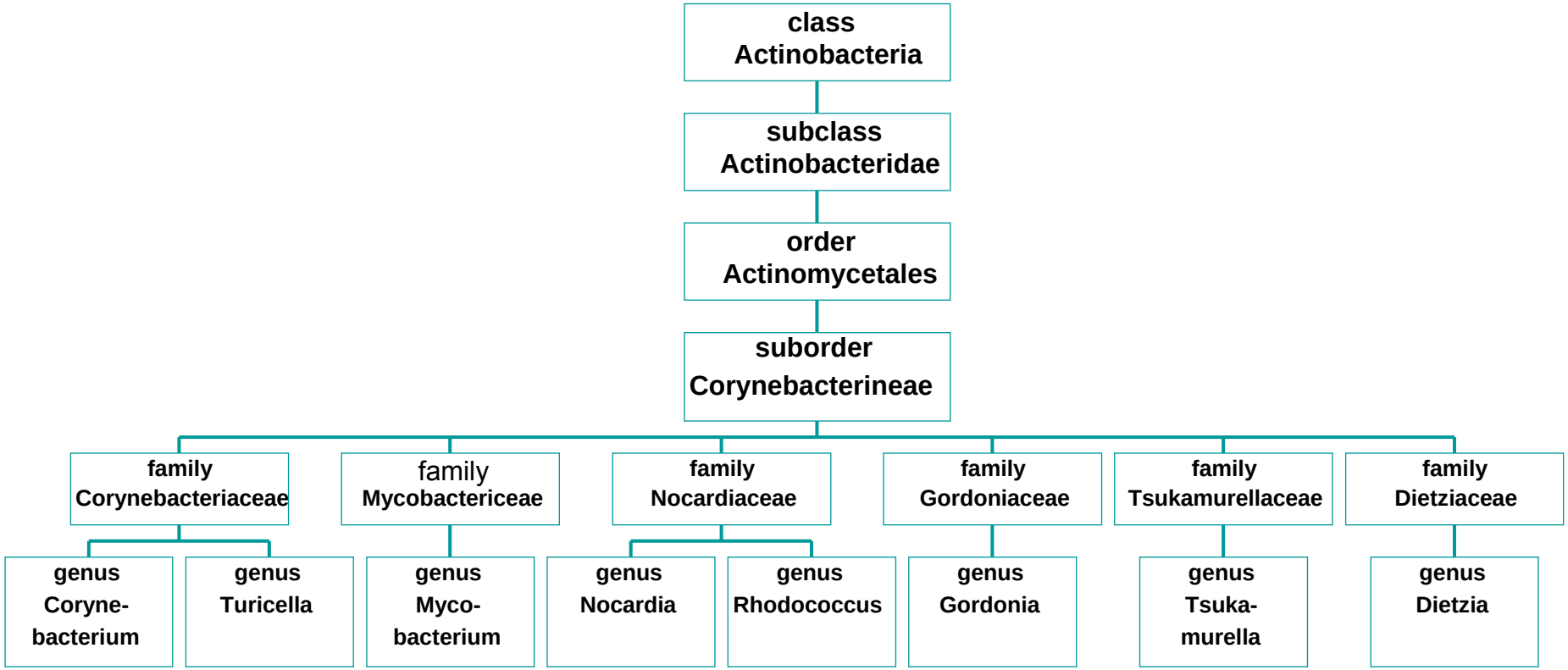
Chronology of published investigations of *Corynebacterium glutamicum* in the department of biotechnology (University of Würzburg)

Tobias Knaf

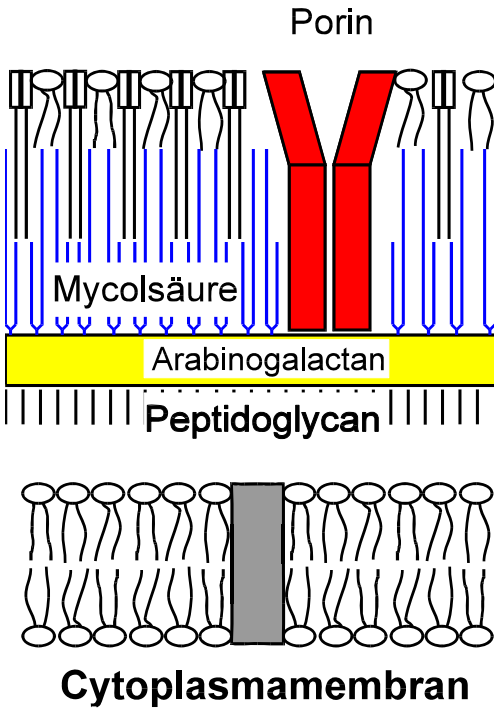
Phylogenetic tree of Actinobacteria



- large amount of lipids in form of mycolic acids in cell wall additional to the thick peptidoglycan layer
- mycolic acids are part of a 2nd bilayer surrounding the peptidoglycan → low permeability

