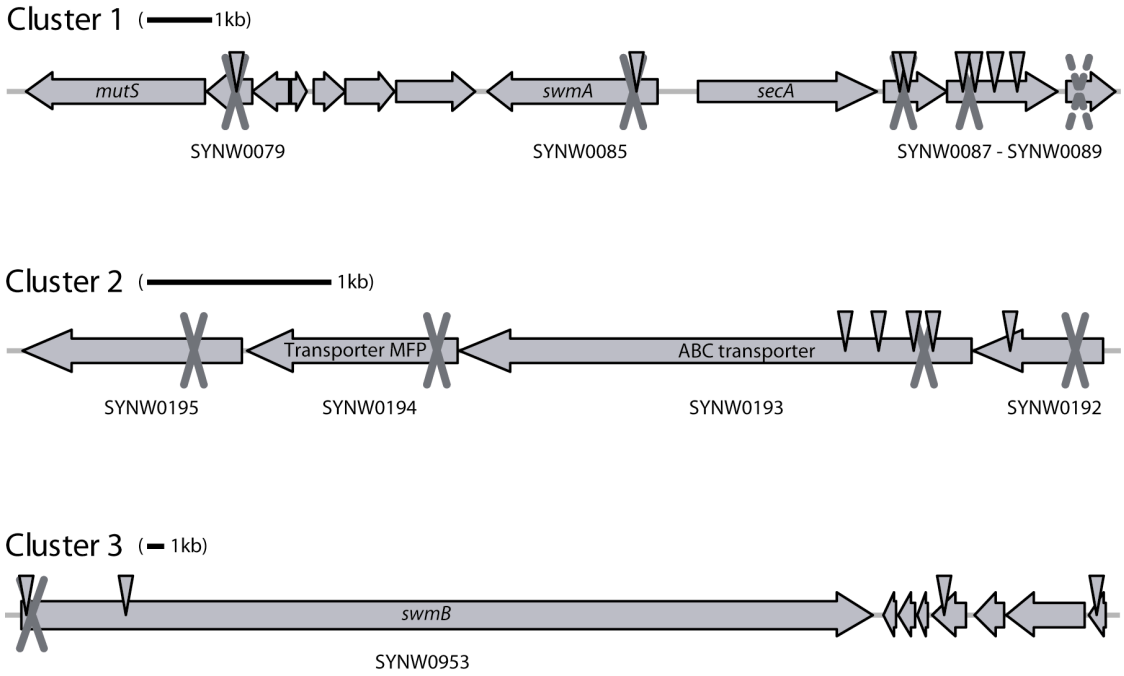

FIG. 1. Chromosomal regions containing clusters of motility genes (8). Arrowheads represent transposon insertions disrupting motility; X, directed inactivation disrupting motility; dashed X, directed inactivation with no effect on motility.

