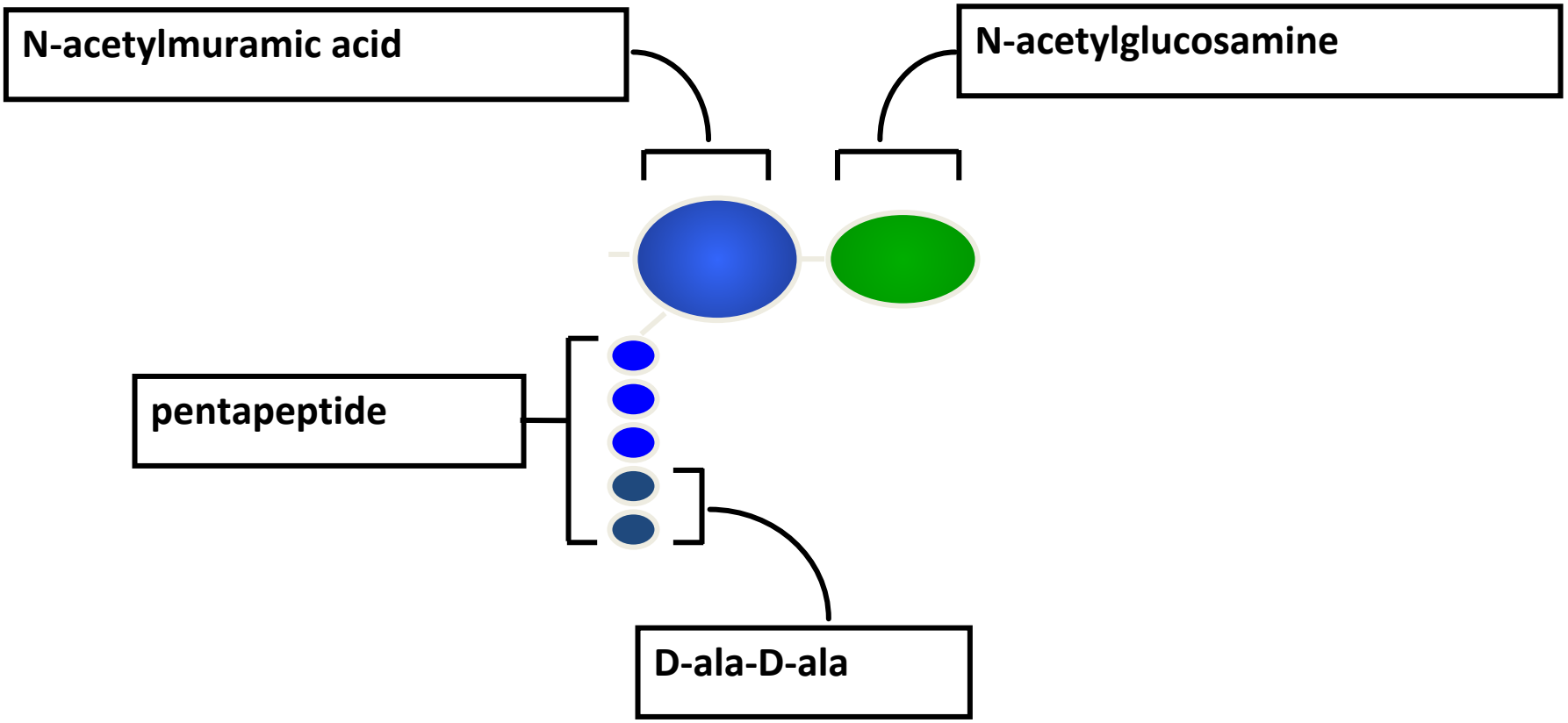
β-Lactum resistance

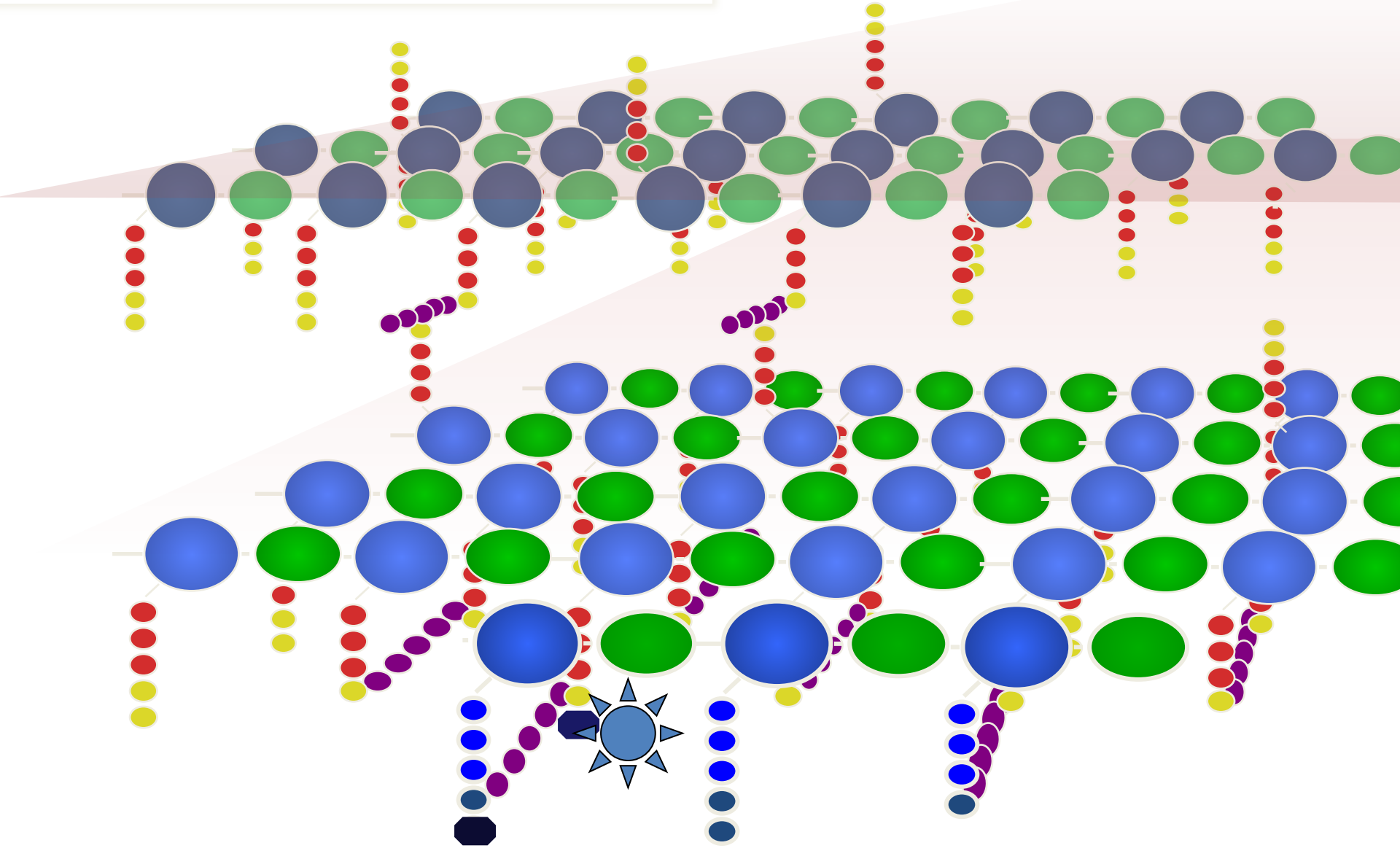
