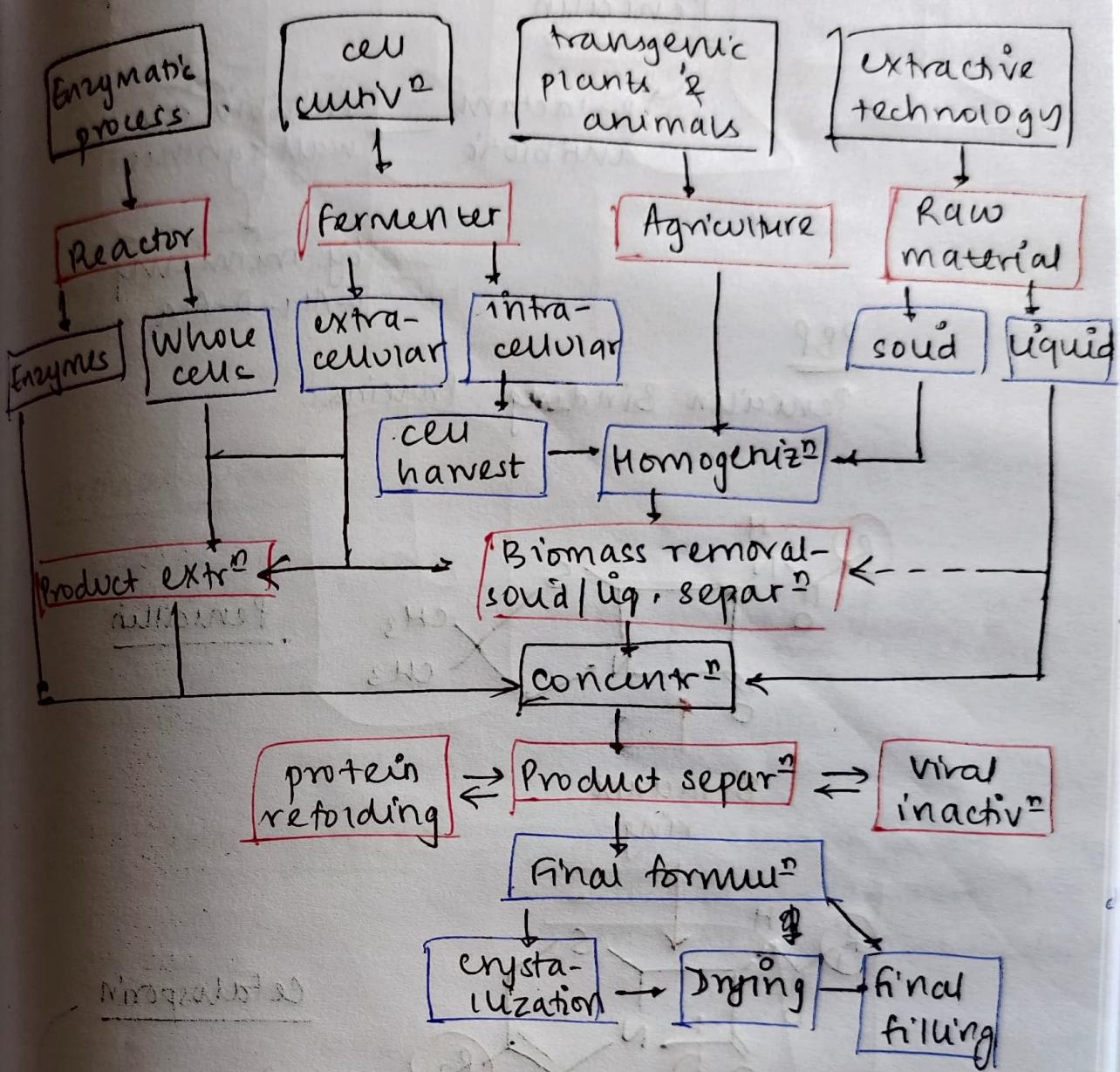


8/8/23



secondary metabolites } not required for the growth & survival of fungi

thus, not produced in max. qty. in log phase

14|8|25

Penicillin

β -lactam antibiotic

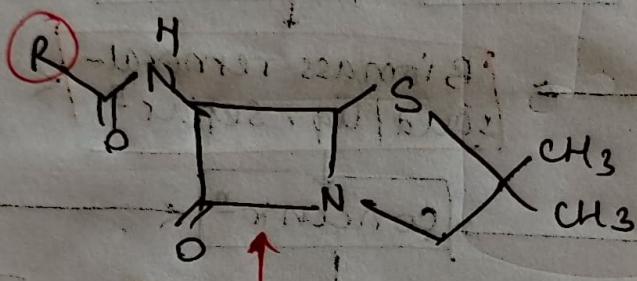
Uso

inhibits cell wall synthesis

by mimicing
D-Ala-D-Ala

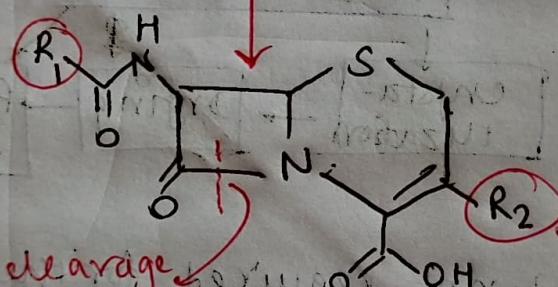
PBP

Penicillin Binding Protein.



Penicillin

β -lactam ring



Cefalosporin

pt. of cleavage
by β -lactamases
leading to AMR

positions where
modifications
can be done

more wide
spectrum
antibiotics

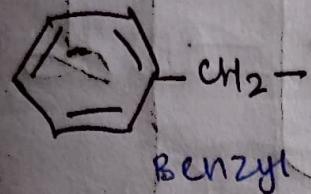
Penicillin G

β -lactamase inhibitors } \rightarrow clavulanic acid

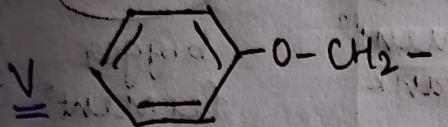
Penicillin V

R Group

G



most common & stable form.



