Observation of BIVR cells grown in the presence of drugs by electron microscopy

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Objective

To elucidate the mechanism of BIVR,
We performed electron microscopy on the BIVR cells grown in the presence of VCM alone and compared with those exposed to VCM and CZX.

Test strain

K744 (BIVR strain)

Culture conditions

(A) Broth

BHIB

(B) Drugs

① Free

② 4mg/L of VCM

③ 4mg/L of VCM + 1mg/L of CZX

④ 1mg/L of CZX

(C) Initial volume of K744

10^7cfu/mL

Method

① Collection of culture sample (BIVR cells) by centrifugation

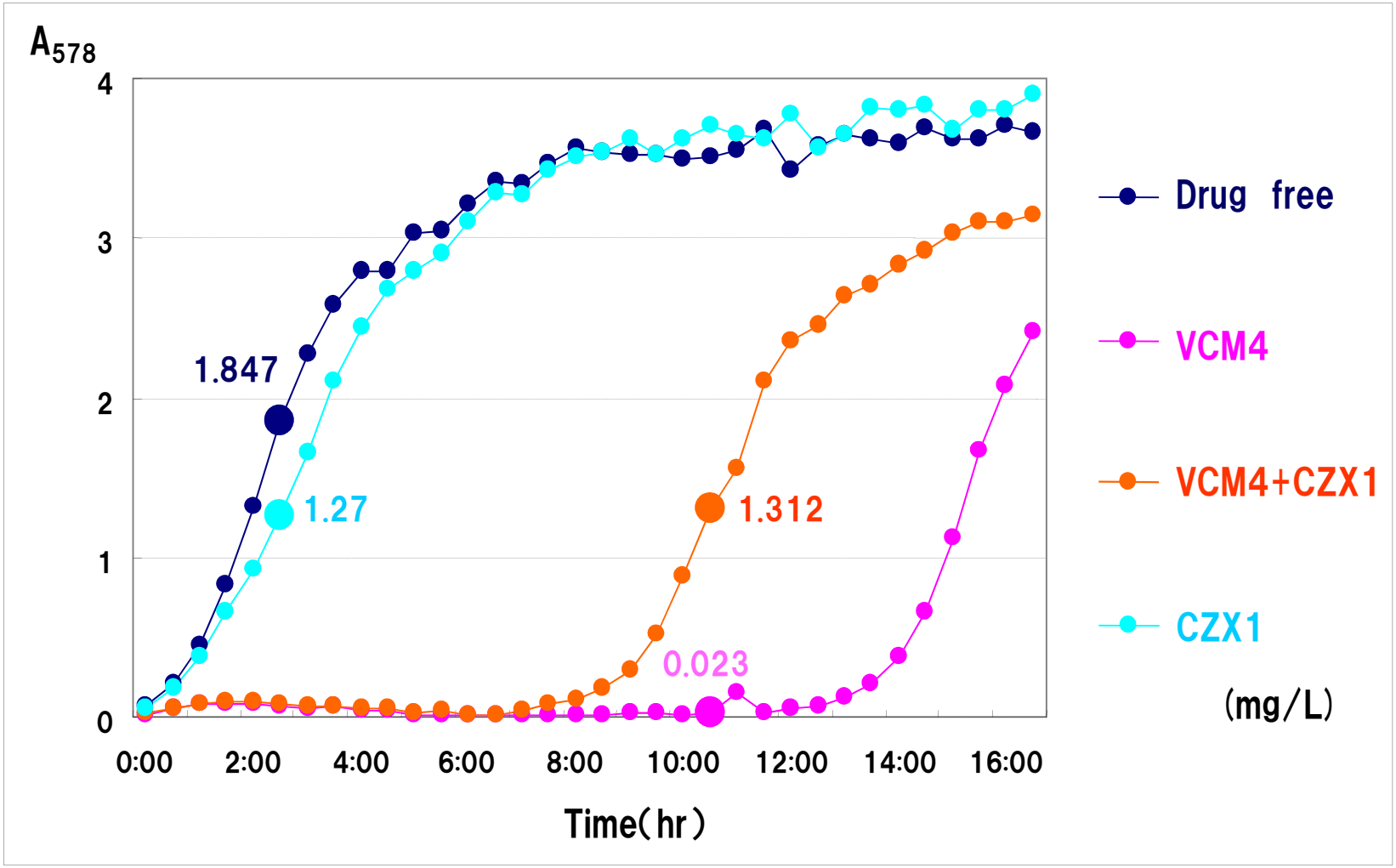
② Washing with PBS buffer (PH7.3)

③ 1% Glutaraldehyde fixation of cells

④ Observation by electron microscopy

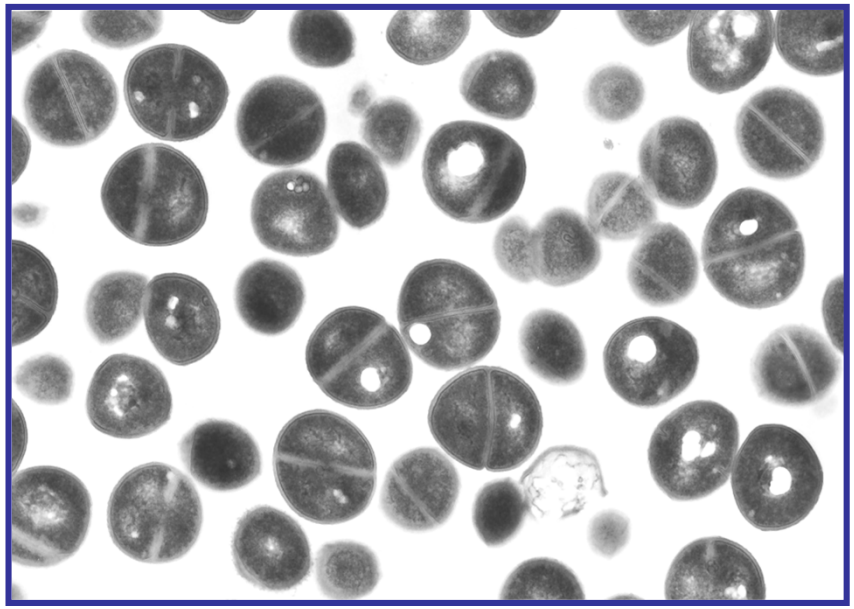
Growth of K744

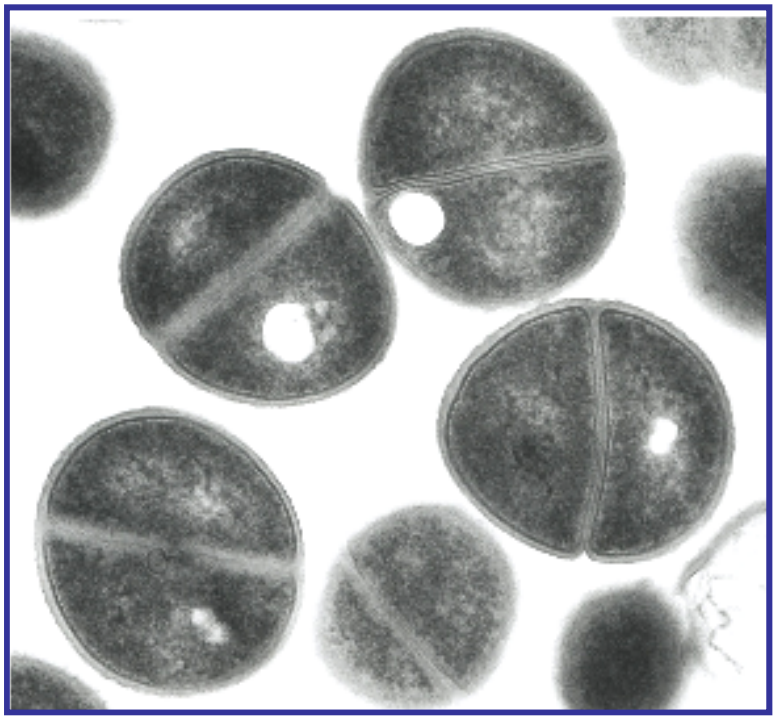
	A_{578}	Time until growing
Drug free	1.847	2.5
VCM	0.023	10.5
VCM+CZX	1.312	10.5
CZX	1.27	2.5