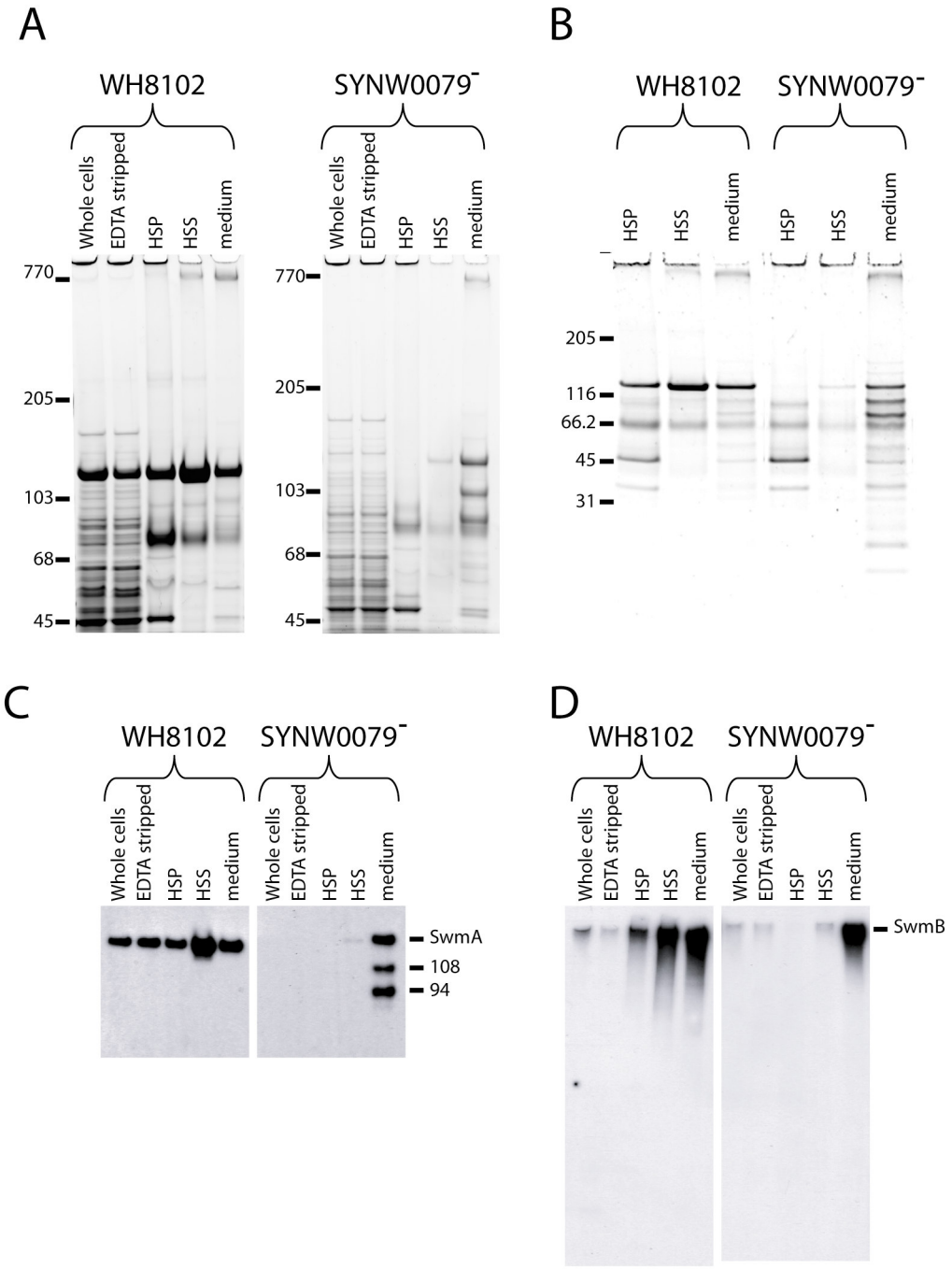

FIG. 2. SYPRO stained 3-8% tris-acetate gels (A), SYPRO stained 10-20% tricine gels (B), anti-SwmA Westerns (C), and anti-SwmB Westerns (D) comparing cellular fractions from wild-type strain WH8102 and SYNW0079- mutant strains. Cells were treated with EDTA to strip outer membranes (EDTA stripped). This material is then subjected to high-speed centrifugation to yield a pellet (HSP) containing the outer membrane and a supernatant (HSS) containing soluble proteins. Spent medium included for comparison as well.

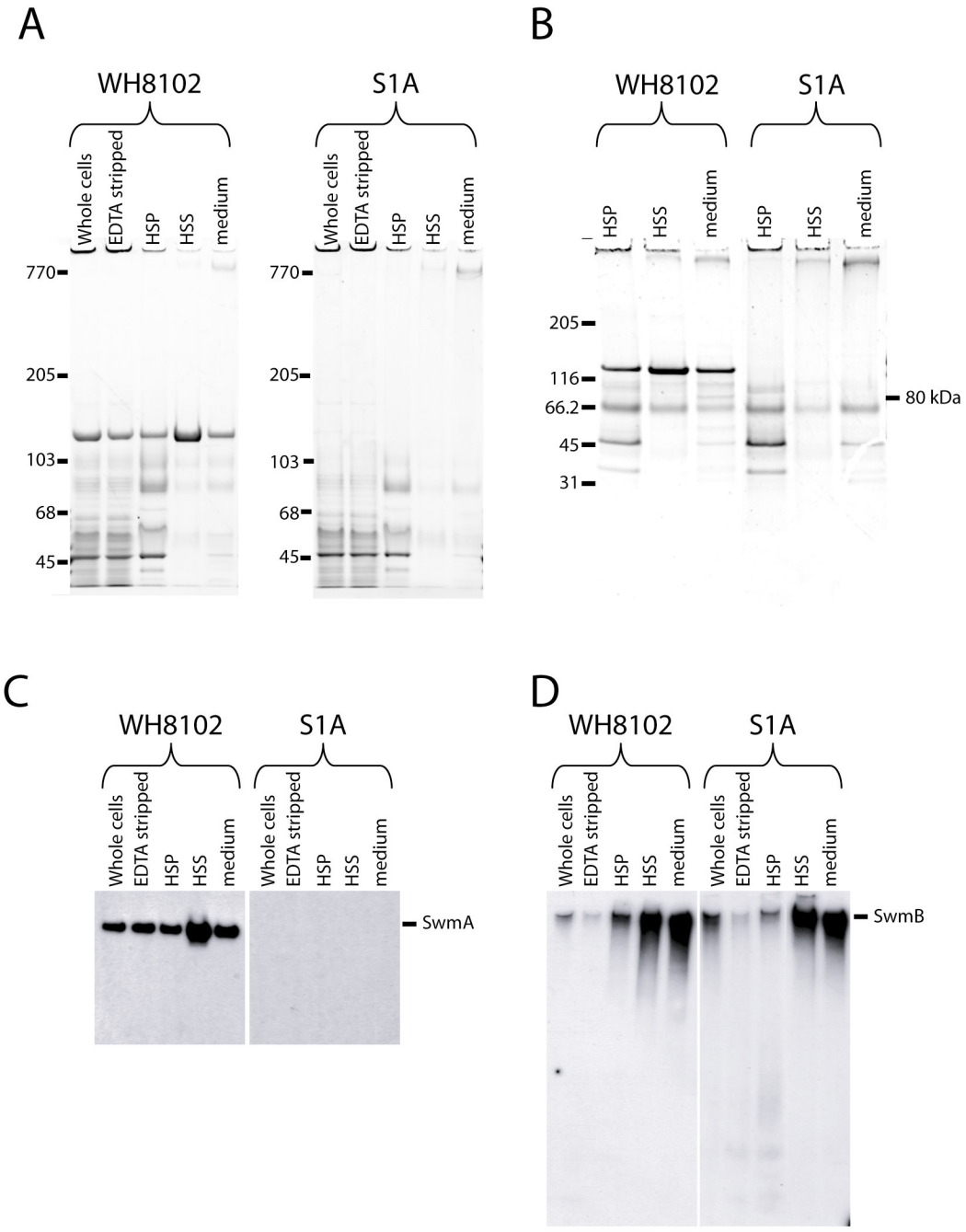

FIG. 3. SYPRO stained 3-8% tris-acetate gels (A), SYPRO stained 10-20% tricine gels (B), anti-SwmA Westerns (C), and anti-SwmB Westerns (D) comparing cellular fractions from wild-type strain WH8102 and S1A mutant strains.