➔ Porins are necessary to allow passage of hydrophilic solutes

Corynebacterineae



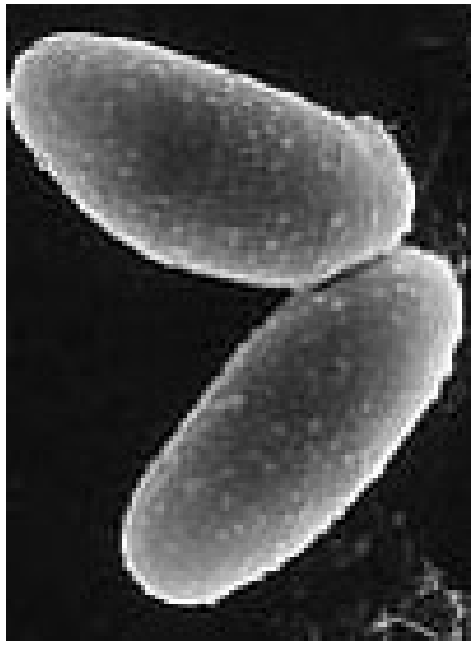
Hünten et al.

- length of mycolic acids varies

Mycobacteria:	60-90 C-atoms
Tsukamurella:	64-74 C-atoms
Gordona:	52-66 C-atoms
Nocardia:	46-58 C-atoms
Corynebacteria:	22-39 C-atoms

- causes dangerous infections

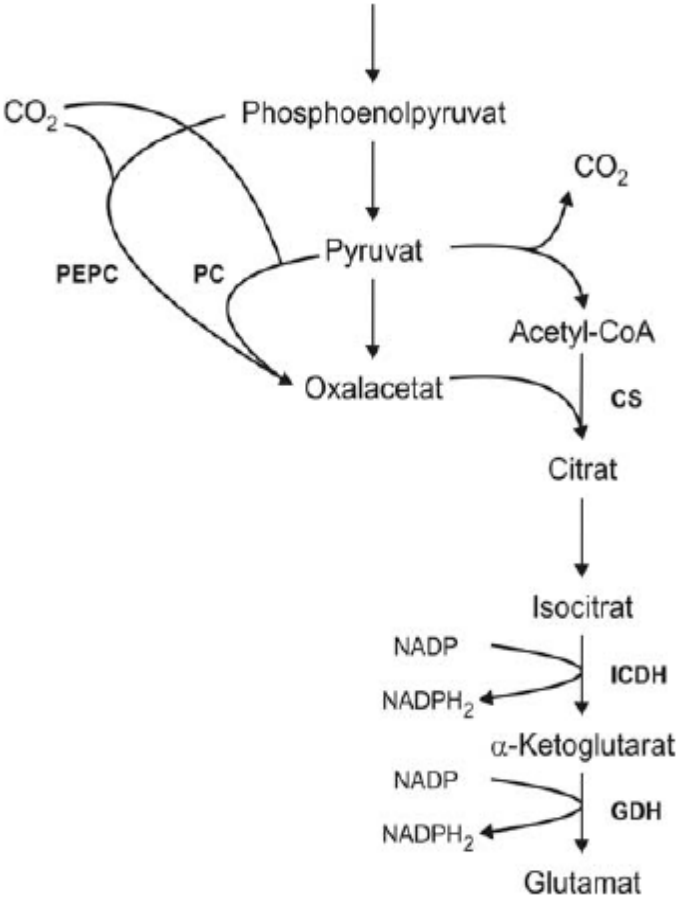
→ *M. tubercolosis*, *M. leprae* and *C. diphteriae*



www.genomenewsnetwork.org

Corynebacteria:
- aerobic, non-sporulating, gram-positive actinomycete
- contain thick peptidoglycan layer covalently bound to arabinogalactan
- mycolic acids linked to arabinogalactan by ester bonds

Synthesis pathway of glutamate in *C. glutamicum*



(dissertation of Kathrin Corinna Stansen, Düsseldorf)