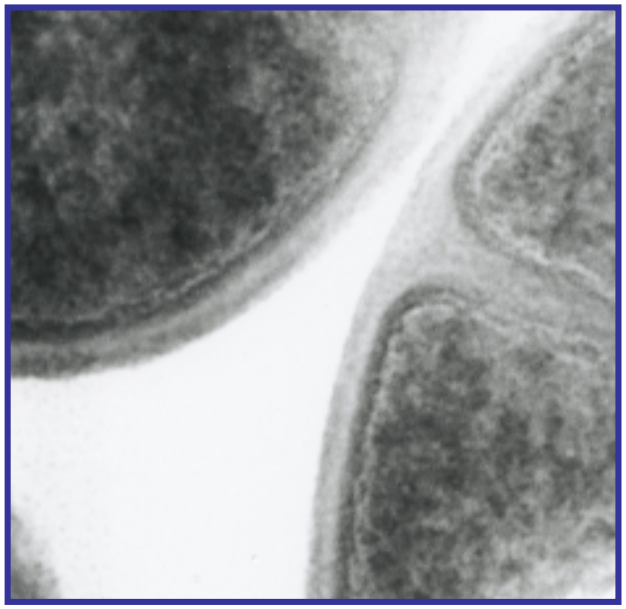


Results ①

～ Drug free ～



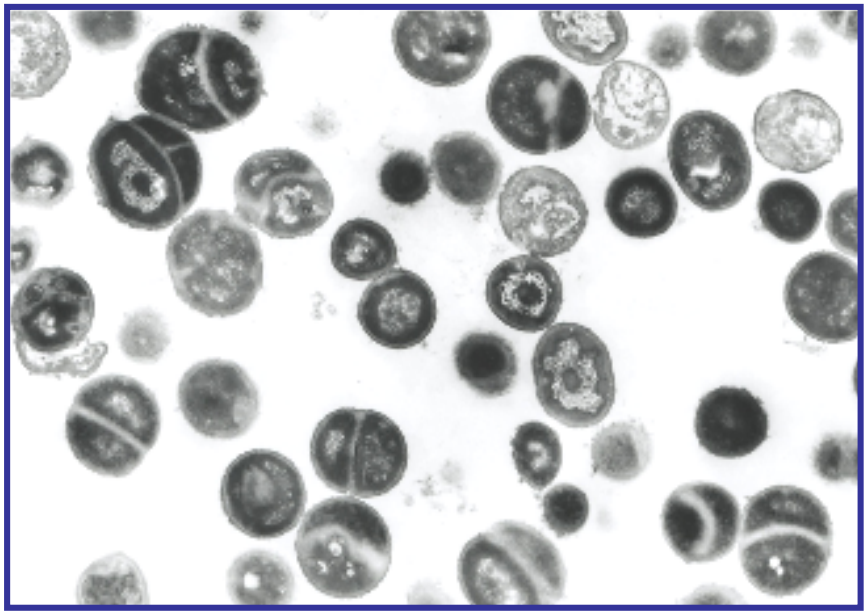


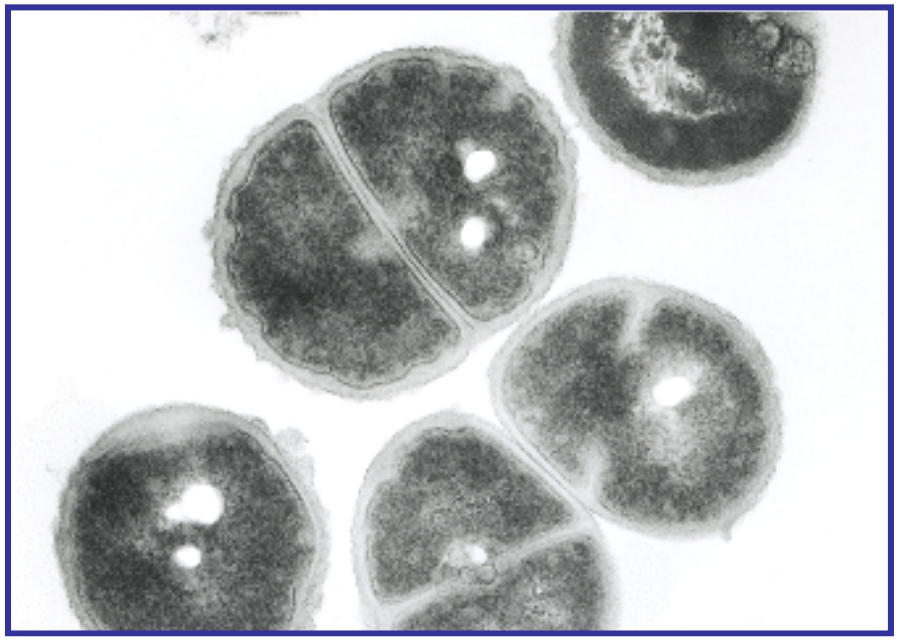


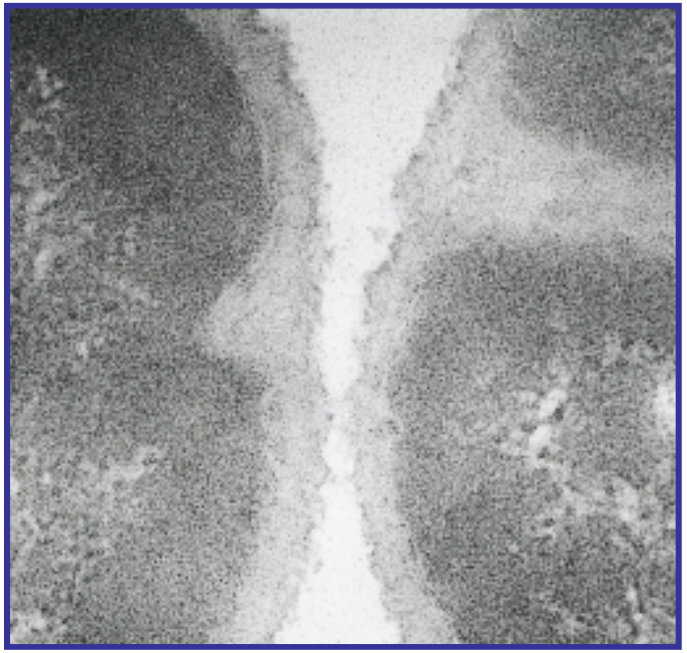
	A_{578}
Drug free	1.847

Results ②

～ VCM ～



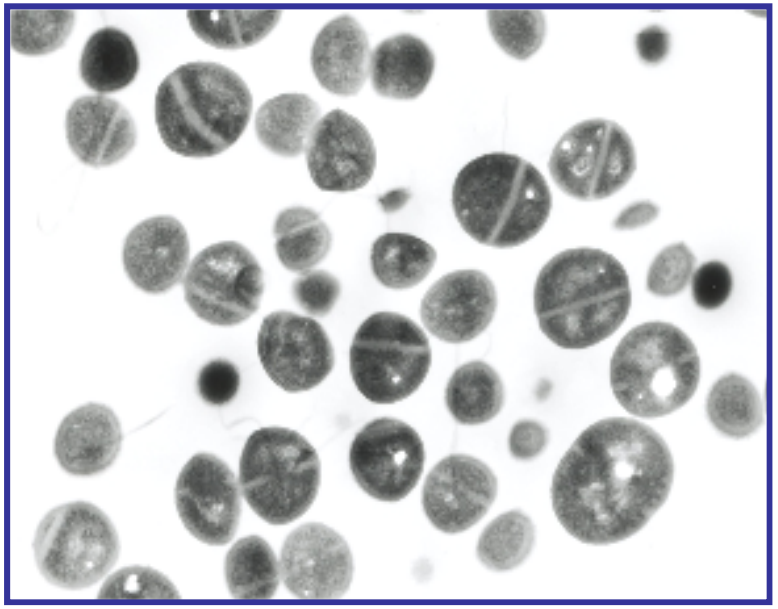