Agneyo Ganguly

Mechanism of Action of β-lactum antibiotics



Cell Wall Assembly

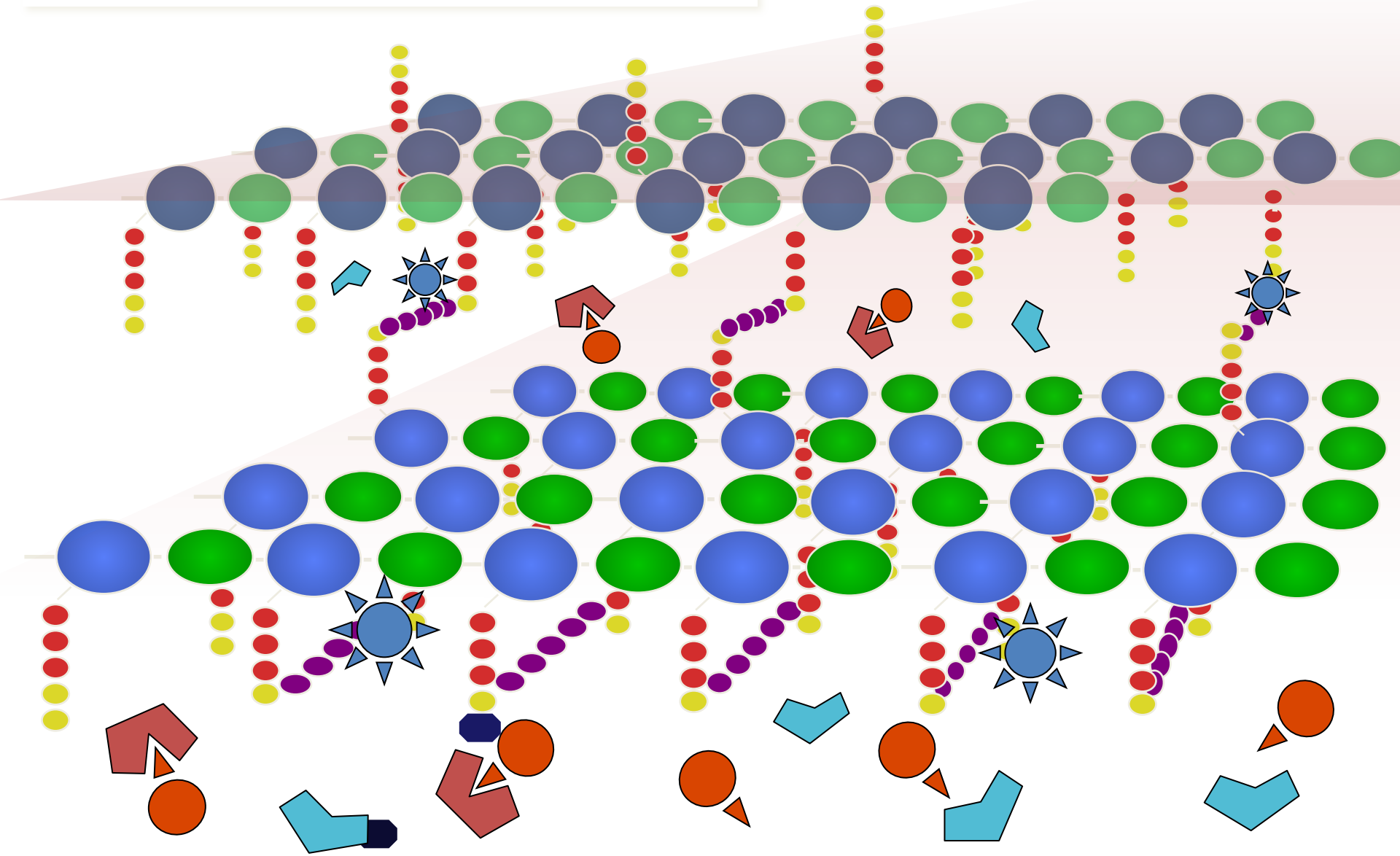
Second layer of cell wall cross-linked to the lower layer