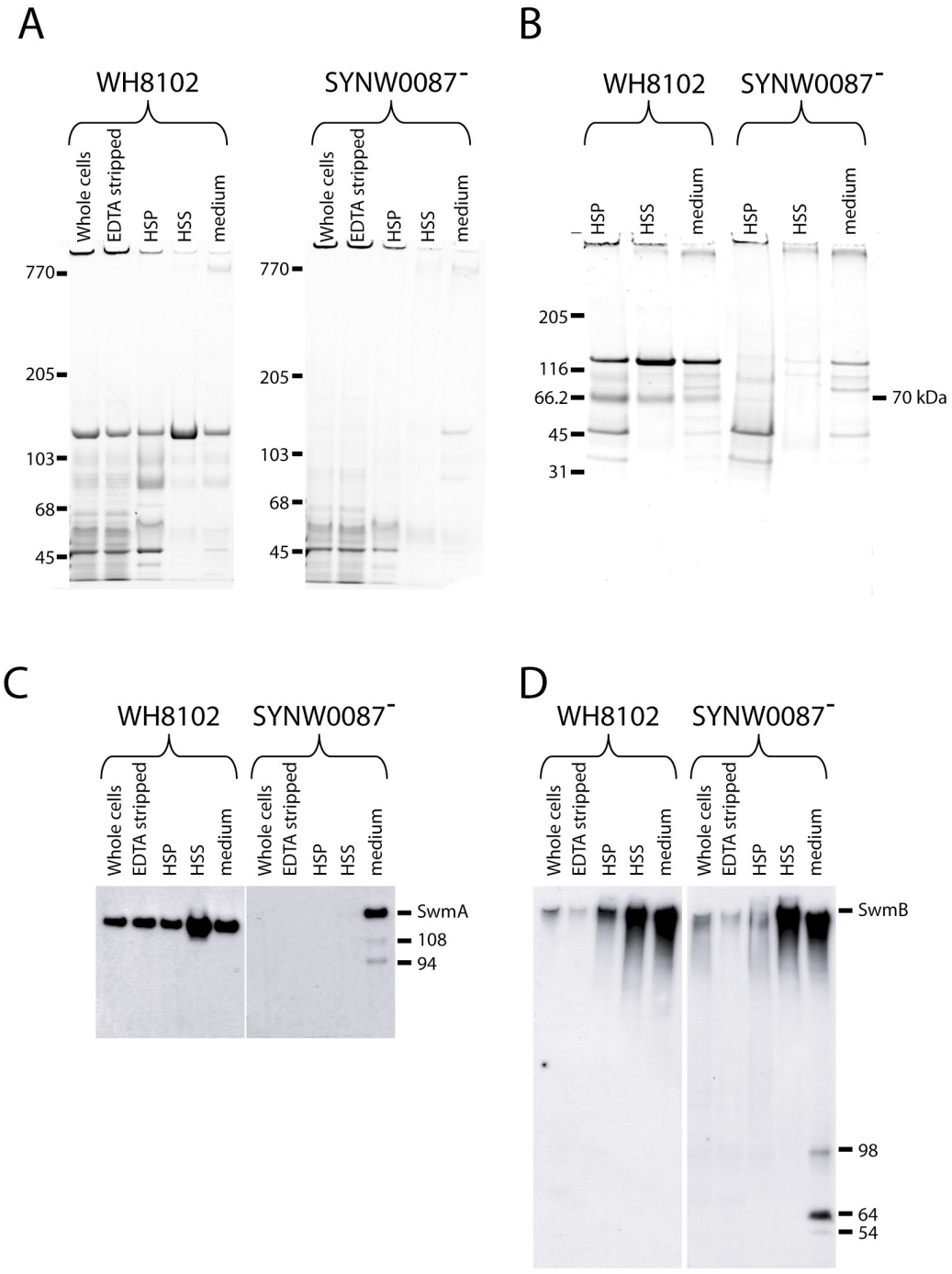

FIG. 4. SYPRO stained 3-8% tris-acetate gels (A), SYPRO stained 10-20% tricine gels (B), anti-SwmA Westerns (C), and anti-SwmB Westerns (D) comparing cellular fractions from wild-type strain WH8102 and SYNW0087⁻ mutant strains.