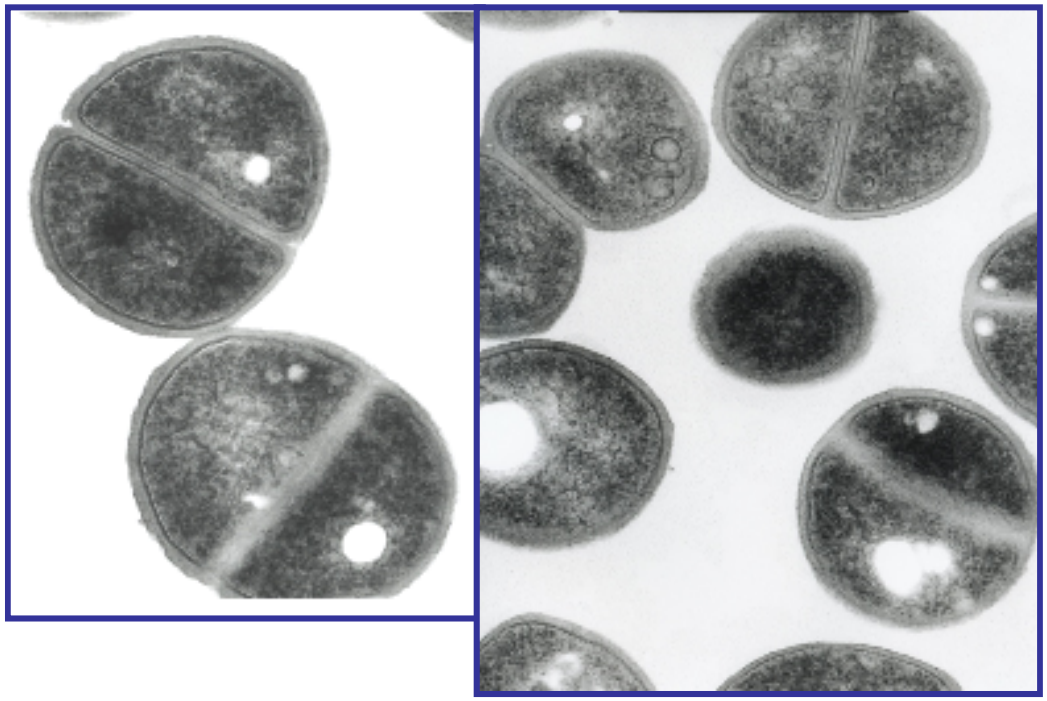


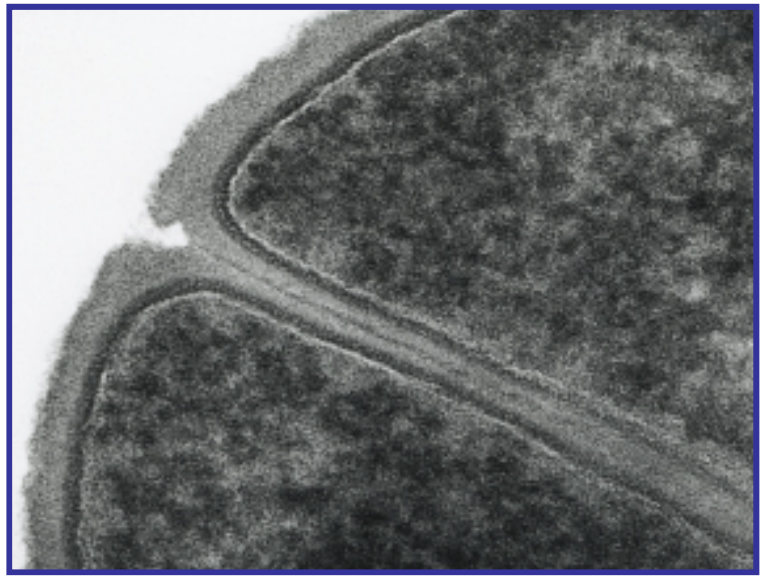
	A_{578}
VCM	0.023

Results ③

～ VCM+CZX ～



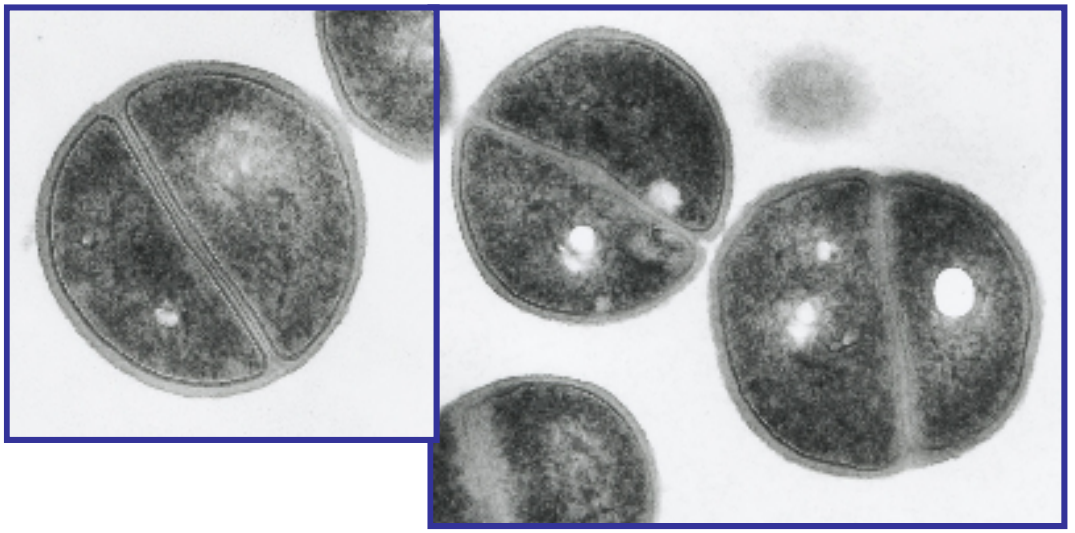


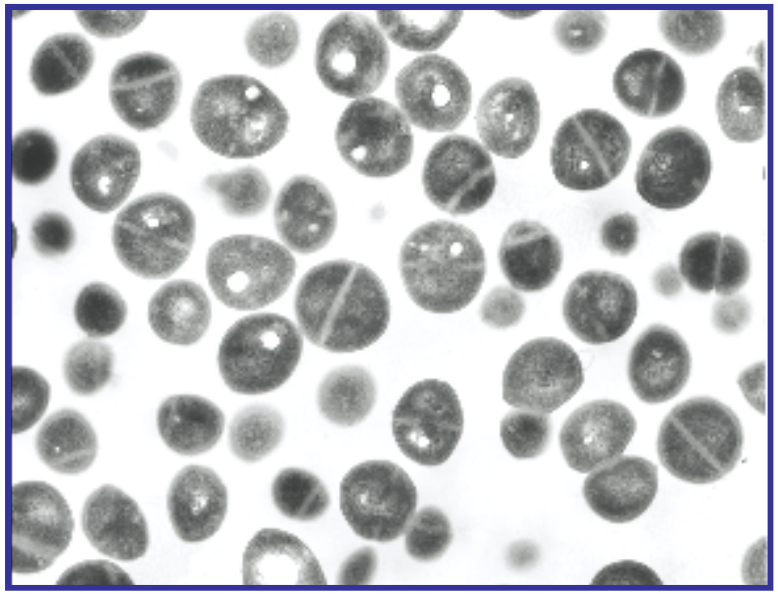


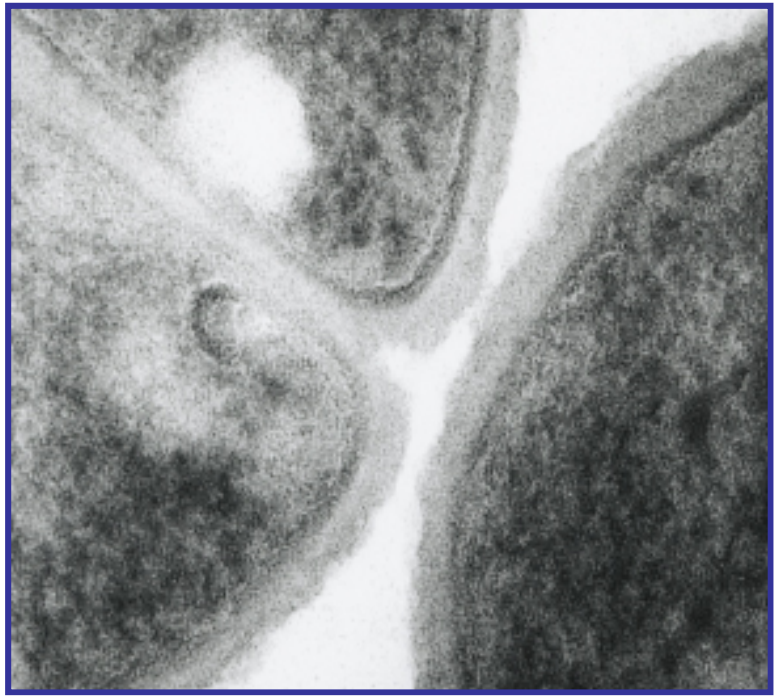
	A_{578}
VCM+CZX	1.312

Results ④

～ CZX ～