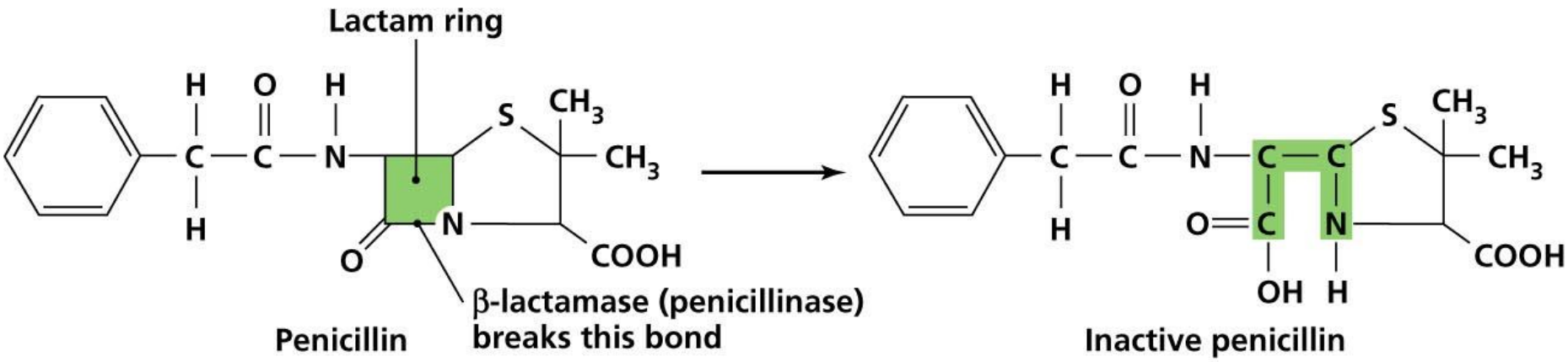


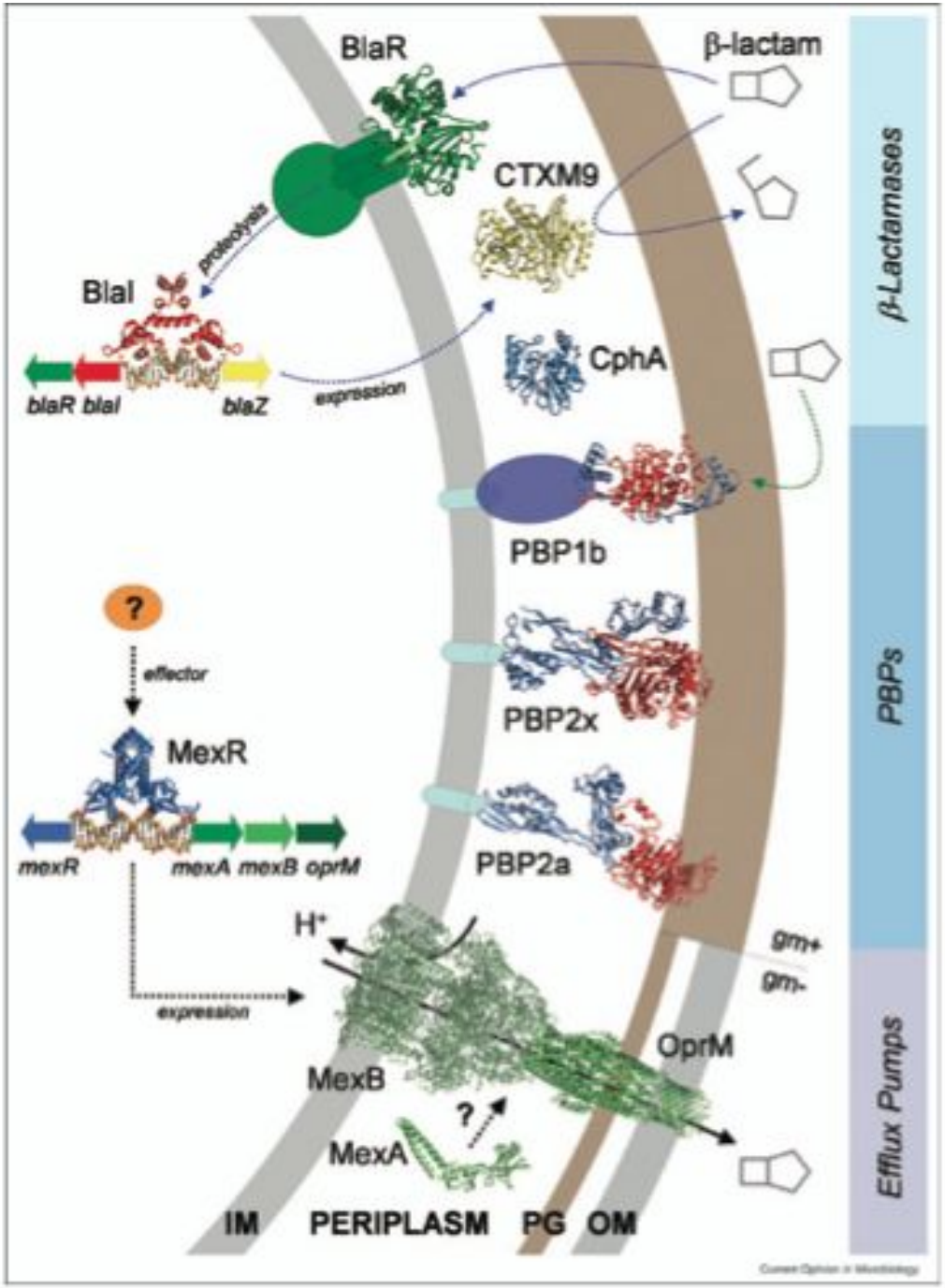
Mechanisms of beta-lactam resistance

- Drug-modifying enzymes (beta-lactamases)
  - Gram-positives(e.g., *S. aureus*) excrete the enzyme
  - Gram-negative (e.g., *E. coli*) retain the enzyme in the periplasm
- Alteration of the PBPs so antibiotic cannot bind
  - e.g., *S. pneumoniae*, gonococcus
- Exclusion from the site of cell wall synthesis
  - Porin mutations in the outer membrane of Gram-negative bacteria only (e.g., *Ps. aeruginosa*)