Cephalosporin:

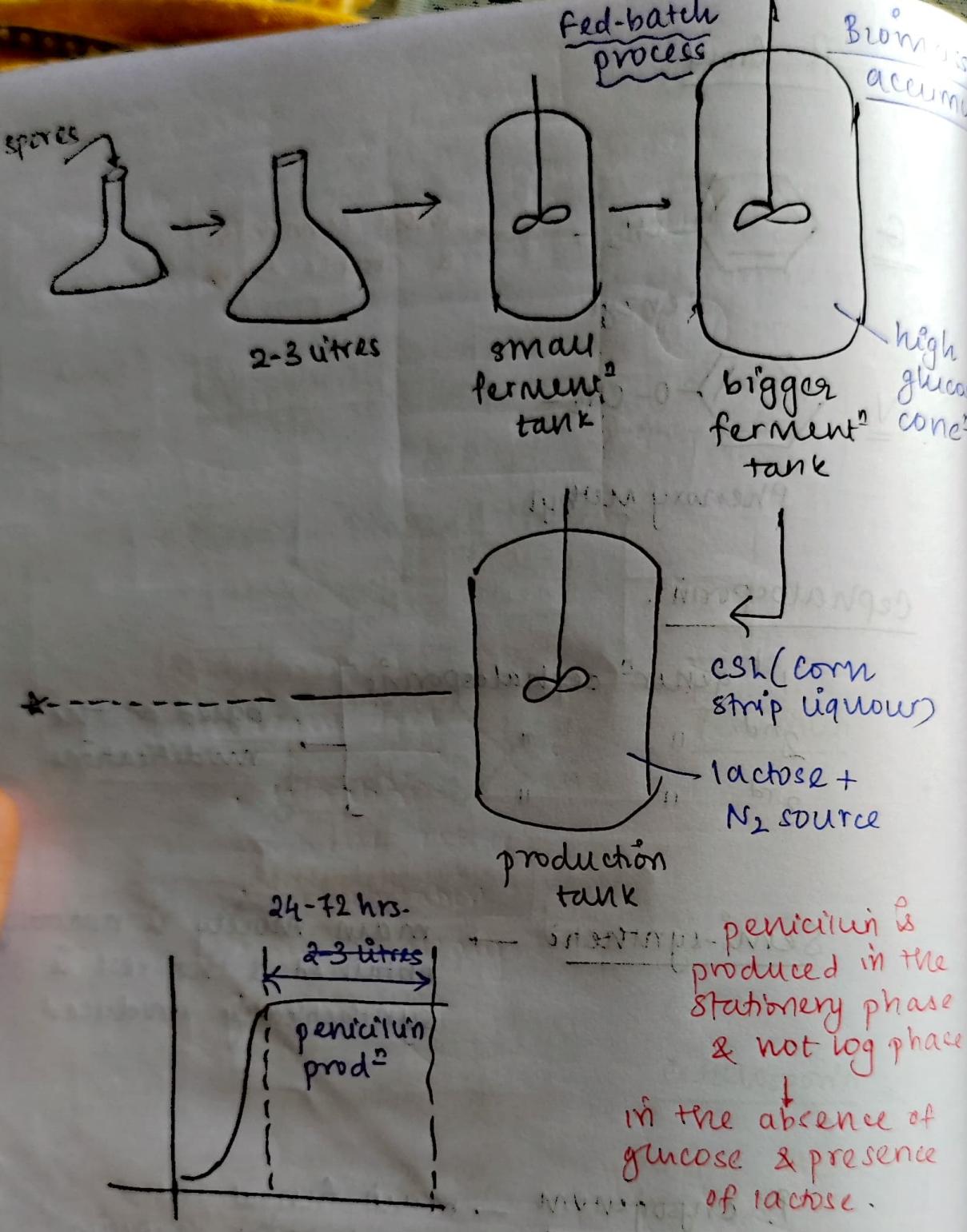
1st generⁿ cephalosporins

2nd
3rd

based on the modifications

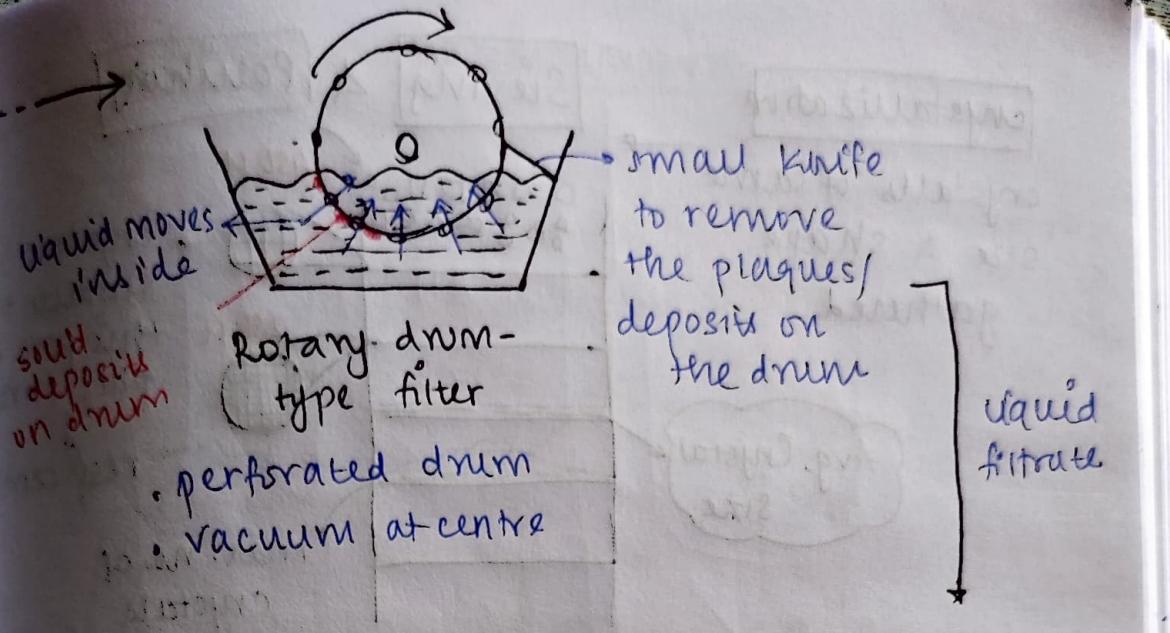
Semi-synthetic → main moiety is naturally obtained & the rest is synthetically produced.

P. chrysogenum → main fungal source for industrial penicillin



CSL → source of phenylethylamine → precursor of penicillin G

N_2 source



~~but penicillin is not very stable at that pH at 25°C.~~

but penicillin is not very stable at that pH at 25°C.

butyl-acetate-
amylacetate (pH ~ 2-3)

SOLVENT EXTRACT

*** pH < 4 → penicillin has a half-life of only 15 mins @ 25°C. \therefore temp. is lowered ($\text{to } 5^\circ\text{C}$)



activated charcoal / carbon treatment
(optional step!)

pH is lowered to ↑ solubility of penicillin in (BAAA). (by addition of H_2SO_4).

Precipitation
→ sodium / potassium acetate
(Na/K-OAc)