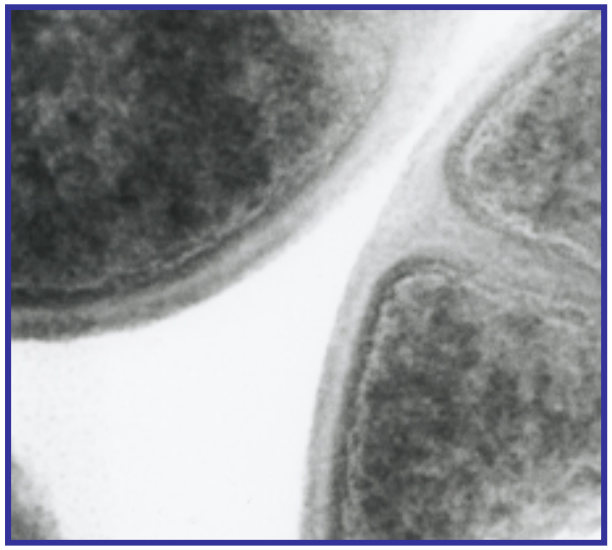




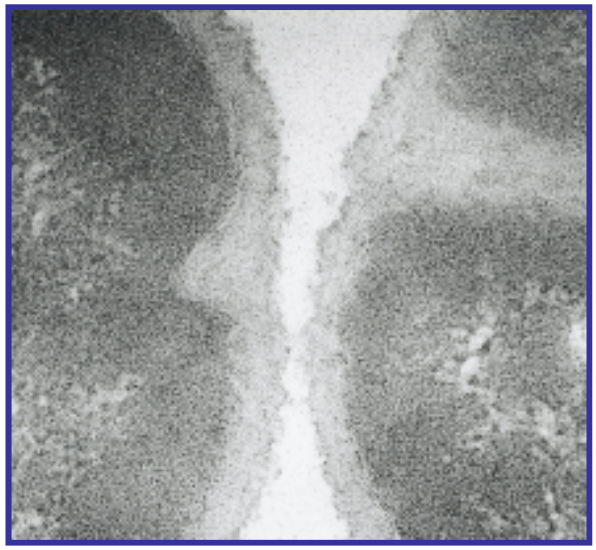


	A_{578}
CZX	1.27

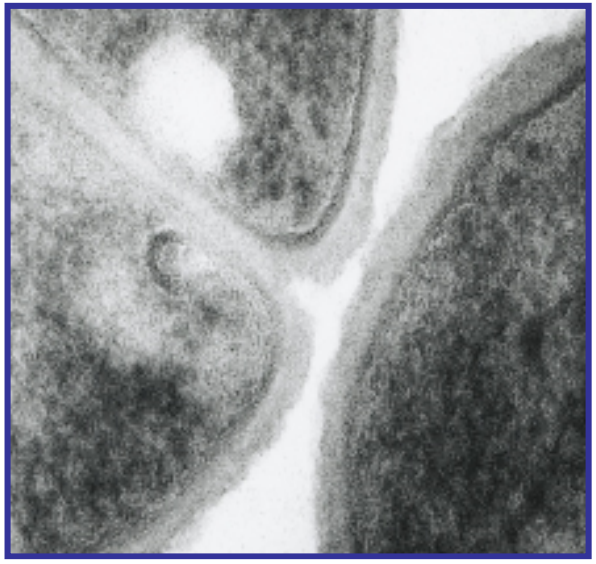
～ Drug free ～



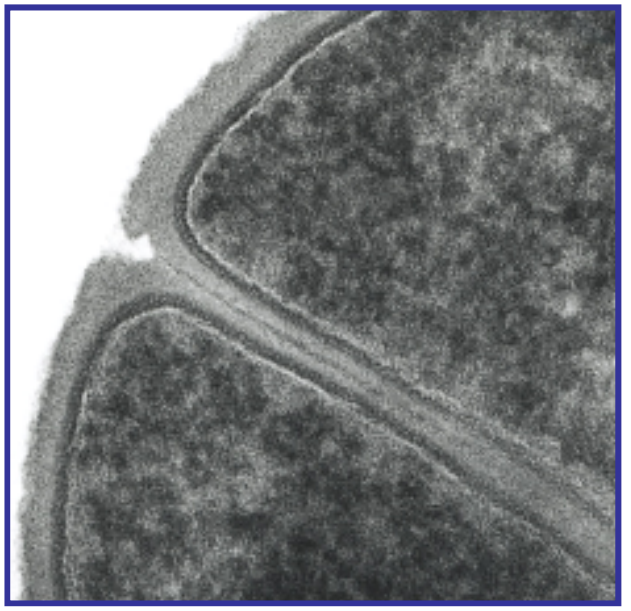
VCM



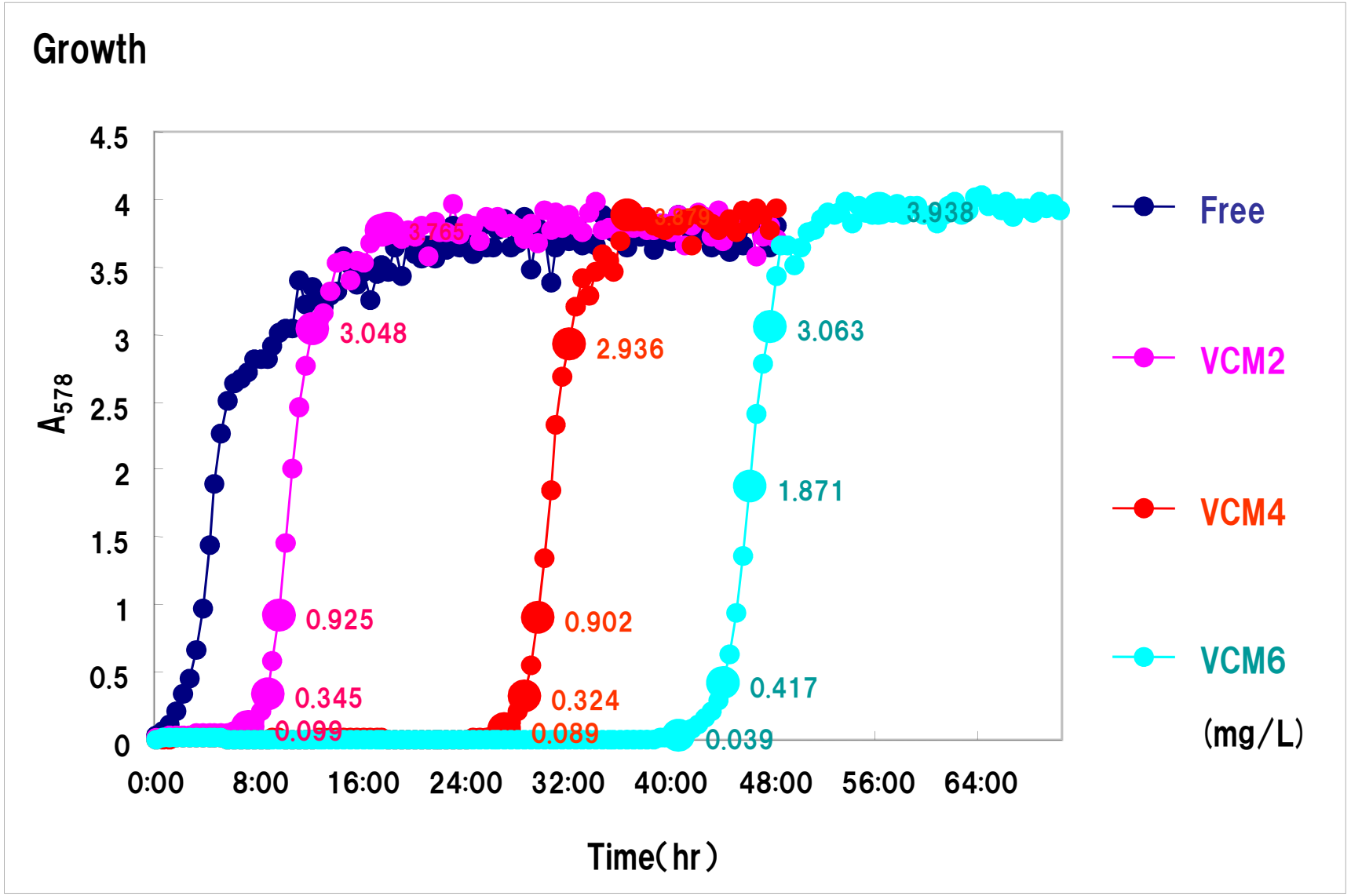
CZX



～ VCM+CZX ～

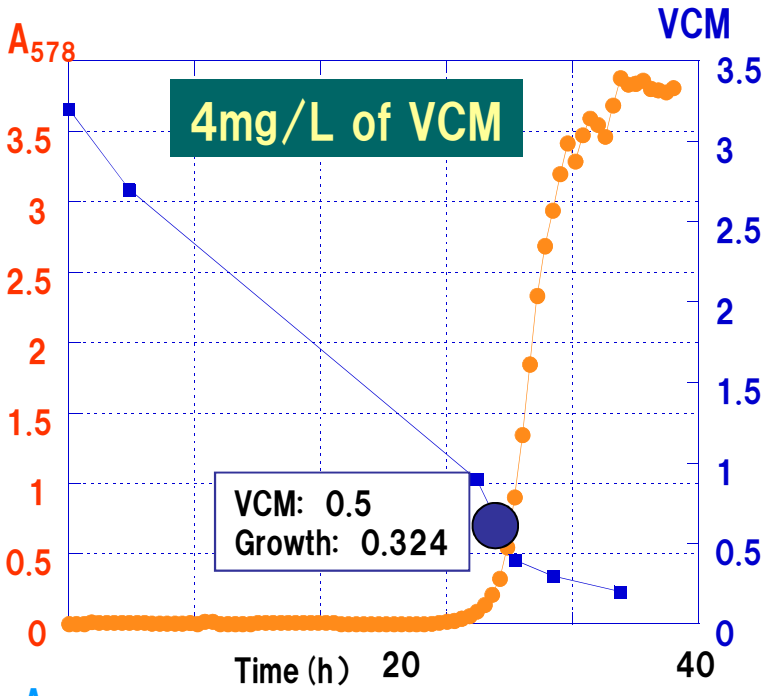