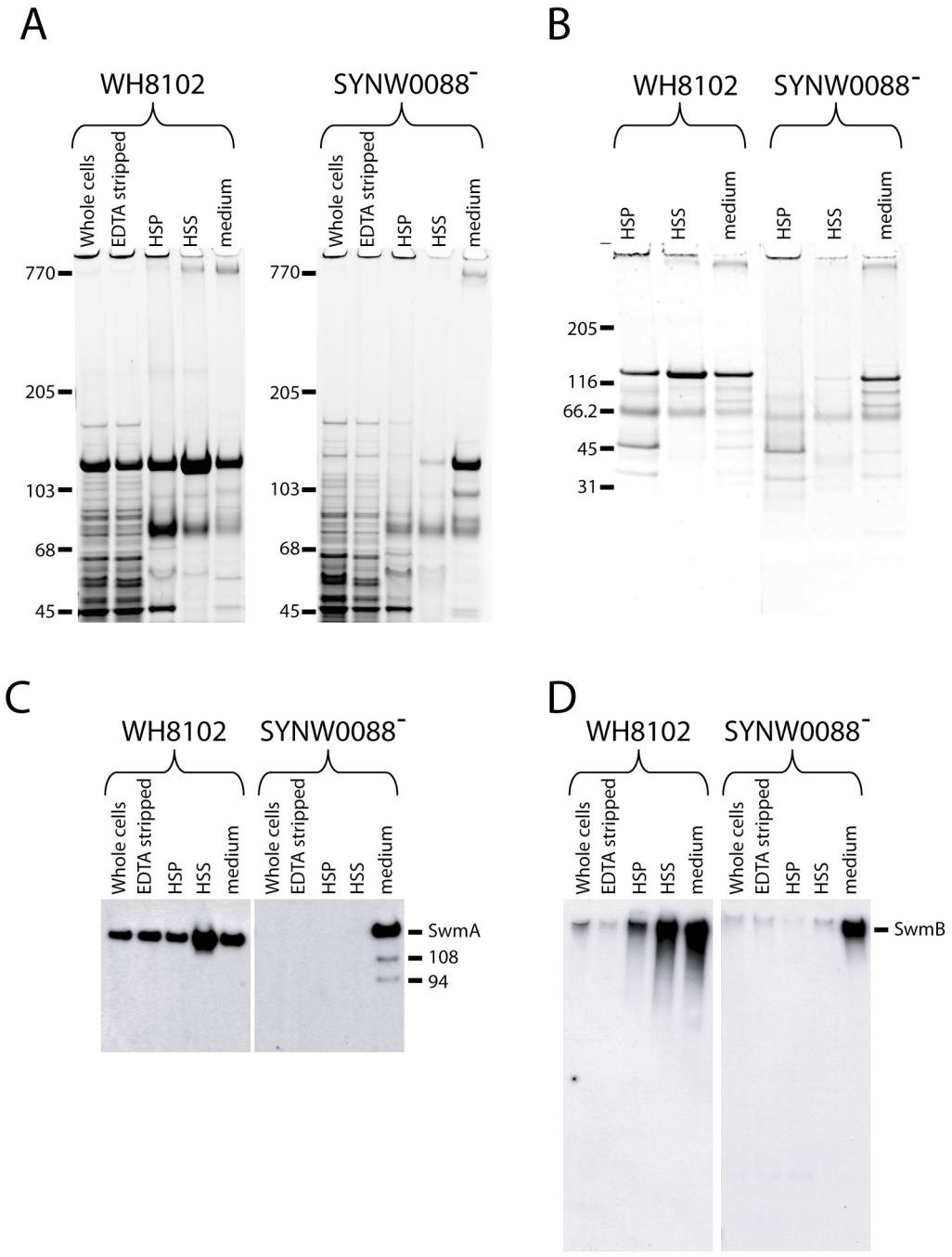

FIG. 5. SYPRO stained 3-8% tris-acetate gels (A), SYPRO stained 10-20% tricine gels (B), anti-SwmA Westerns (C), and anti-SwmB Westerns (D) comparing cellular fractions from wild-type strain WH8102 and SYNW0088^{-} mutant strains.