if accidentally pigments, etc. comes into broth

crystallization

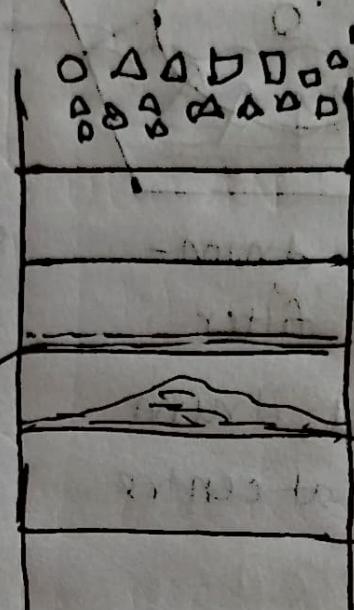
Penicillin G
in diff. crystalline forms

Crystallization

crystals of same size & shape gathered

Sieving & Polishing

Avg. Crystal size



450μ

250μ

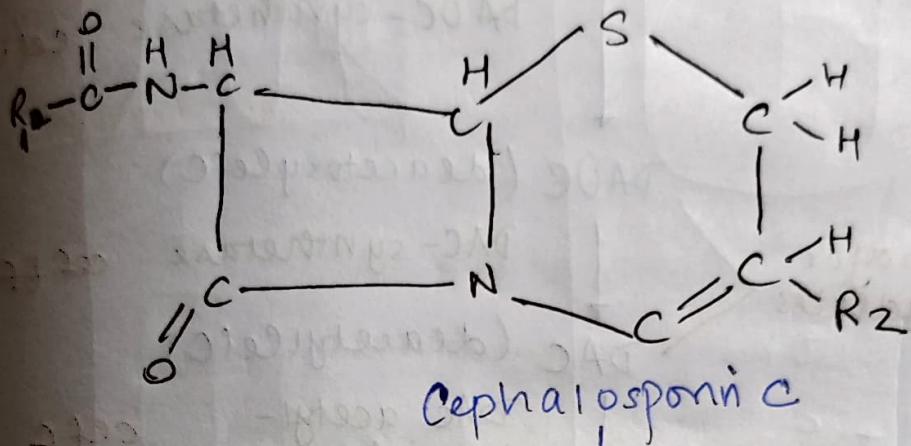
max. no. of crystals

Prominant Crystals

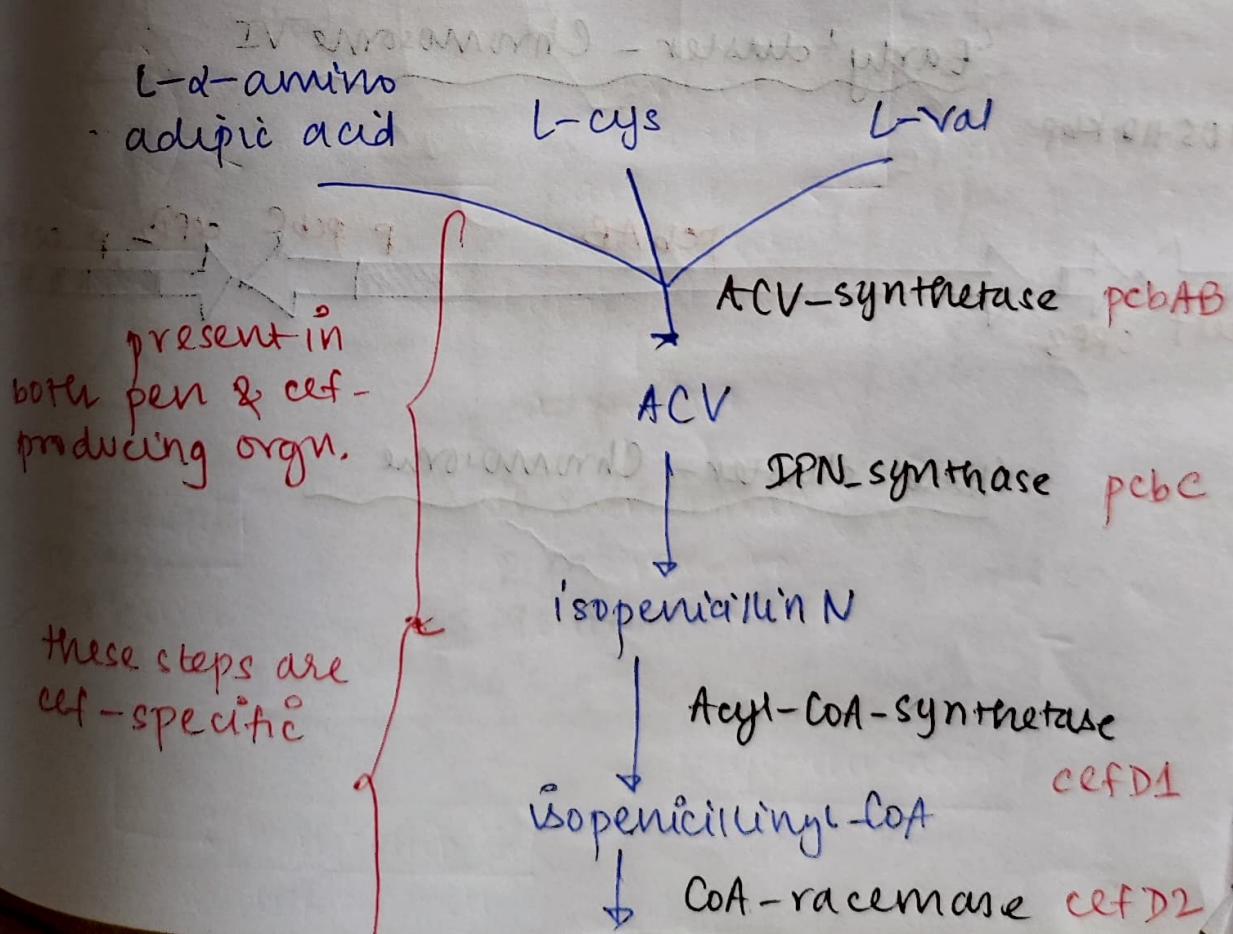
Downstream Operⁿ:

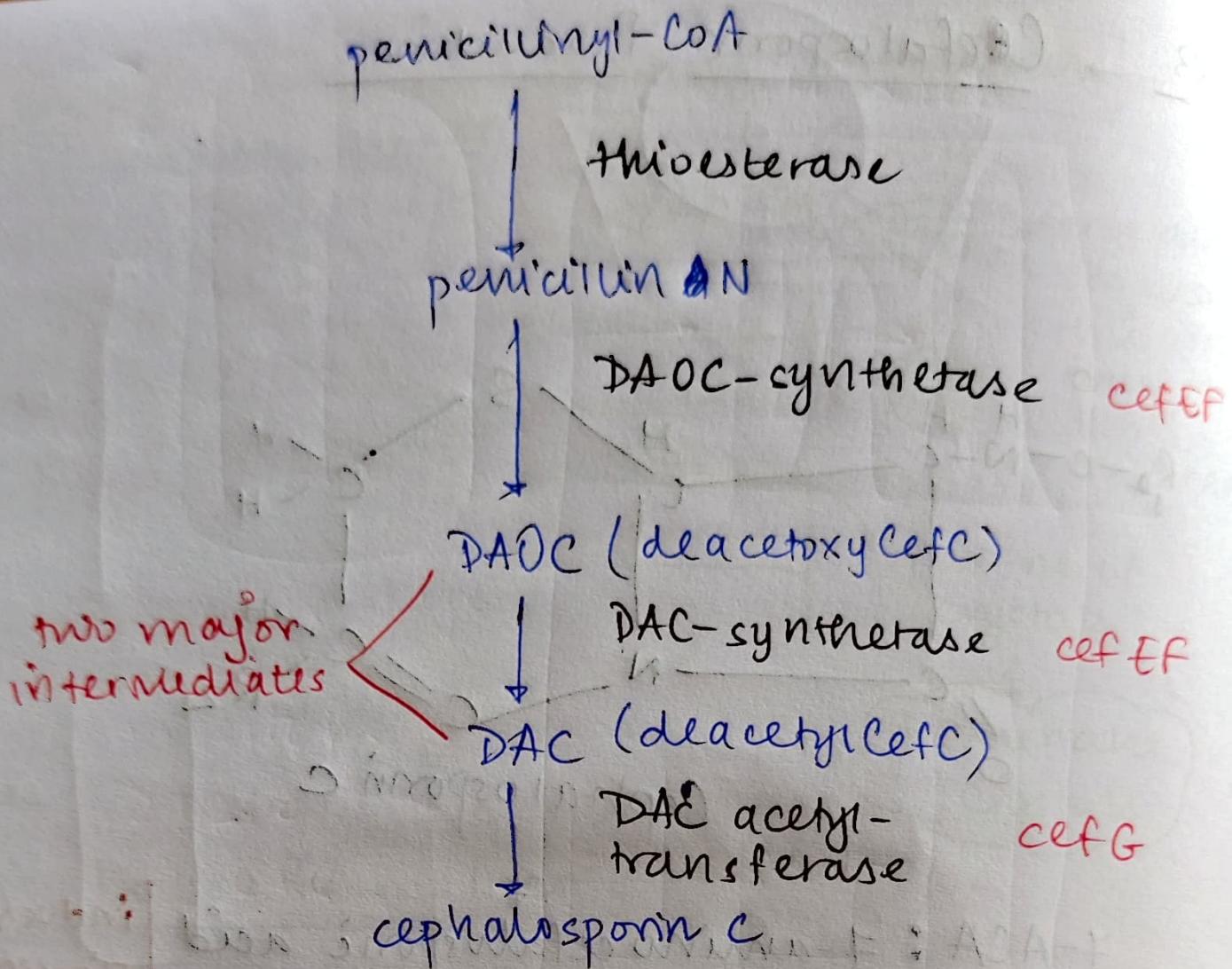
- Filtration
- Solvent extraction
- Precipitation
- Sieving analysis
- Polishing.