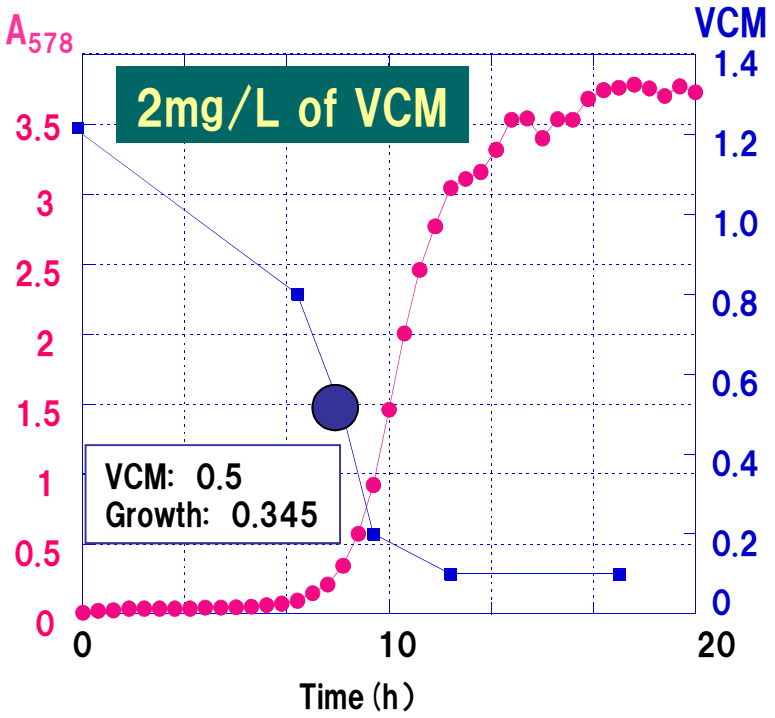
Growth of K744 (BIVR) in the presence of VCM

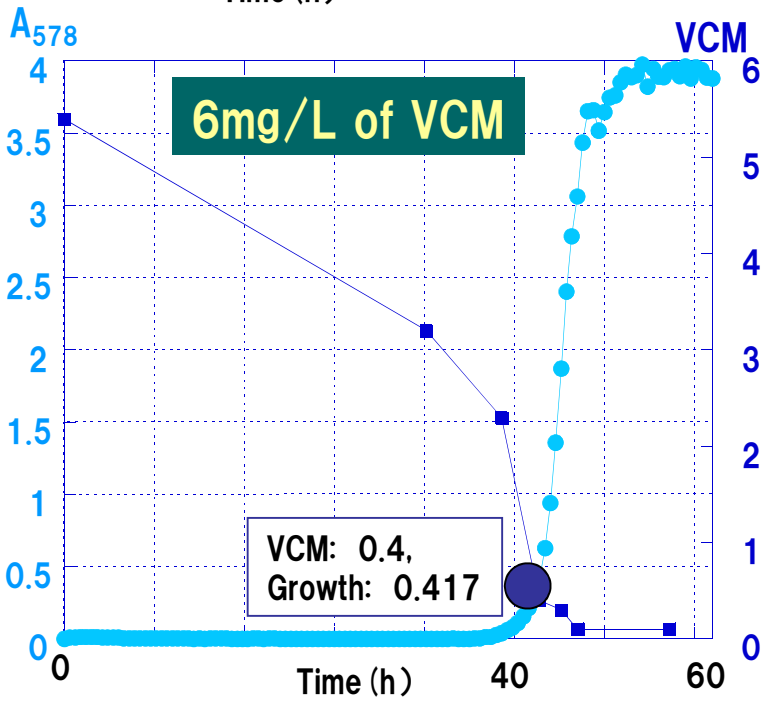


Correlation between the growth of K744 strain and amount of free VCM remaining in the culture

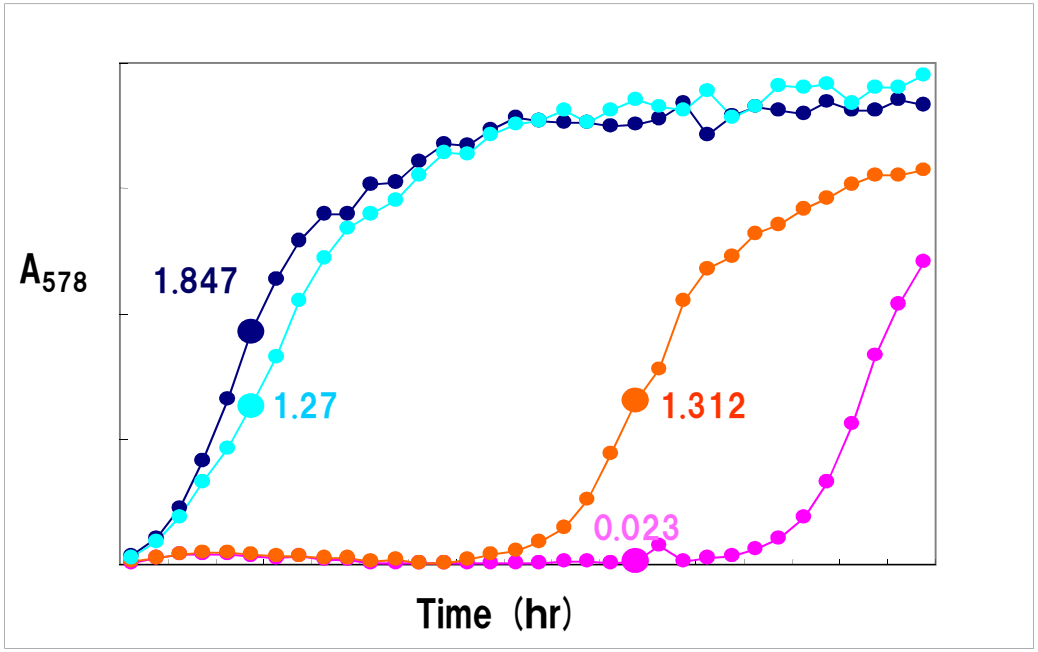
VCM ≦0.5 ⇒ Growth (A_{578}=0.3-0.4)