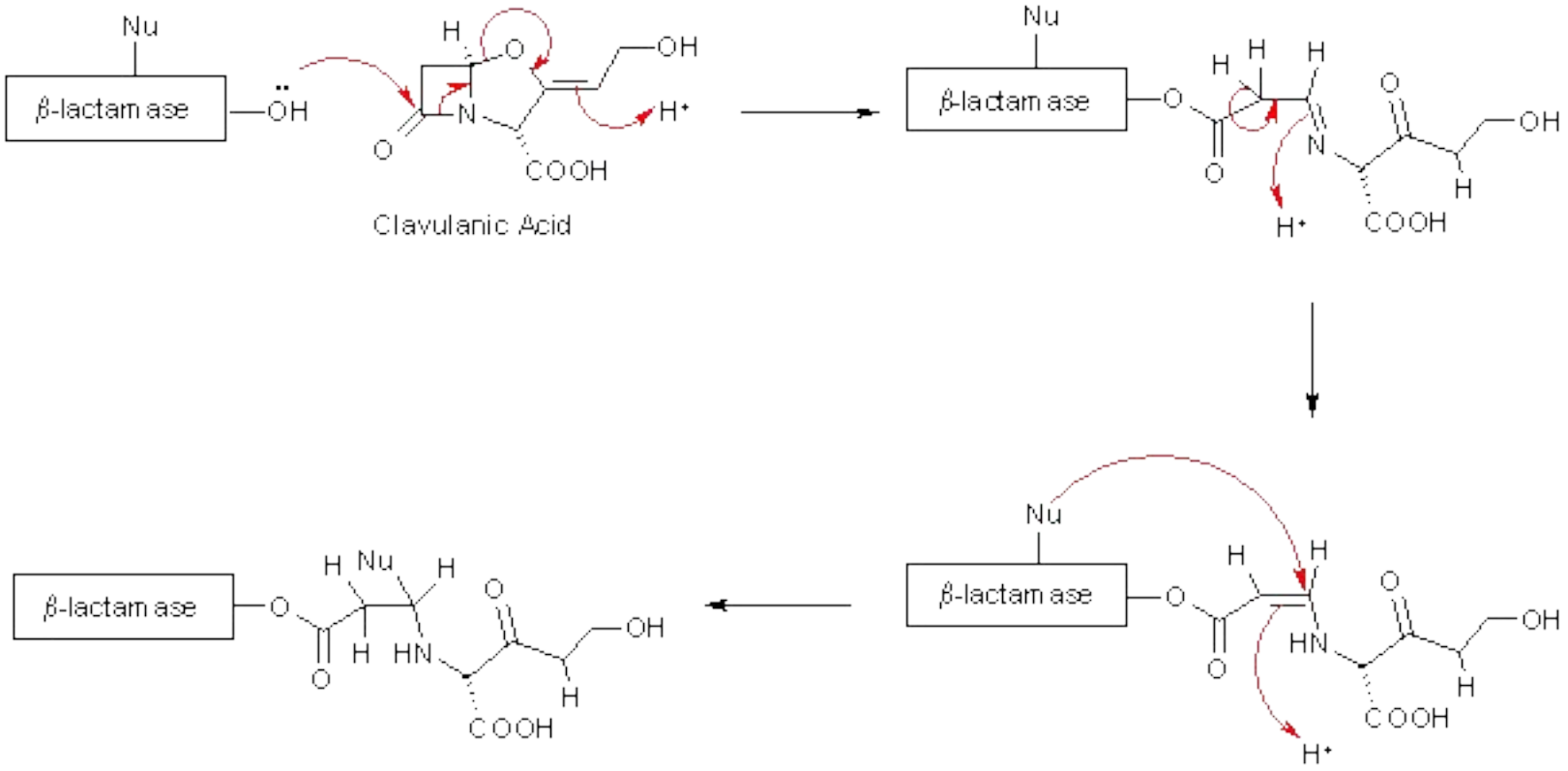
Beta-lactamase activity






BlaR
β-lactam
CTXM9
proteolysis
BlaI
CphA
blaR blaI
blaZ
expression
β-Lactamases
PBP1b
PBPs
?
effector
MexR
PBP2x
mexR
mexA mexB oprM
PBP2a
H+
gm+
gm-
expression