Cefalosporin Production



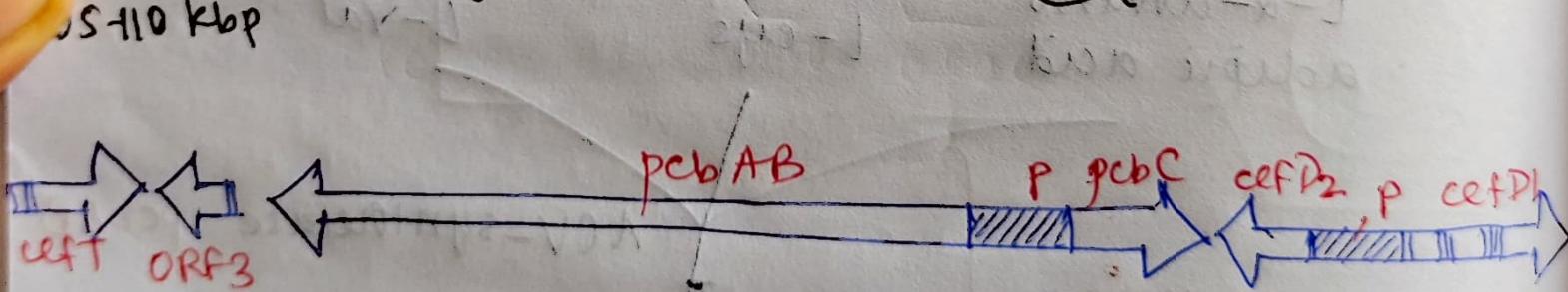
7-ACA: 7-aminocephalosporanic acid [industrially significant]
 ↴ active
 stands in pharmaceutical (API). ingredient





Cephalosporin gene cluster.

'Early' cluster - Chromosome VI



'late' cluster - Chromosome

L-Cys

S-containing
amino acid

methionine methylene is
a precursor.

methionine ↑
(regulator).

rxn. is produced rxn. is
pushed to produce
more L-cys

↑ penicillin
& cef prod².

Cefalosporin prod²: Steps involved:

A. cryogenum

slant from
cell bank



Germinⁿ
stage

~30 mL
72 hrs.
25-28°C



seed stage

1-1.5 mL
70-90 hrs.
28°C



vegetative I stage

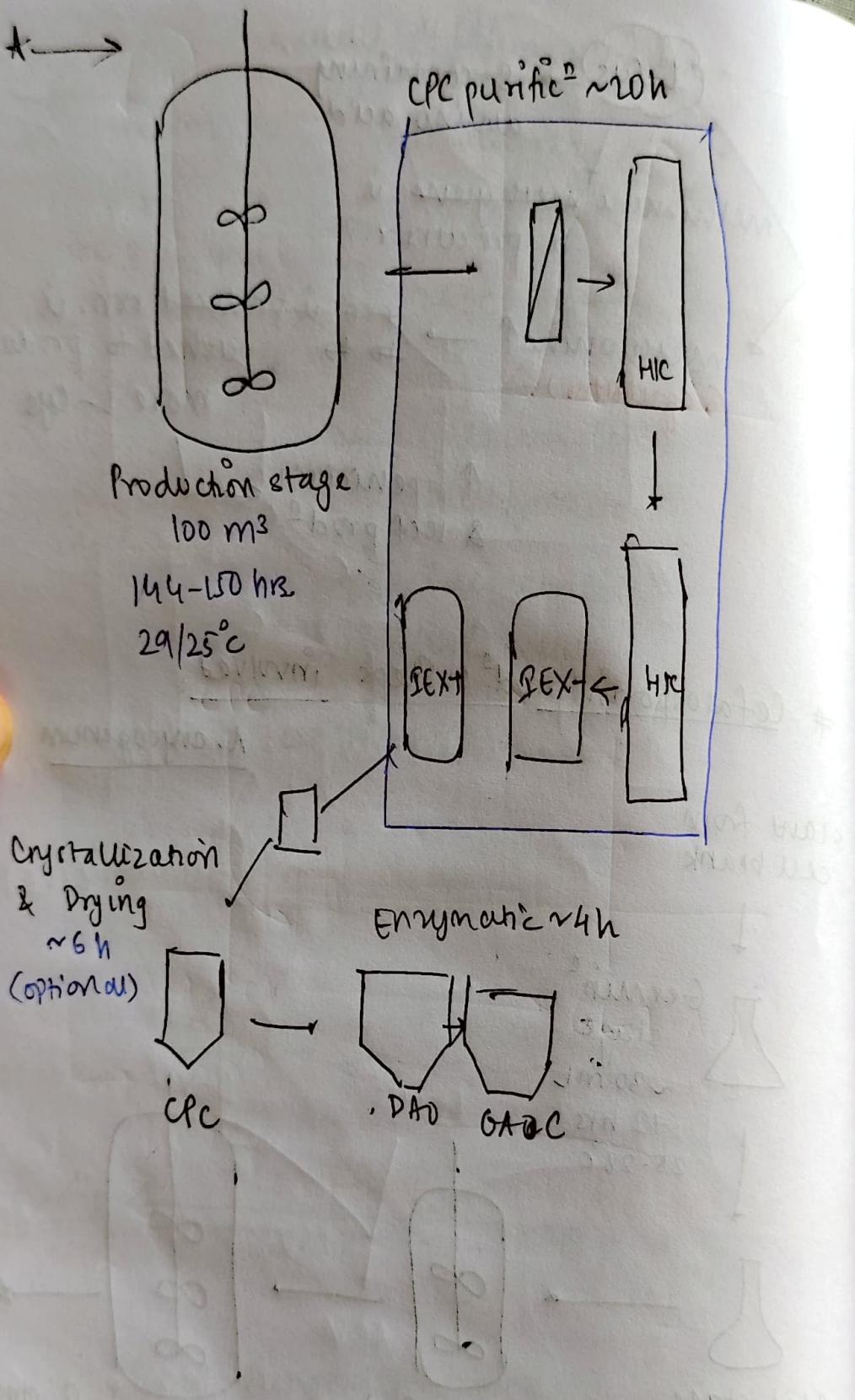
1-2 m³
85-96 hrs.
28°C



vegetative II stage

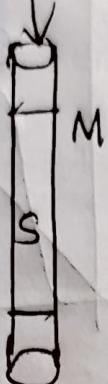
20 m³
45-50 hrs.
28°C





chromatography

Analyte



- ① Binding
- ② Elution

$$K = \frac{C_S}{C_M}$$

conc. in the stationary phase

partitioning coefficient

function

conc. in the mobile phase

partitioning → charge based
 → affinity based
 → etc.

A) Ion-Exchange Chromatography

analyte charge
based on PI

the resin should
be of opp. charge

B) Hydrophobic interⁿ Chrom.
(HIC).

CPC purification:

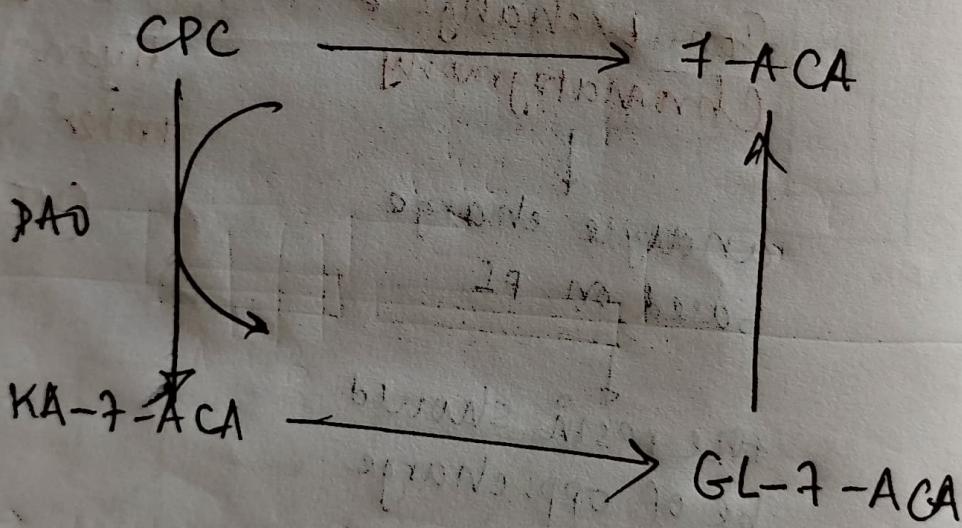
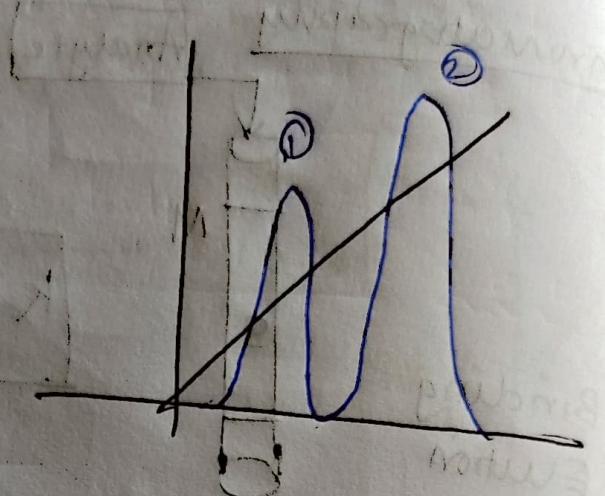
A. cryogenum fermentⁿ broth

Filtration

Large scale HIC columns for
removal of proteins, peptides

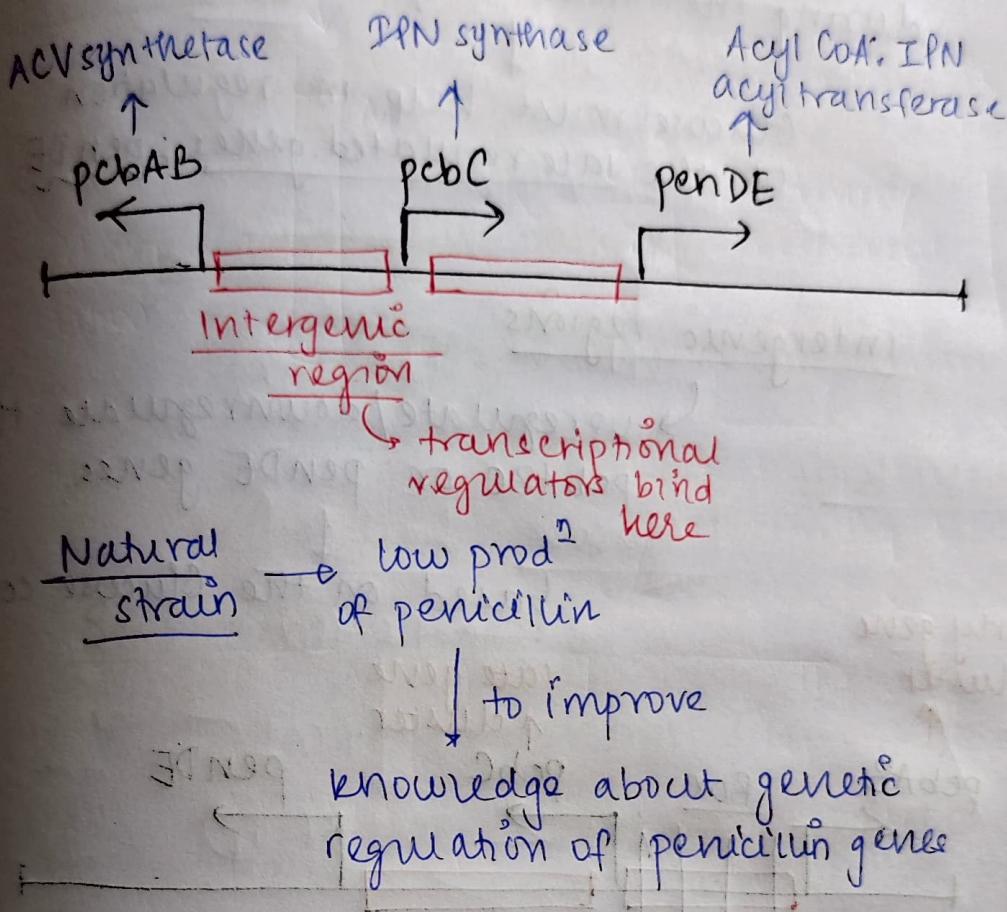
200mM
NaCl

2M
NaCl

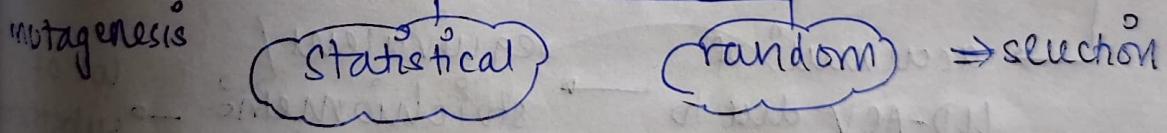


28/8/23

Regulation of Penicillin Biosynthesis



strain improvement



Current industrial strains → GM strains.

