

**S4 Table.** LC-MS analysis of SA culture supernatants from wt *S. clavuligerus* and  $\Delta$ *cpe-INTF* mutant strains for detecting clavulanic acid and pathway intermediates.

Strain (plasmid) <sup>a</sup>	Metabolite <sup>b,c</sup>						
	Clavaminc acid ( <i>m/z</i> =223/155)	<i>N</i> -acetyl clavaminic acid ( <i>m/z</i> =265/197)	<i>N</i> -glycyl clavaminic acid ( <i>m/z</i> =280/212)	<i>N</i> -acetyl glycyl clavaminic acid ( <i>m/z</i> =322/254)	<i>C</i> -glycyl clavaminic acid ( <i>m/z</i> =324/256)	Clavaldelyde ( <i>m/z</i> =222/154)	Clavulanic acid ( <i>m/z</i> =224/156)
wt	-	-	-	-	-	-	+
$\Delta$ <i>cpe-INTF</i>	-	-	-	-	-	-	-
$\Delta$ <i>cpe-INTF</i> (pSET: <i>cpe</i> <sup>Sc</sup> )	-	-	-	-	-	-	+
$\Delta$ <i>cpe-INTF</i> (pSET: <i>cpe</i> <sup>Sc-Trp91Ala</sup> )	-	-	-	-	-	-	+
$\Delta$ <i>cpe-INTF</i> (pSET: <i>cpe</i> <sup>Sc-Ser173Ala</sup> )	-	-	-	-	-	-	-
$\Delta$ <i>cpe-INTF</i> (pSET: <i>cpe</i> <sup>Sc-Lys176Ala</sup> )	-	-	-	-	-	-	-
$\Delta$ <i>cpe-INTF</i> (pSET: <i>cpe</i> <sup>Sc-Ser234Ala</sup> )	-	-	-	-	-	-	-
$\Delta$ <i>cpe-INTF</i> (pSET: <i>cpe</i> <sup>Sc-Gln321Ala</sup> )	-	-	-	-	-	-	+
$\Delta$ <i>cpe-INTF</i> (pSET: <i>cpe</i> <sup>Sc-Tyr359Ala</sup> )	-	-	-	-	-	-	-
$\Delta$ <i>cpe-INTF</i> (pSET: <i>cpe</i> <sup>Sc-Lys375Ala</sup> )	-	-	-	-	-	-	-
$\Delta$ <i>cpe-INTF</i> (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. Some clavaminic acid derived 5S metabolites reported to accumulate in certain *S. clavuligerus* mutant strains were also included in the analysis.

<sup>c</sup> (+) and (-) indicates that ions corresponding the metabolite were either detected or not detected in culture supernatants during analysis, respectively.