OprM
MexB
?
MexA
IM
PERIPLASM
PG
OM
Efflux Pumps

β-lactamase inhibitor : Clavulanic acid



Clavulanic acid biosynthesis in *S. clavuligerus*

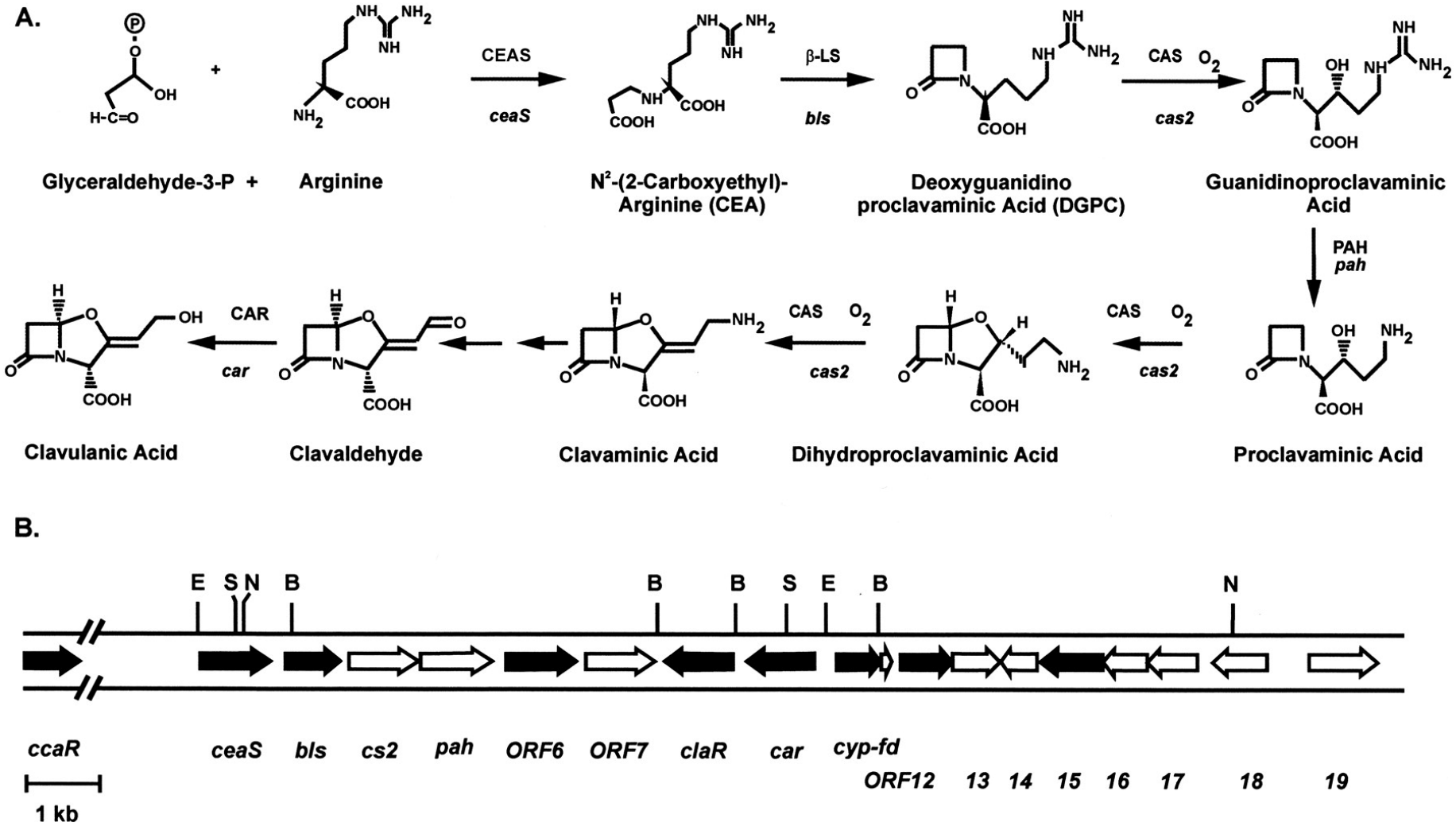
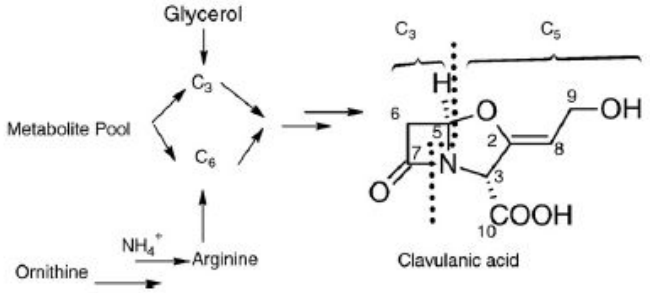