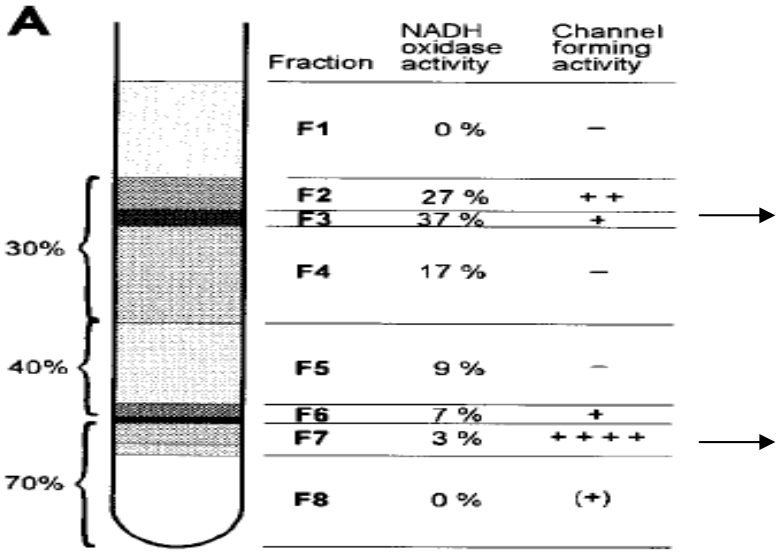
- fermentation of 10^6 tons glutamate and $5{,}5*10^5$ tons L-lysine per year
- glutamate is used as a flavoring agent in food
  L-lysine as an animal food supplement
- glutamate-production by amination of α-ketoglutarate

http://www.chemistry.emory.edu/justice/seminar/images/fig10.03.gif

Identification of Channel-Forming Activity in the Cell Wall of *Corynebacterium glutamicum*

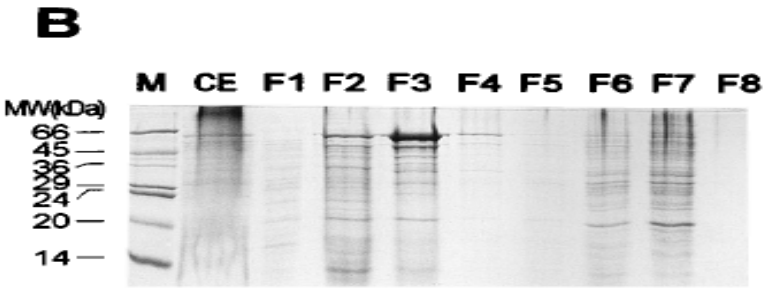
- Niederweis et al, 1995 -

Fractions formed in a sucrose-step gradient of the cell envelope from *C. glutamicum*

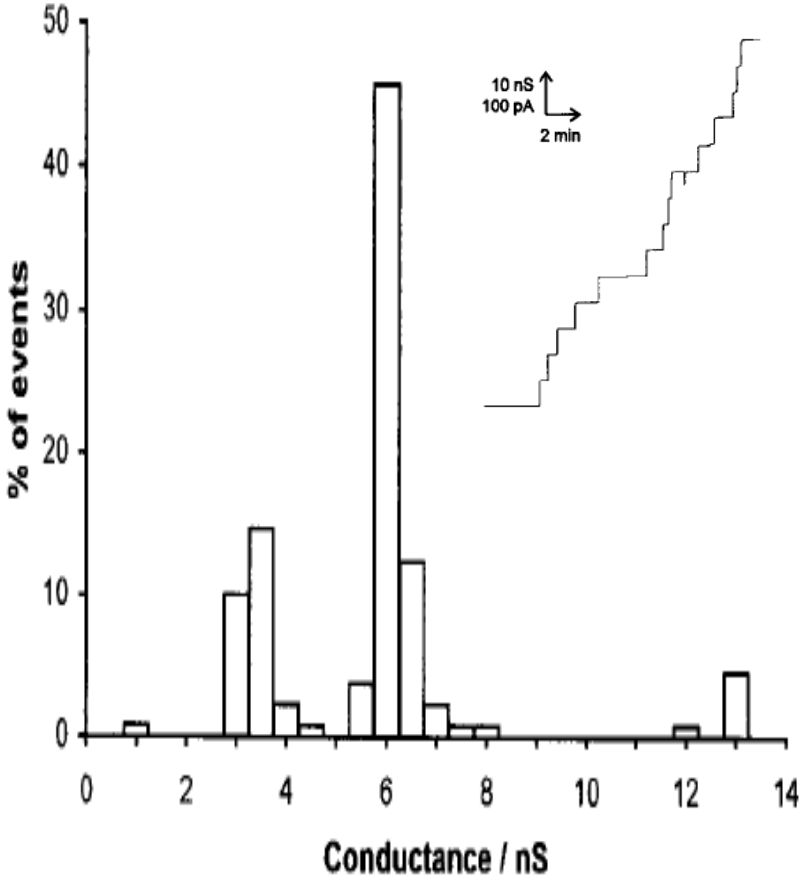


major fraction of the cytoplasmic membrane

highest channel-forming activity;
free of cytoplasmic membrane



Single-channel recording and histogram of fraction F7 of the sucrose-step gradient