Important factors :

- pH of the medium (\uparrow at slightly alkaline pH: 7.5-8.5)
- Glucose-lactose balance

↳ ↑ Glucose \uparrow production.

It was seen that when Glucose was + during the later stages, pen prodⁿ ↑↑

Glucose must help in regulation of the late regulated genes: penDE

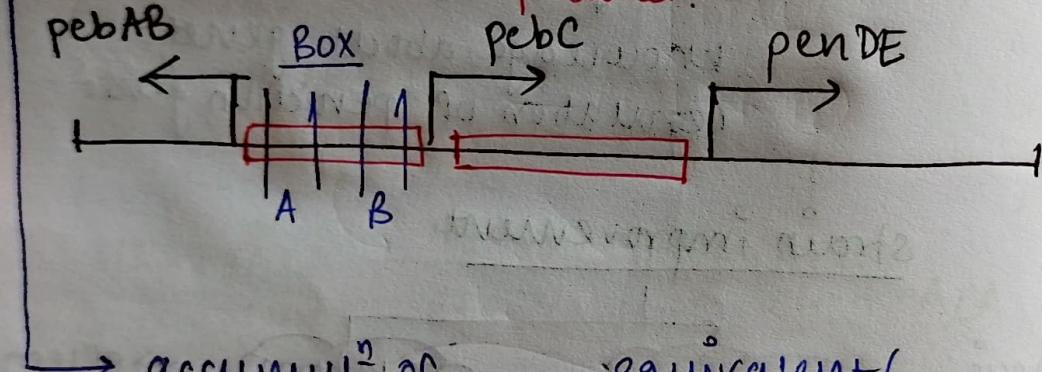
Intergenic regions

↳ upregulate/downregulate the *pcbABC* or *penDE* genes.

↓ based on the Glucose conc.

early gene cluster

late gene cluster

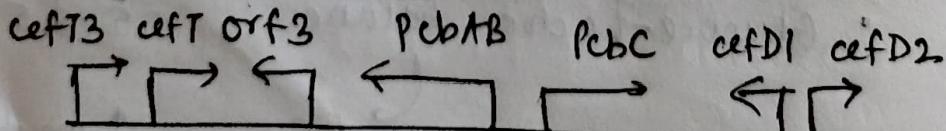


→ accumulⁿ of UD-ACV due to ↑ prodⁿ of *pcbAB* protein

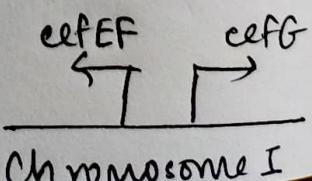
equivalent/stoichiometric amt. of *pbcC* & *penDE* have to be produced to produce penicillin

lot of substrate will be available w/o the enzyme

Cef gene cluster



Chromosome VIII



Chromosome I

Cre sequence: Glucose suppressors

CreA → regulate glucose

Cre-independent pathways:

PTA1

Protein Transcriptional
Activator

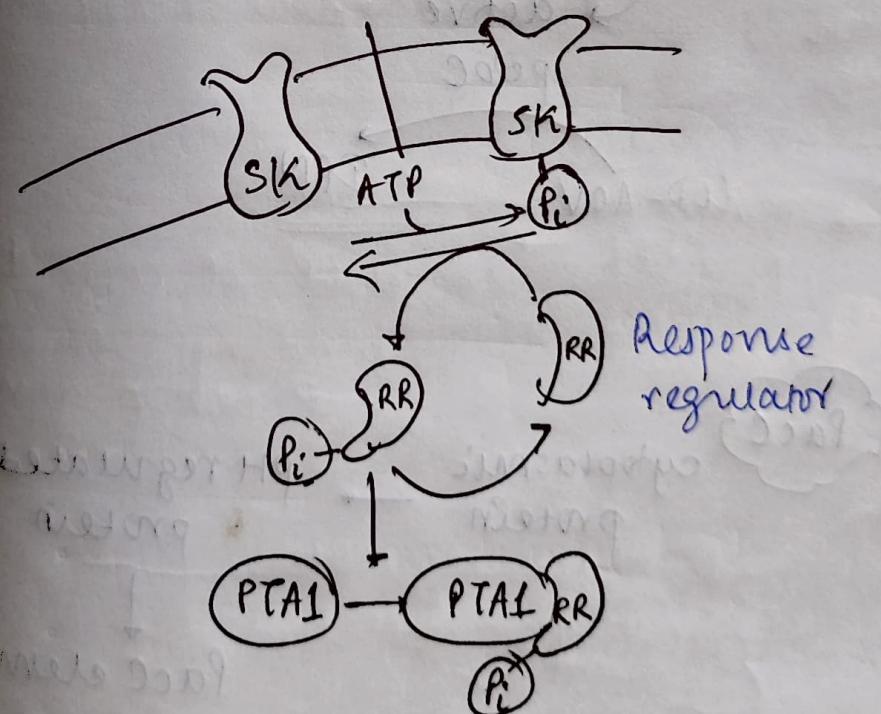
A-box

SK: sensory kinase

phosphorylⁿ site

SK<sup>absence
of
Glucose</sup>

SK-P



Glucose + pcBC activated

↑ Glucose

pcbAB synth.

box A

box B

LUD-ACV formed.



IPN

pcbc reg.

After log:

↓ Glucose

Lactose ↑

RR → RR- P_i

PTA1

transcripⁿ
activator

bind to the
site in A box

PTA1-RR- P_i

active
pcbc

LUD-ACV

IPN

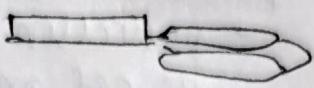
Pacc

cytoplasmic
protein

pH regulated
protein

Pacc elements
upstream of
both pcbAB & pbcC

under normal conditions → PacC does not bind



PacC "closed form"

↓ pH alkaline

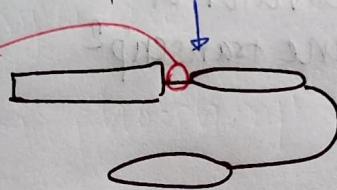
↓ alkaline proteases

(PalB)

cleaves

N-terminal
form of
packed C

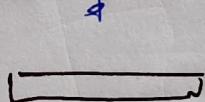
Alkaline-
activated
protease



PacC
"open
form"