Table 2
Production of clavulanic acid by different strains of *Streptomyces clavuligerus* grown on different carbon and nitrogen sources

Strain	Carbon source	Nitrogen source	Clavulanate concentration (mg/l)	Specific concentration (mg/g of cell)	Volumetric productivity (mg/l-h)	Reference
NRRL 3585	Glycerol	Proline, glutamic acid	12.9	9.9	0.36	Romero et al. (1984)
NRRL 3585	Glycerol	Proline, glutamic acid Proline, ornithine	–	8.9	–	Romero et al. (1984)
ATCC 27064	Glycerol	Malt extract, Bacteriological peptone	–	16.5	–	Belmar-Beiny and Thomas (1991)
DSM 738	Glycerol	Soy meal extract, meat peptone	300	37.5	3.16	Mayer and Deckwer (1996)
		Soy meal, meat peptone	500	45.5	5.26	
NRRL 3585	Sucrose	Proline, glutamic acid	0.0	0.0	0.0	Lee and Ho (1996)
	Glycerol		6.4	1.5	–	
N21	Sucrose		0.0	0.0	0.0	
	glycerol		11.2	5.5	–	
	crude palm oil		11.3	7.3	–	
NRRL 3585	Glycerol	NH_4Cl	35	13	0.47	Ives and Bushell (1997)
NRRL 3585	Glycerol	Soy meal, Bacteriological peptone	472	98.3	4.92	Gouveia et al. (1999)
		Samprosoy 90NB, Bacteriological peptone	920	191.6	12.78	
ATCC 27064	Glycerol	Soy meal extract, peptone, ornithine, arginine	311	–	–	Chen et al. (2003)
ATCC 27064	Glycerol	Samprosoy 90NB, Malt extract, Yeast extract	404	–	8.8	Baptista-Neto et al. (2005a)
ATCC 27064	Glycerol	Samprosoy 90NB, Malt extract, Yeast extract	614	–	11.4	Rosa et al. (2005)
ATCC 27064	Glycerol	Soybean flour, ornithine	672	–	–	Wang et al. (2005)
ATCC 27064	Glycerol	Samprosoy 90NB, Yeast extract	475		11.7	Baptista-Neto et al. (2005b)
ATCC 27064	Glycerol, Soybean oil	Soybean flour	753			Maranesi et al. (2005)
ATCC 27064	Glycerol Soybean oil	Soybean flour	906	–	–	Ortiz et al. (2007)
ATCC 27064	Glycerol	Samprosoy 90NB, Malt extract, Yeast extract	719	–	–	Teodoro et al. (2006)

Values estimated from original works.



- The primary metabolic precursors of clavulanic acid are d-glyceraldehyde-3-phosphate (G3P) and l-arginine.

- Supplemented fermentations of *S. clavuligerus* with arginine increases only the intracellular pool size of this precursor, but not the production of clavulanic acid.

