

# Isolation and Molecular Characterization of *Bacillus thuringiensis* Harboring Putative *ps* Genes from Bangladesh

Nasima Aktar<sup>a</sup>, Md. Asaduzzaman Shishir<sup>a,b</sup>, Shakila Nargis Khan<sup>a</sup>, and Md. Mozammel Hoq<sup>a\*</sup>

**Supplementary Table 1:** Isolates with desired protein band

Protein (PS)	Molecular weight (KDa)	Isolates
PS 1	81	3 (DSf2, DSf3, DSg1)
PS 2	37	11 (32L, DSA1, DSc5, DSd2, DSg1, DSi2, DSi4, RHSc3, RHSd3, RHSd4, FHSe4)
PS 3	88	2 (DSf2, DSf3)
PS 4	31-34	12 (23S, 31S, 59S, 32L, DSA1, DSd2, DSf2, DSg1, DSh1, SSe1, SSf3, JSa2)

**Supplementary Table 2:** Sequence infor of different parasporin protein

Name	Cry no.	Accession Number	Authors	Source Strain
PS1Aa1	Cry31Aa1	AB031065	Mizuki et al. 2000	A1190
PS1Aa2	Cry31Aa2	AY081052	Jung&Côté ; 2002	M15
PS1Aa3	Cry31Aa3	AB250922	Uemori et al. 2006	B195
PS1Aa4	Cry31Aa4	AB274826	Yasutake et al. 2006	Bt 79-25
PS1Aa5	Cry31Aa5	AB274827	Yasutake et al. 2006	Bt 92-10
PS1Aa6	submitting	AB375062	Nagamatsu et al. 2010	CP78A
PS1Ab1	Cry31Ab1	AB250923	Uemori et al. 2006	B195
PS1Ab2	Cry31Ab2	AB274825	Yasutake et al. 2006	Bt 31-5
PS1Ac1	Cry31Ac1	AB276125	Yasutake et al. 2006	Bt 87-29
		AB731600		
PS2Aa1	Cry46Aa1	AB099515	Ito & Kitada et al. 2004	A1547
PS2Aa2	Cry46Aa2	AB454419	Ishikawa et al. 2008	A1470
PS2Ab1	Cry46Ab1	AB186914	Yamagiwa et al. 2004	TK-E6
PS3Aa1	Cry41Aa1	AB116649	Yamashita et al. 2005	A1462
PS3Ab1	Cry41Ab1	AB116651	Yamashita et al. 2005	A1462
PS4Aa1	Cry45Aa1	AB180980	Okumura & Saitoh. 2004	A1470

**Supplementary table 3:** Properties of parasporin gene specific primers.

Parasporin	Primer	Primer Sequence	Length (bp)	GC Content
PS 1	Forward	5'-CAGAACTCGCAGCATTA-3'	20	45.00%
	Reverse	5'-TAATTTGGTCCGAAAGTTGG-3'	20	40.00%
PS 2	Forward	5'-CAGCATTAGGTCAAGTCCA-3'	20	45.00%
	Reverse	5'-GACGAGGAAAATTAGCACCA-3'	20	45.00%
PS 3	Forward	5'-CCAAGATGCAGCTGATGATT-3'	20	45.00%
	Reverse	5'-GGTTCTCTATGGCATCTATCGTA-3'	23	43.50%
PS 4	Forward	5'-GCTGCAAGGGGATACACATATT-3'	22	50.00%
	Reverse	5'-ATGCCCTGCTATGTCAGCTT-3'	20	50.00%