N-term.
exposed

PalB



"PacC
active
form"

bind to the PacC
site in the B-box

can activate both
pcbAB & pcbC

Human Genome

Molecular Biology

CCAAT BOX

upstream of **pcbAB** & **pcbc**

penicillin as well as cef production

Gene Regulatory Complex

Complex I

PENRI

Penicillin Regulator I

+ve regulator of penDE
lowest abundant enzyme

activator of gene transcrⁿ

PENRII

repressor of gene transcrⁿ

Deletion of CCAAT box :

8 fold ↑ in ~~pcbDE~~ **pcbC**

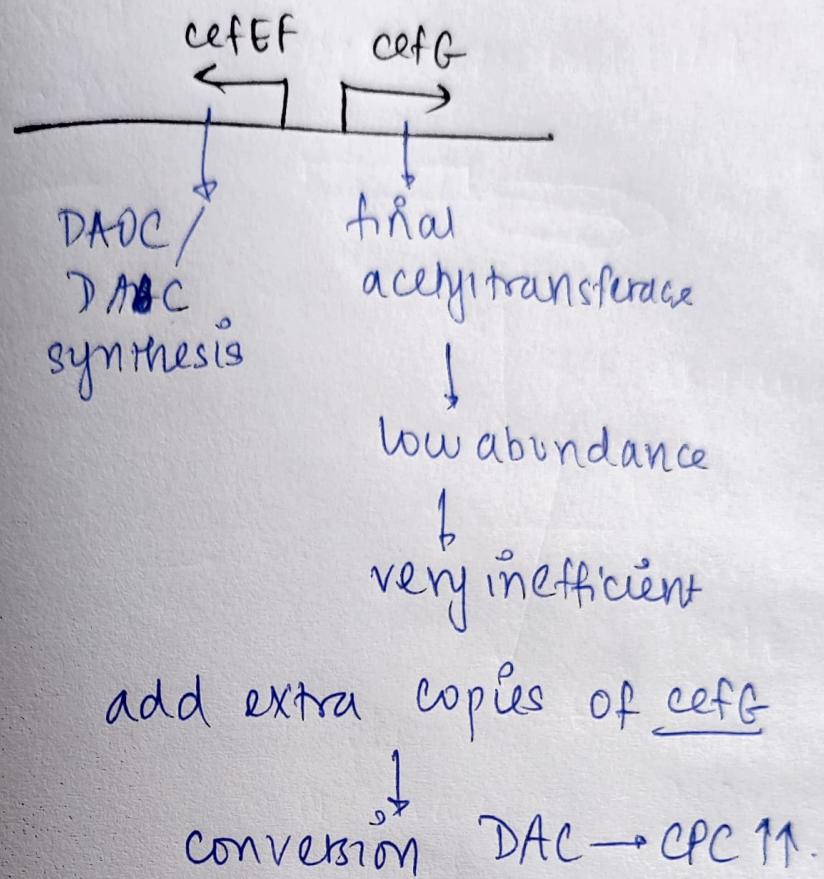
30% decrease in ~~pcbE~~ **pcbC** **pcbAB**

if we can ↑ penDE prodⁿ

↳ penicillin prodⁿ ↑ → solution :

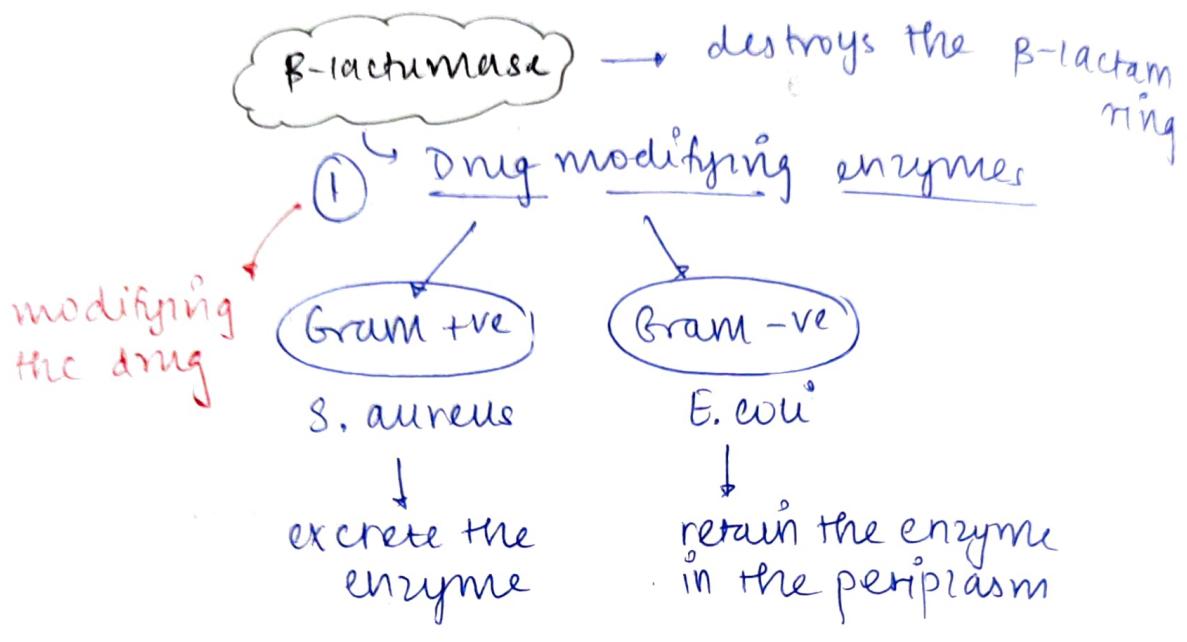
addⁿ of more PENRI binding sites upstream of penDE gene.