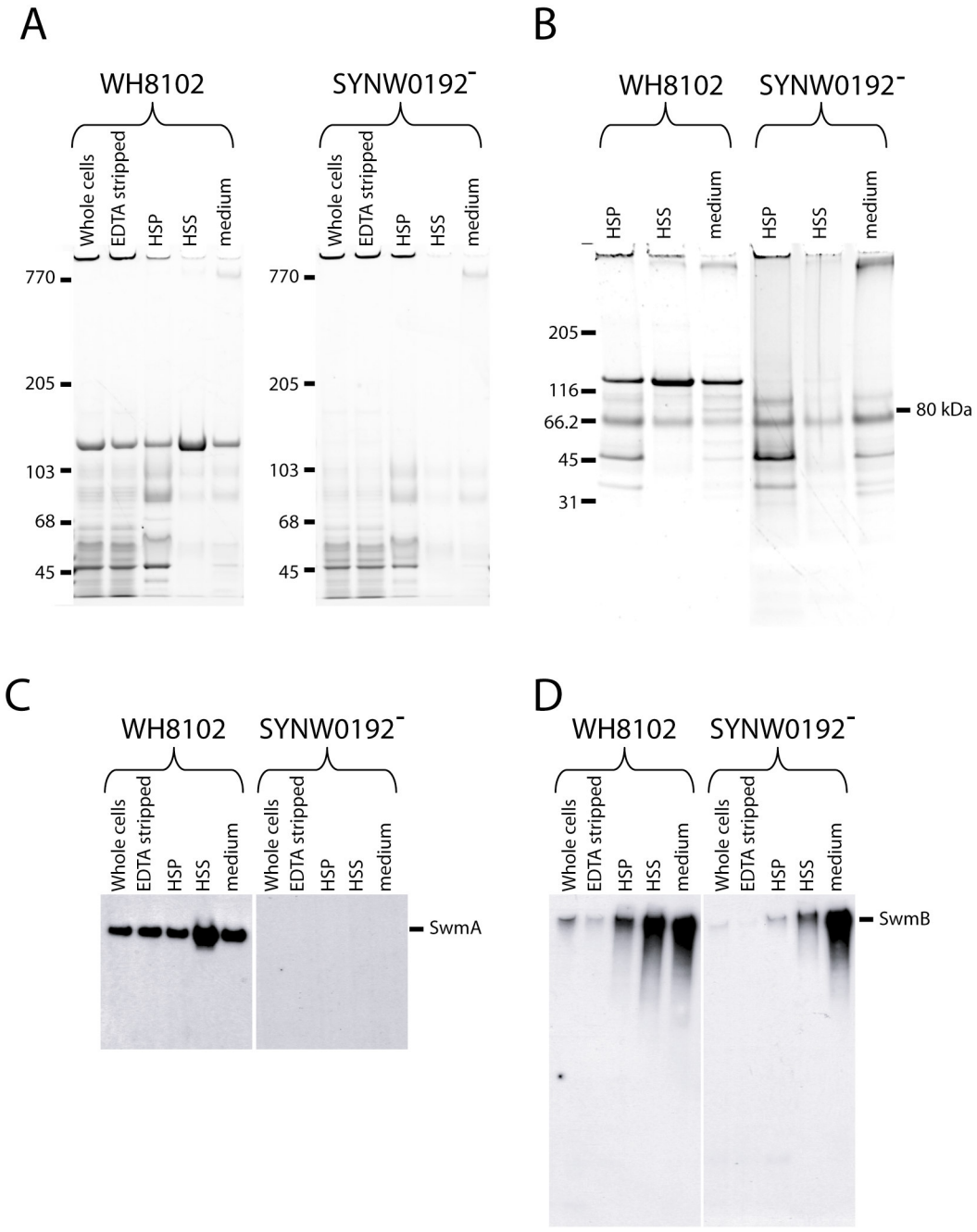

FIG. 6. SYPRO stained 3-8% tris-acetate gels (A), SYPRO stained 10-20% tricine gels (B), anti-SwmA Westerns (C), and anti-SwmB Westerns (D) comparing cellular fractions from wild-type strain WH8102 and SYNW0192^{-} mutant strains.

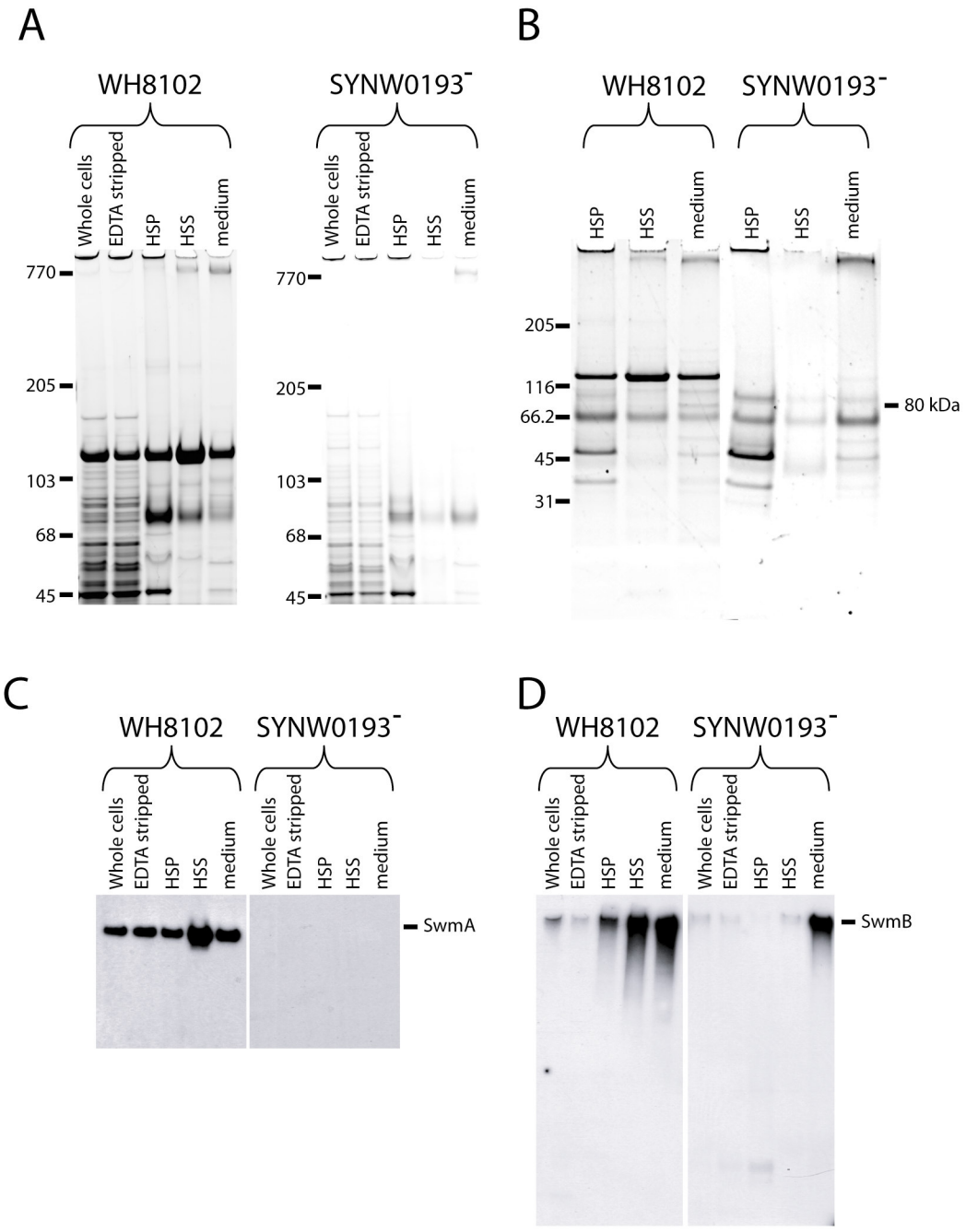

FIG. 7. SYPRO stained 3-8% tris-acetate gels (A), SYPRO stained 10-20% tricine gels (B), anti-SwmA Westerns (C), and anti-SwmB Westerns (D) comparing cellular fractions from wild-type strain WH8102 and SYNW0193^{-} mutant strains.