**Supplementary Table 4:** Biochemical Test results of the non-hemolytic *Bacillus thuringiensis* isolates

Isolates	Gram Staining	Oxidase production	Catalase production	Urea hydrolysis	Starch hydrolysis	Casein hydrolysis	Citrate utilization	Indole Production	VP Test	Motility
Reference HD-73	+	+	+	+	+	+	+	+	+	-
23S	+	+	+	+	-	+	+	+	+	-
59S	+	+	+	+	+	+	+	+	+	-
32L	+	+	+	+	-	+	+	+	+	+
DSa1	+	+	+	+	+	+	+	+	+	-
DSb2	+	+	-	+	+	+	+	+	+	-
DSb3	+	-	+	+	+	-	+	+	+	+
DSb7	+	+	-	+	+	+	+	+	-	-
DSc5	+	+	+	+	+	+	+	+	-	-
DSd2	+	+	+	+	+	+	+	+	+	-
DSd3	+	+	+	+	+	+	+	+	+	+
DSf2	+	+	+	+	-	-	+	+	-	-
DSf3	+	+	+	+	-	+	+	+	+	-
DSg1	+	+	+	-	+	+	+	+	+	-
DSg3	+	+	+	+	+	+	+	+	+	+
DSh1	+	+	+	+	+	+	+	+	+	-
DSh3	+	+	-	+	+	+	+	+	+	-
DSi2	+	+	+	+	+	+	+	+	-	+
DSi4	+	+	+	+	-	-	-	+	-	-
DDa1	+	+	+	+	+	-	-	+	-	+
DDa3	+	+	+	+	+	+	+	+	-	-
SSe1	+	+	+	+	+	-	+	+	+	-
SSF3	+	+	+	+	+	+	+	+	-	-
JSa2	+	+	+	+	+	+	+	+	+	-
RHSc3	+	+	-	+	+	+	+	+	+	-
RHSd1	+	+	+	+	+	+	+	+	+	-
RHSd3	+	+	+	+	+	+	+	+	+	-
RHSd4	+	+	+	+	+	+	+	+	+	-
FHSe4	+	+	+	+	+	+	+	+	+	-

**Supplementary Table 5: Fermentation of Monosaccharides and Disaccharides**

<b>Isolates</b>	<b>Glucose fermentation</b>	<b>Sucrose Fermentation</b>	<b>Lactose Fermentation</b>
HD-73	+	-	-
23S	+	+	-
59S	+	+	-
32L	+	-	-
DSa1	+	-	-
DSb2	+	-	-
DSb3	+	-	-
DSb7	+	-	-
DSc5	+	-	-
DSd2	+	-	-
DSd3	+	-	-
DSf2	+	-	-
DSf3	+	-	-
DSg1	+	-	-
DSg3	+	-	-
DSh1	+	-	-
DSh3	+	-	-
DSi2	+	-	-
DSi4	+	-	-
DDa1	+	-	-
DDa3	+	-	-
SSe1	+	-	-
SSf3	+	-	-
JSa2	+	-	-
RHSc3	+	-	-
RHSd1	+	-	-
RHSd3	+	-	-
RHSd4	+	-	-
FHSe4	+	-	-

**Supplementary Table 6:** Summary of SDS-PAGE analysis of the isolates

Isolates	Concentration of protein (mg/ml)	Molecular weight of crude protein (KDa)	MW of protein after proteinase digestion (KDa)
23S	0.568	44.6, 30.8, 29, 25.2, 22.7	27
31S	0.522	44.3, 30.3, 27.3	27
59S	0.5	29.5, 26.7	26
32L	0.632	43.7, 37.9, 29.8, 26.7	26
DSa1	0.506	65.1, 26.5	27
DSb3	0.516	No band	No band
DSb7	0.565	66.9, 26.7	26
DSc5	0.579	66.3, 26.3	
DSd2	0.1184	64.8, 43.9, 31.4	27
DSd3	0.3837	46.6	26
DSf2	0.5041	92.7, 44.8	30, 64
DSf3	0.2245	90.9, 45.1	27, 30, 64
DSg1	0.1776	31.3, 75	30, 70
DSg3	0.1653	65.3, 46.9	27
DSh1	0.0979	68.6, 29.7	27
DSh3	-	-	-
DSi2	0.0449	66.5, 46.6, 37	27
DSi4	0.0857	39.7	27
DDa1	0.3693	66, 61, 57	27
DDa3	0.0755	66, 47	27
SSe1	0.1347	45, 29, 23	27
SSf3	0.0898	45, 29, 23	27
JSa2	0.1408	44, 29	27
RHSc3	1.33	38	26
RHSd1	0.59	32	27
RHSd3	0.8	44, 37	27
RHSd4	0.91	57, 44, 40, 35	27
FHSe4	0.54	44, 38, 36	26