- N₂ source regulators
- methyltransferases



4/9/23

β -Lactam Resistance



② Alteration of the PBPs so antibiotic cannot bind

not modifying the drug, but the target

S. pneumoniae
gonococcus

③ Exclusion from the site of cell wall synthesis

drug thrown out

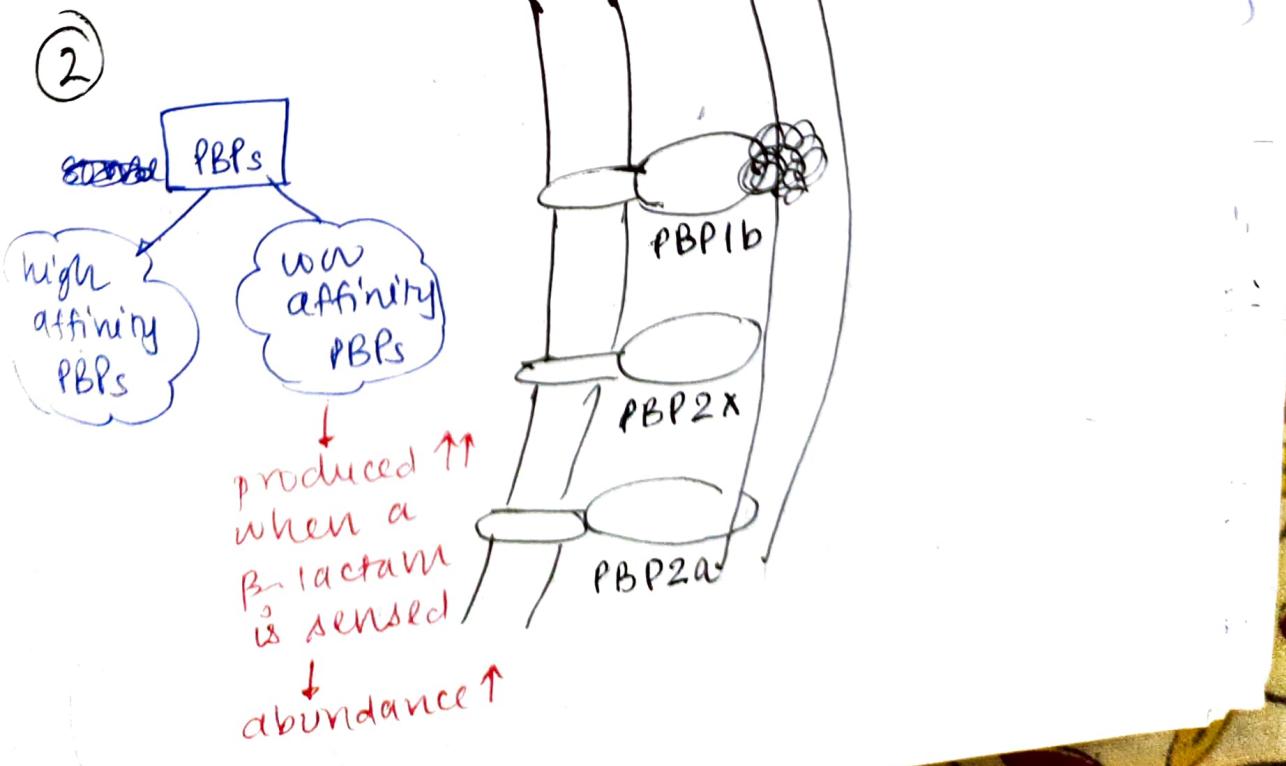
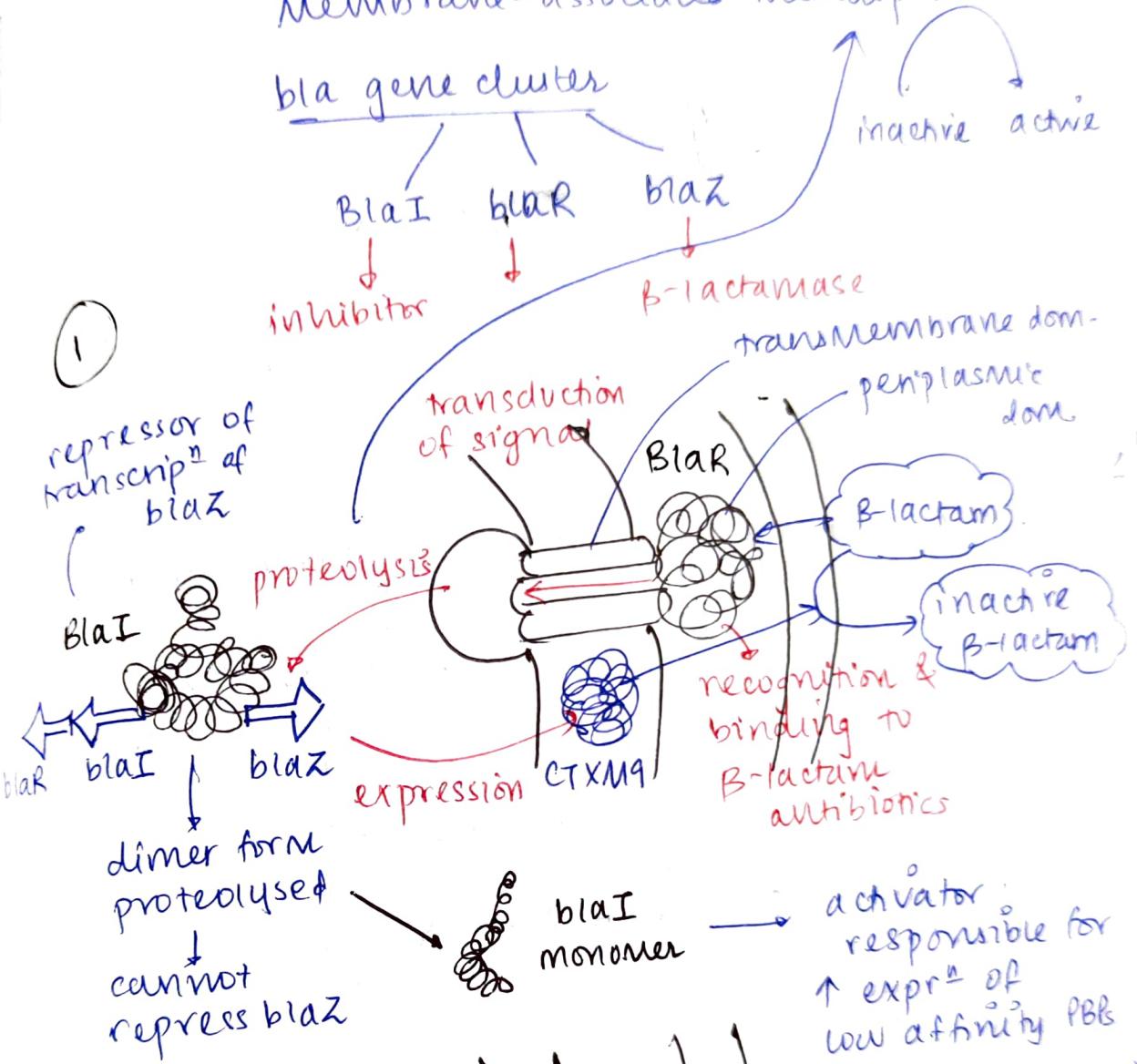
Efflux pumps

Penicillinase

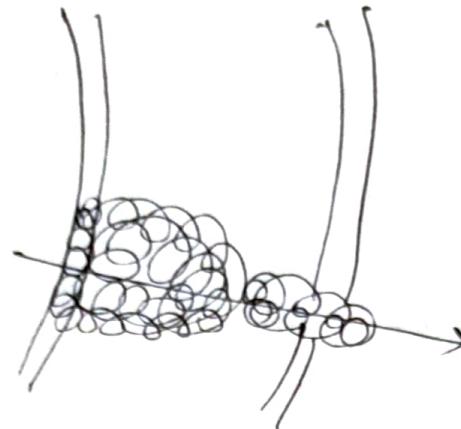
cyclic structure destruction

β -lactamase:

Membrane-associated metalloproteases.



3



when efflux pump
come into play,
 β -lactamase
inhibitors do not
work

β -lactamase inhibitor: Clavulanic acid

β -lactam-like
ring structure

β -lactamase cannot
distinguish b/w an
antibiotic & the
inhibitor

after cleaving
the clavulanic
acid, the β -
lactamase
becomes trapped

cannot inactive
antibiotics anymore

Clavulanic acid biosynthesis,

S. claviger

Glyceraldehyde

3-P (G3P) + Arginine

Clavulanic
acid