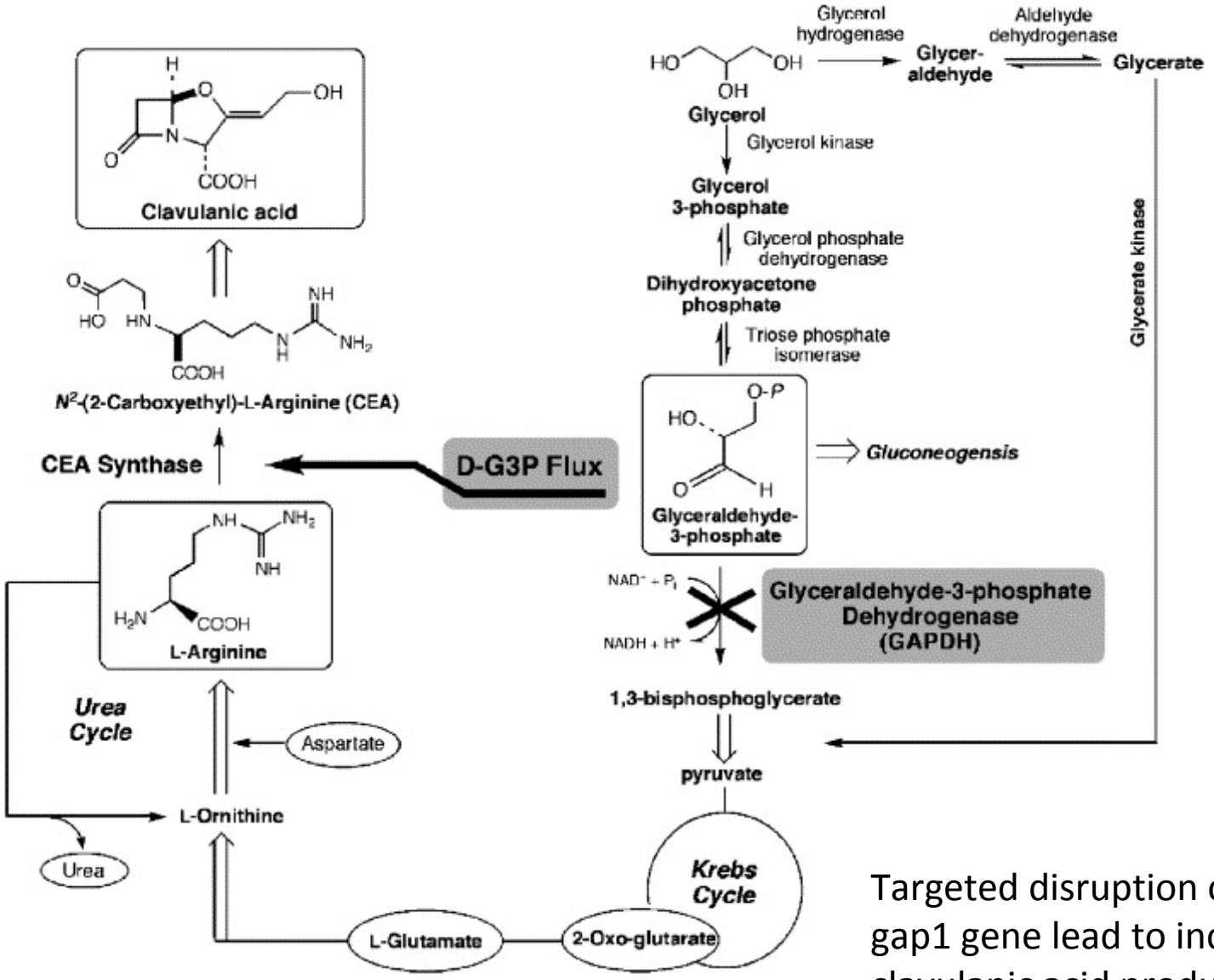
- Metabolic flux analysis has further suggested that a limiting factor for clavulanic acid biosynthesis is the C_3 precursor, G3P.

- This deduction was supported by the observation of a stimulatory effect on clavulanic acid production by supplementing cultures of *S. clavuligerus* with glycerol.

- G3P is an intermediate of the glycolytic pathway and also the entry point in the gluconeogenesis pathway for the synthesis of glucose.

- Metabolic analysis has further shown that in wild-type *S. clavuligerus* the favored direction of G3P flux (~80%) is consistently towards the glycolytic pathway, and the rest (⩽20%) enters the gluconeogenesis and clavulanic acid pathways

Clavulanic acid production: Strain improvement



Targeted disruption of the gap1 gene lead to increased clavulanic acid production