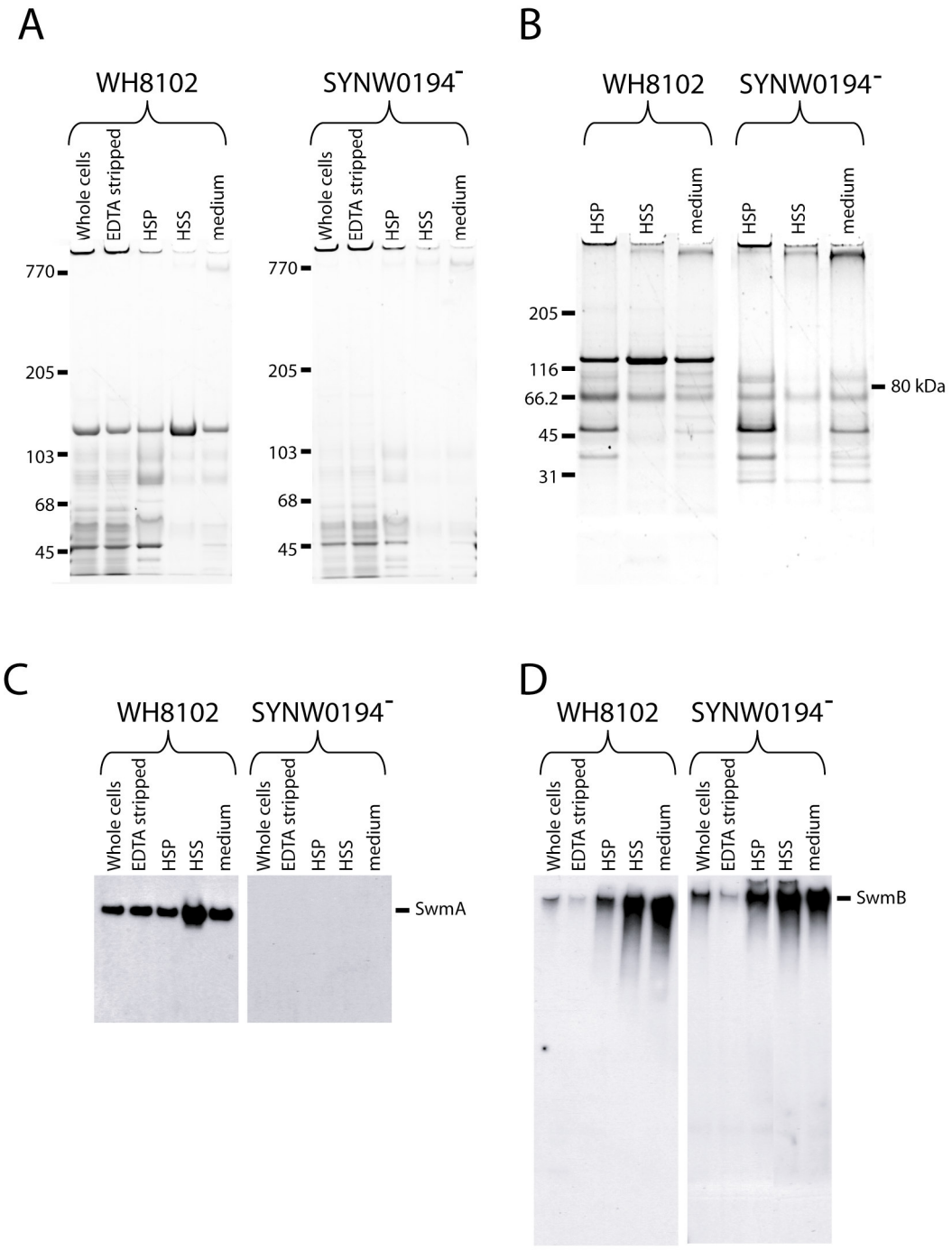

FIG. 8. SYPRO stained 3-8% tris-acetate gels (A), SYPRO stained 10-20% tricine gels (B), anti-SwmA Westerns (C), and anti-SwmB Westerns (D) comparing cellular fractions from wild-type strain WH8102 and SYNW0194^{-} mutant strains.