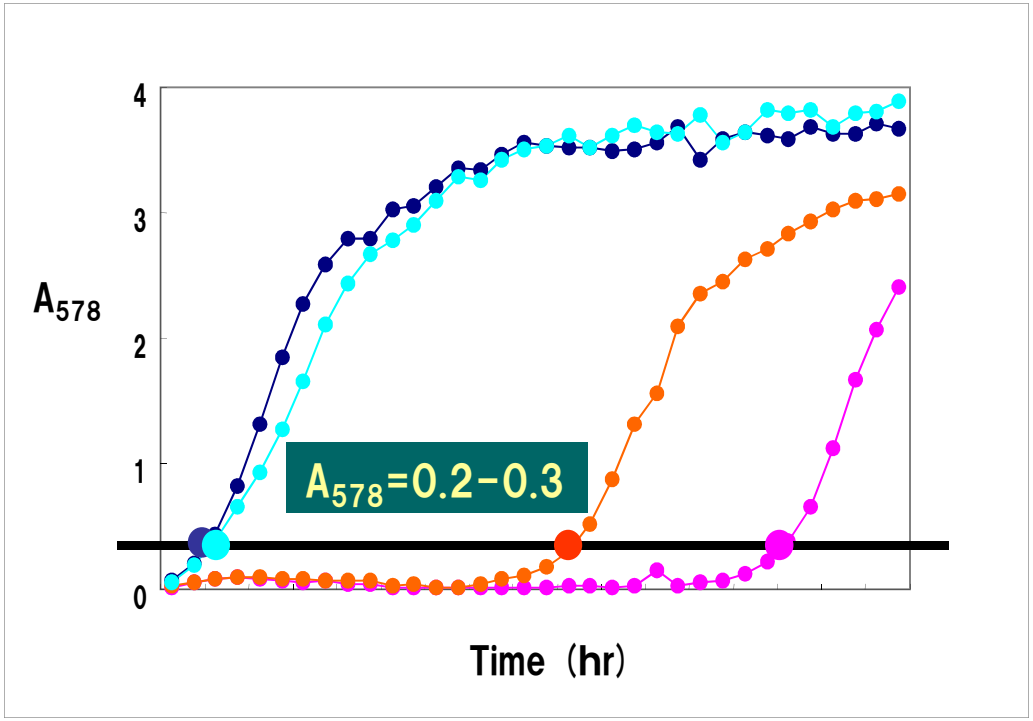






Examination





Drug free

VCM4

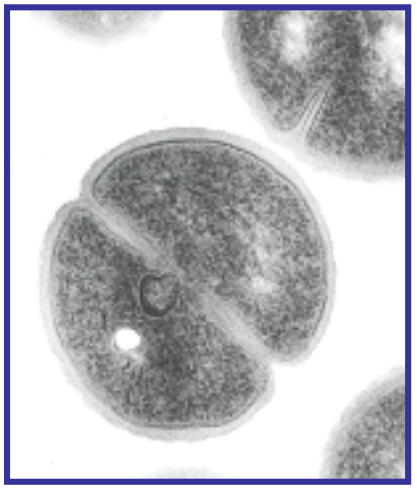
VCM4+CZX1

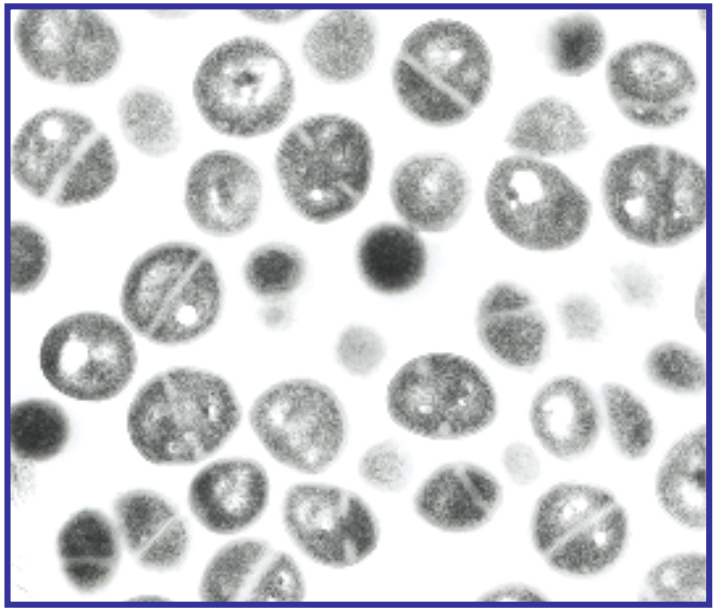
CZX1

Results ①

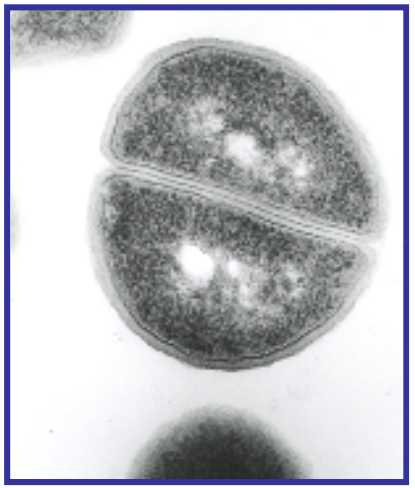
Culture conditions	A_{578}
Drug free	0.228
CZX	0.206

