

**S5 Table.** LC-MS analysis of soy medium culture supernatants from wt *S. clavuligerus* and  $\Delta cpe-1NF$  mutant strains for detecting 5S clavam production.

Strain (plasmid) <sup>a</sup>	Metabolite <sup>b,c</sup>		
	2-Hydroxymethyl clavam (m/z=144/212)	Clavam-2-carboxylate (m/z=138/226)	Clavulanic acid (m/z=156/224)
wt	+	+	+
$\Delta cpe-1NF$	+	+	-
$\Delta cpe-1NF$ (pSET: <i>cpe</i> <sup>Sc</sup> )	+	+	+
$\Delta cpe-1NF$ (pHM: <i>cpe</i> <sup>Ct</sup> )	+	+	-

<sup>a</sup> Select strains were fermented for 96 hours and culture supernatants were subjected to LC-MS analysis after imidazole derivatization.

<sup>b</sup> Metabolites and respective *m/z* values of corresponding imidazole derivatives/fragmentation products/ions are indicated, where (+ and -) indicate that ions corresponding to the metabolite were either present or was not detected in culture supernatants, respectively.

<sup>c</sup> The presence of 2-hydroxymethyl clavam and clavam-2-carboxylate was used to monitor 5S clavam production since the metabolites produced as part of a common biosynthetic pathway in *S. clavuligerus*.