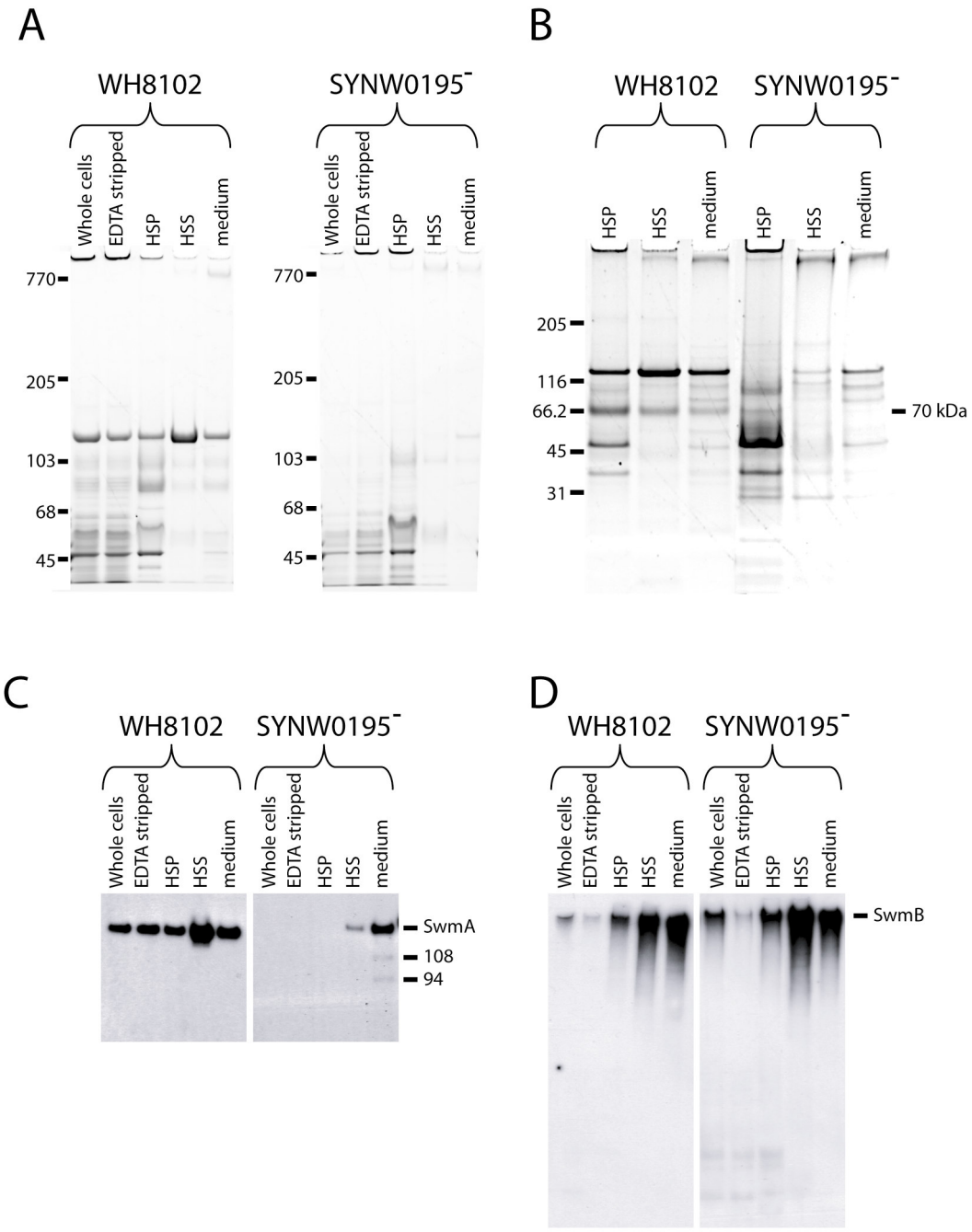

FIG. 9. SYPRO stained 3-8% tris-acetate gels (A), SYPRO stained 10-20% tricine gels (B), anti-SwmA Westerns (C), and anti-SwmB Westerns (D) comparing cellular fractions from wild-type strain WH8102 and SYNW0195^{-} mutant strains.