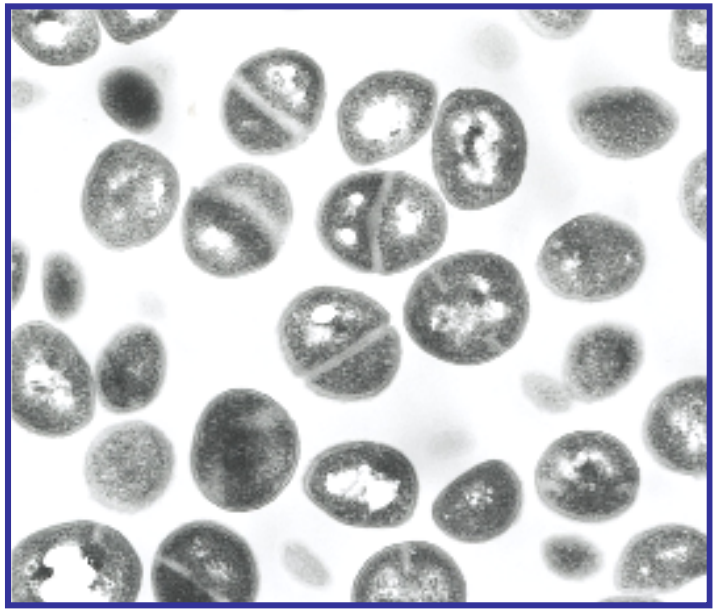
Drug free





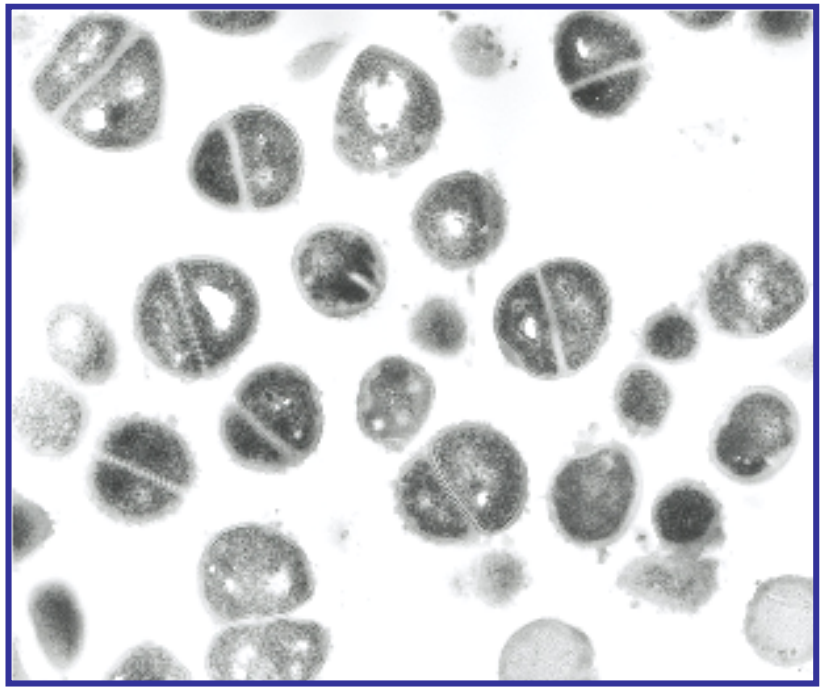
CZX

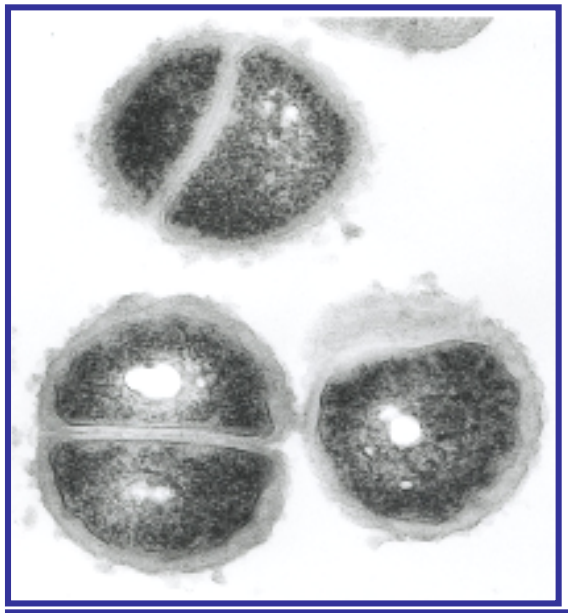


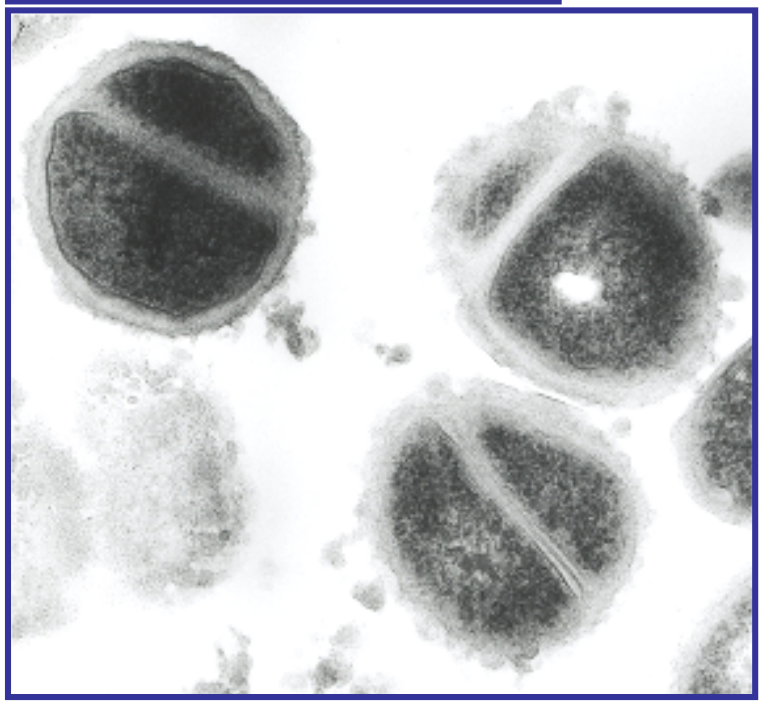


Results ②

～ VCM ～



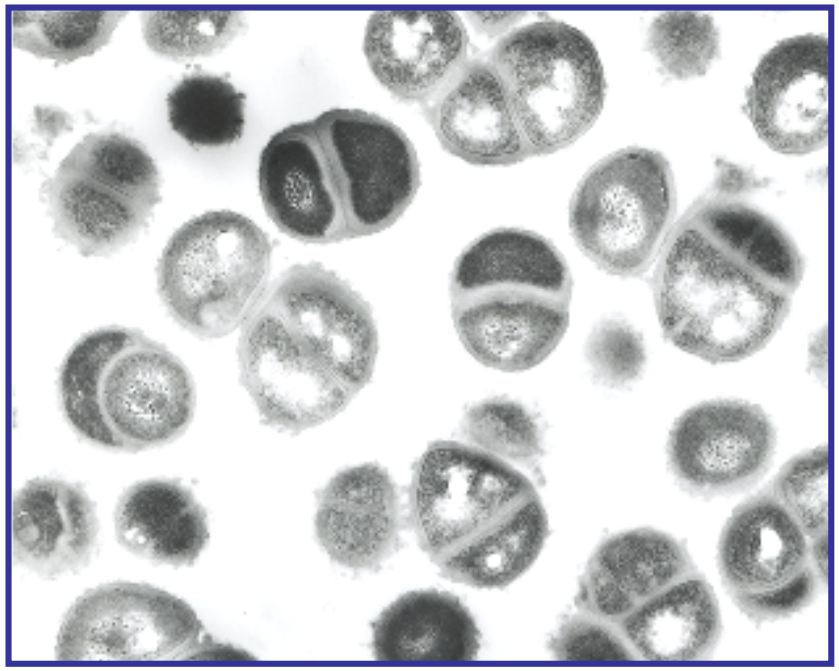


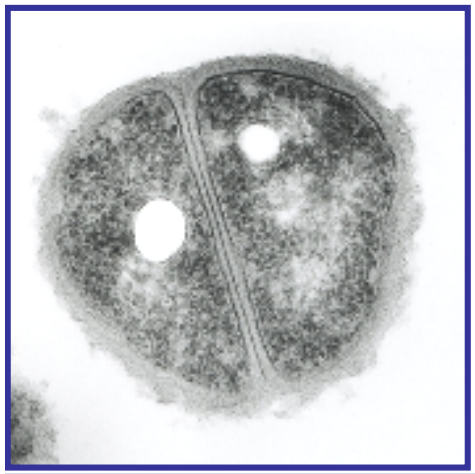


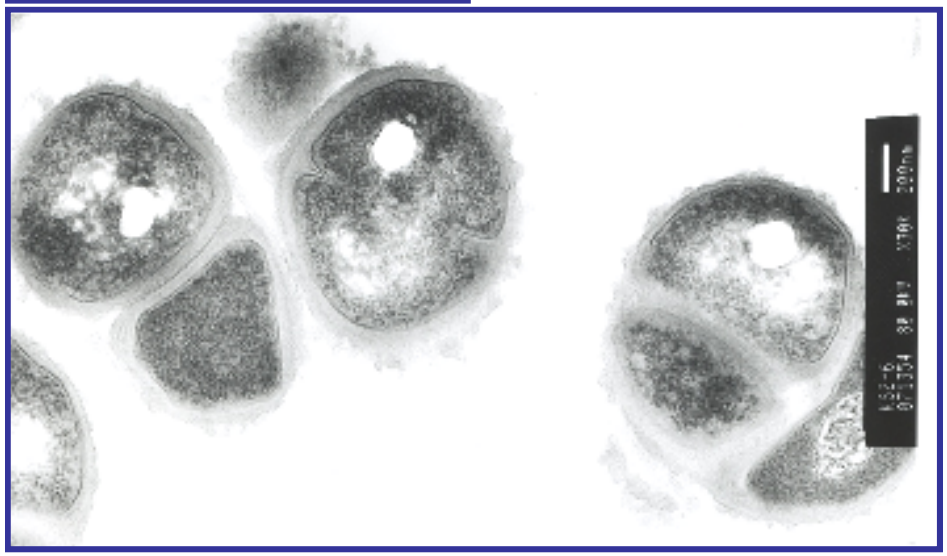
Culture conditions	A_{578}
VCM	0.210

Results ③

～ VCM+CZX ～



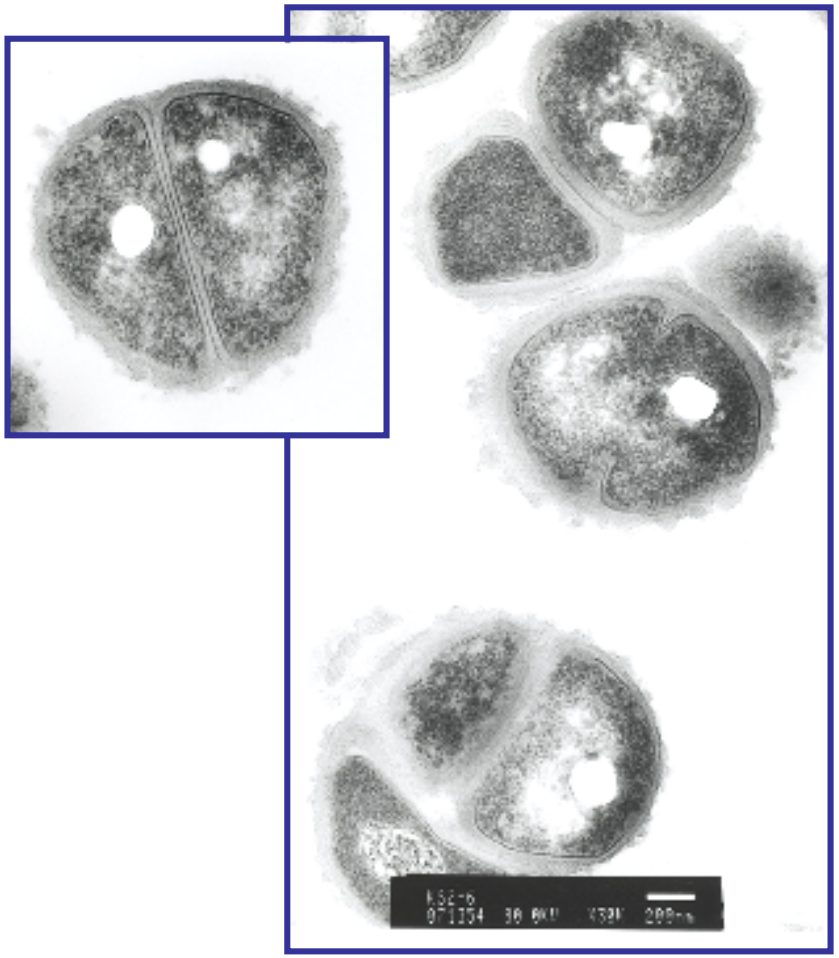




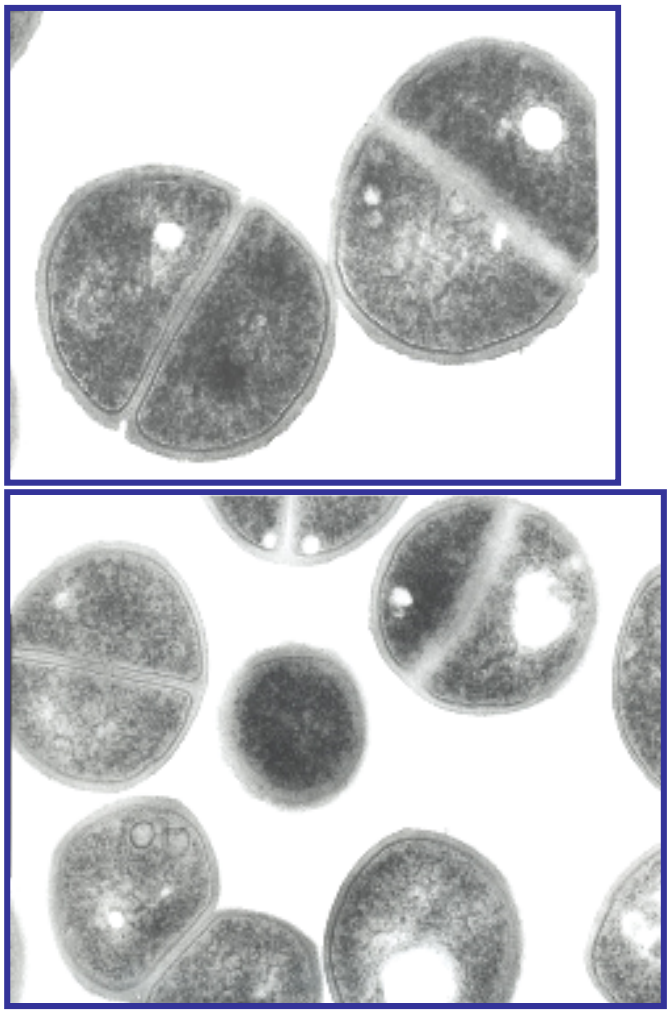
Culture conditions	A_{578}
VCM+CZX	0.202

Cells grown in the presence of VCM plus CZX

VCM+CZX (A_{578}=0.202)

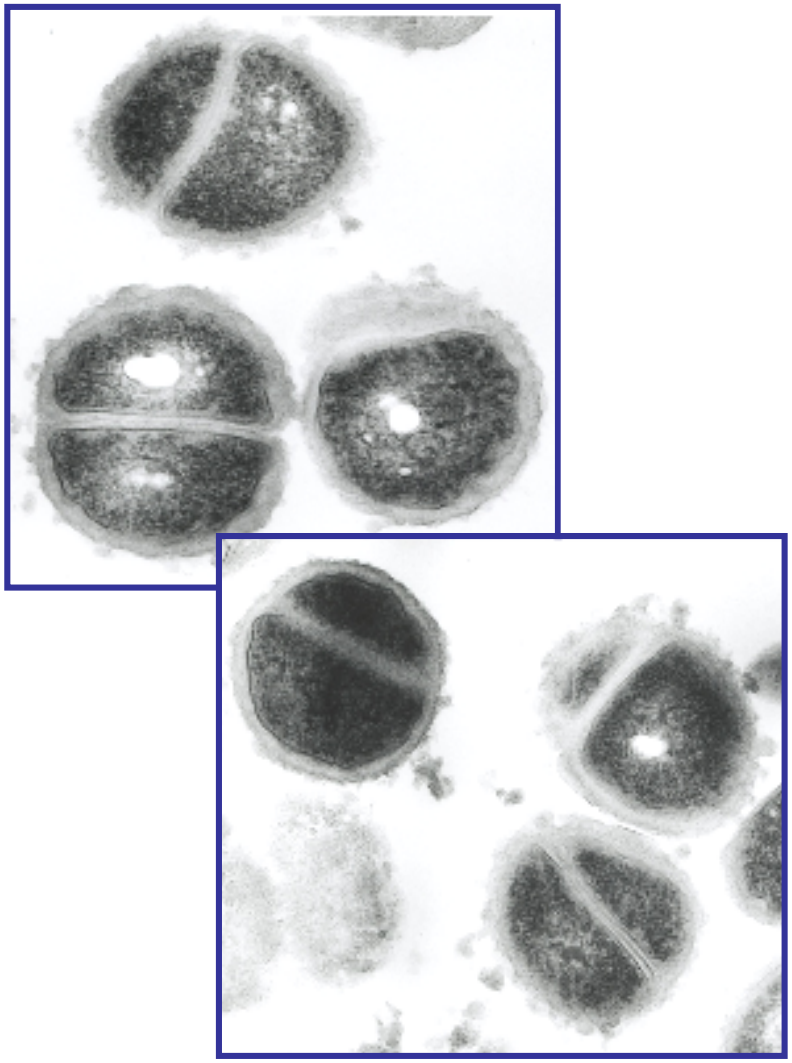


VCM+CZX (A_{578}=1.312)

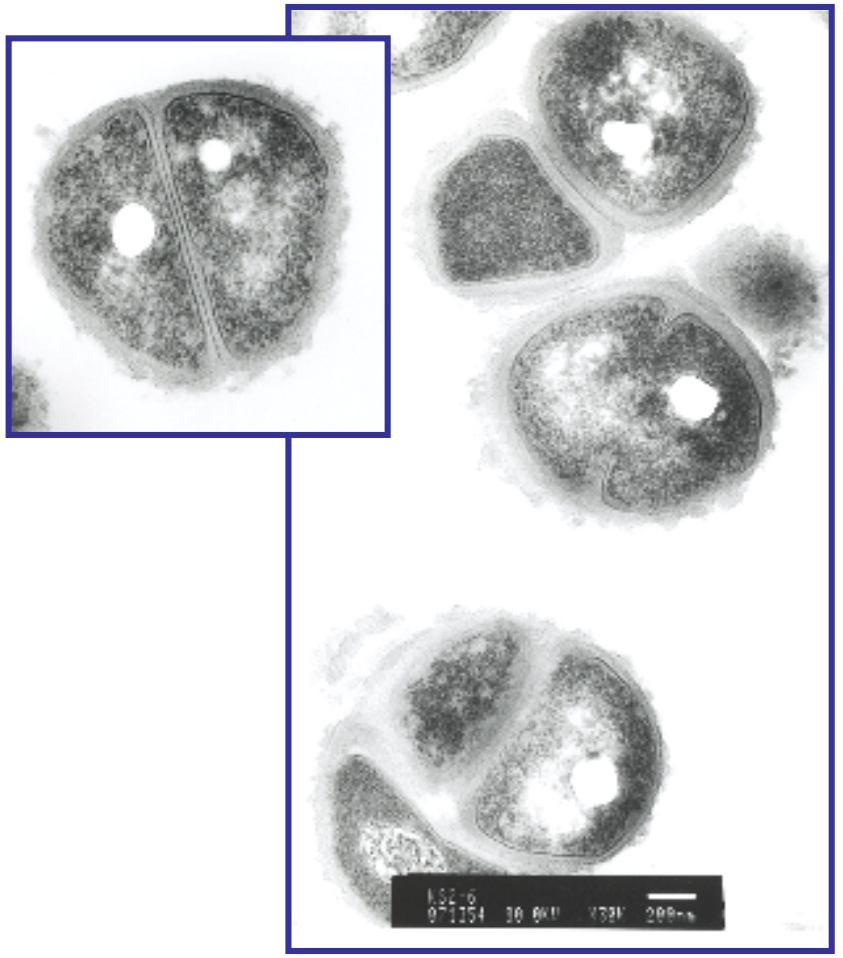


Comparison of cells grown with VCM alone and those with VCM plus CZX

VCM (A_{578}=0.210)



VCM+CZX (A_{578}=0.202)



Summary

*Cell wall thickening under the exposure to VCM was confirmed.

*The non-growing cells in the broth containing VCM with or without CZX exhibited thickened cell wall, while the same cells grown to A_{578} over 1.0 showed normal cell wall thickness.

* The thickness of cell wall in both cells grown in the presence of VCM with or without CZX were almost same degree.

* It is hard to regard cell wall thickness as the VCM-resistant mechanism of BIVR.

Future plan

* Determination of the VCM binding site by immunoelectron microscope using gold-colloid.