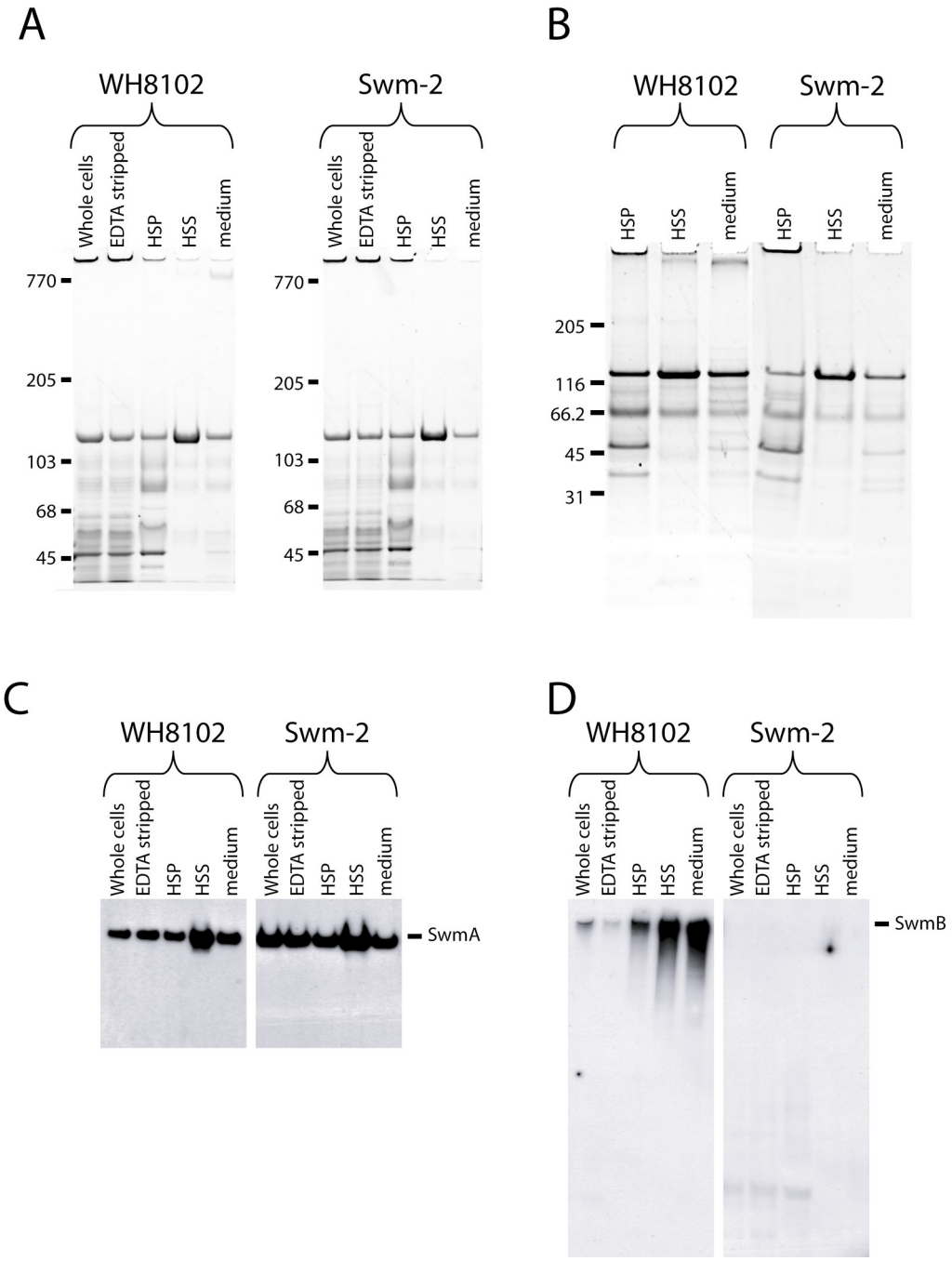

FIG. 10. SYPRO stained 3-8% tris-acetate gels (A), SYPRO stained 10-20% tricine gels (B), anti-SwmA Westerns (C), and anti-SwmB Westerns (D) comparing cellular fractions from wild-type strain WH8102 and Swm-2 mutant strains.

TABLE 1. Bacterial strains and plasmids used in this study

Strain or Plasmid	Relevant Characteristics[a]	Source or reference[b]
Strains		
Synechococcus sp.		
WH8102	Motile strain, recipient in conjugations with pMUT100 constructions	J. Waterbury
S1A	*swmA* mutant, inactivated with pBB1000	(8)
Swm-2	*swmB* mutant, inactivated with pJM20	(8)
SYNW0079$^-$	SYNW0079 (conserved hypotherical) inactivated with pJM37	(8)
SYNW0087$^-$	SYNW0087 (putative glycosyltransferase) inactivated with pJM62	(8)
SYNW0088$^-$	SYNW0088 (putative glycosyltransferase) inactivated with pJM18	(8)
SYNW0192$^-$	SYNW0192 (putative PPIase) inactivated with pJM59	(8)
SYNW0193$^-$	SYNW0193 (ABC transporter) inactivated with pJM40	(8)
SYNW0194$^-$	SYNW0194 (MFP transport accessory) inactivated with pJM60	(8)
SYNW0195$^-$	SYNW0195 (putative glycosyltransferase) inactivated with pJM61	(8)
E. coli		
MC1061	Host for pRK24, pRL528; used as a donor in pJM construction conjugations	(4)
DH5α	Recipient in transformations	BRL
Plasmids		
pRK24	Tc^r, Amp^r; conjugal plasmid, RK2 derivative	(2, 11)
pRL528	Cm^r; helper plasmid, encodes *mob*	(2, 6)
pCR2.1-TOPO	Kan^r, Amp^r; PCR product cloning vector	Invitrogen
pJM37	pMUT100 containing SYNW0079 fragment 78600..78939	(8)
pBB1000	pMUT100 containing *swmA* fragment 84507..84926	(8)

TABLE 1 (continued). Bacterial strains and plasmids used in this study

pJM62	pMUT100 containing SYNW0087 fragment 88793..89004	(8)
pJM18	pMUT100 containing SYNW0088 fragment 89414..89635	(8)
pJM19	pMUT100 containing SYNW0089 fragment 91118..91367	This work
pJM59	pMUT100 containing SYNW0192 fragment 193443..193661	(8)
pJM40	pMUT100 containing SYNW0193 fragment 194020..194287	(8)
pJM60	pMUT100 containing SYNW0194 fragment 197115..197342	This work
pJM61	pMUT100 containing SYNW0195 fragment 198491..198714	This work
pJM20	pMUT100 containing *swmB* fragment 913153..913396	(8)

[a] nt, nucleotide. Numbering according to (12).
[b] BRL, Bethesda Research Laboratories, Gaithersburg, MD; Invitrogen, Carlsbad, CA

Table 2. Protein content of cellular fractions for all motility mutants.

Strain	Putative function of inactivated gene	SwmA				SwmB				70 kDa	80 kDa
		WC	HSP	HSS	Med	WC	HSP	HSS	Med	All	Med
WH8102		X	X	X	X	X	X	X	X	X	X
SYNW0079$^-$	conserved hypothetical				X^+	X		29%	X	X	X
S1A	cell surface motility component					X	X	X	X	X	
SYNW0087$^-$	glycosyltransferase				X^+	X	X	X	X^+		X
SYNW0088$^-$	glycosyltransferase				X^+	X		26%	X	X	X
SYNW0192$^-$	chaperonin					X	X	X	X	X	
SYNW0193$^-$	ABC transporter					X		23%	X	X	
SYNW0194$^-$	MFP					X	X	X	X	X	
SYNW0195$^-$	glycosyltransferase			22%	X^+	X	X	X	X		X
Swm-2	cell surface motility component	X	X	X	X					X	X

SwmA and SwmB specifically assayed for by Western analysis. 70 kDa and 80kDa proteins visualized by SYPRO staining. WC, whole cells; HSP, EDTA stripped high-speed pellet; HSS, EDTA stripped high-speed supernatant; Med, spent media concentrate; X, present at wild-type levels; %, present but at a fraction of the level present in wild-type cells (by densitometry); X^+, present at wild-type size along with other smaller bands detected by western analysis.

The text of Chapter VI, in full, is being prepared for publication. The dissertation author was the primary author, and co-author B. Brahamsha directed and supervised the research, which forms the basis for this chapter.