- channel-lifetime: several minutes
- G = 6nS in 1M KCl
- zero-current-membran potential: 40mV
- P_{cat}/P_{an}: 9-11

→cation-selectivity

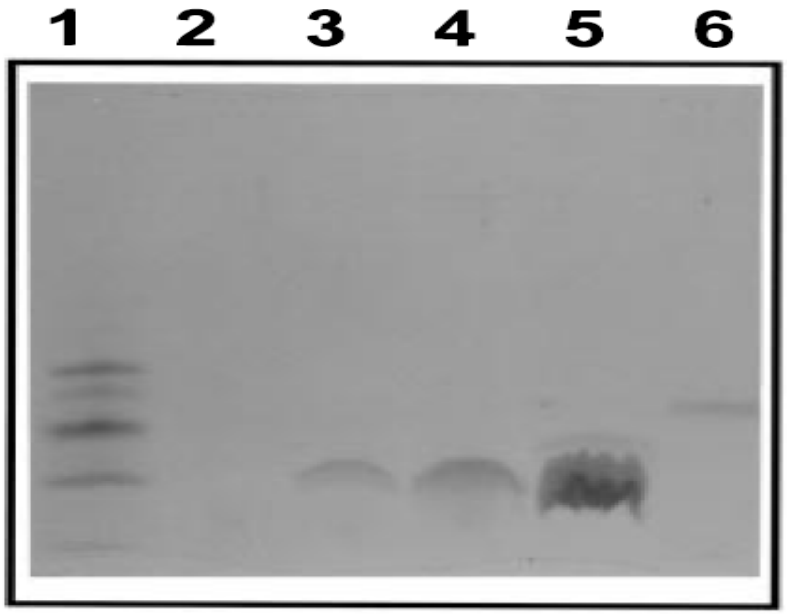
➔existence of a hydrophilic pathway through the mycolic acids

Biochemical and Biophysical Characterization of the Cell Wall Porin of *Corynebacterium glutamicum*: The Channel is Formed by a Low Molecular Mass Polypeptide

- Lichtinger et al, 1998 -

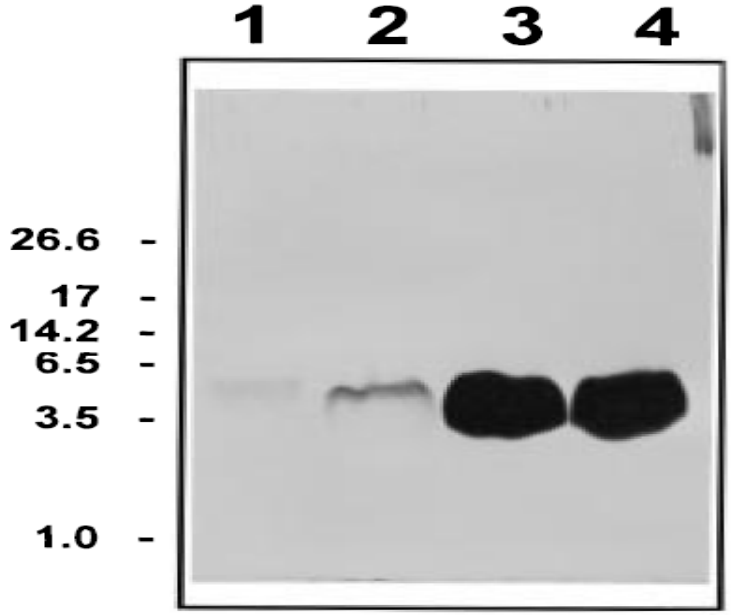
10% tricine containing SDS-PAGEs of the cell wall channel protein of *C. glutamicum*

coomassie



F9 F11 F16 F25

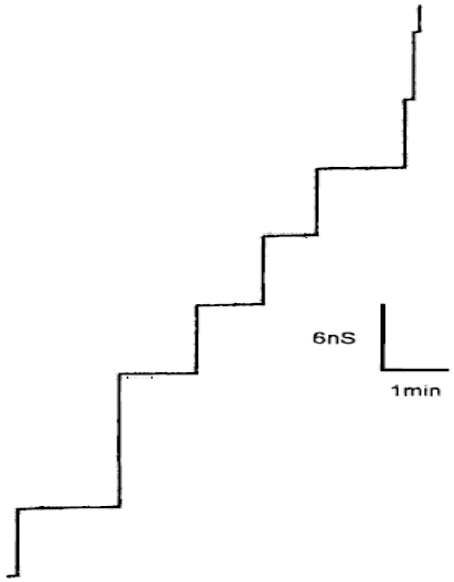
silver stain (purity)



F9 F13 F16 F17

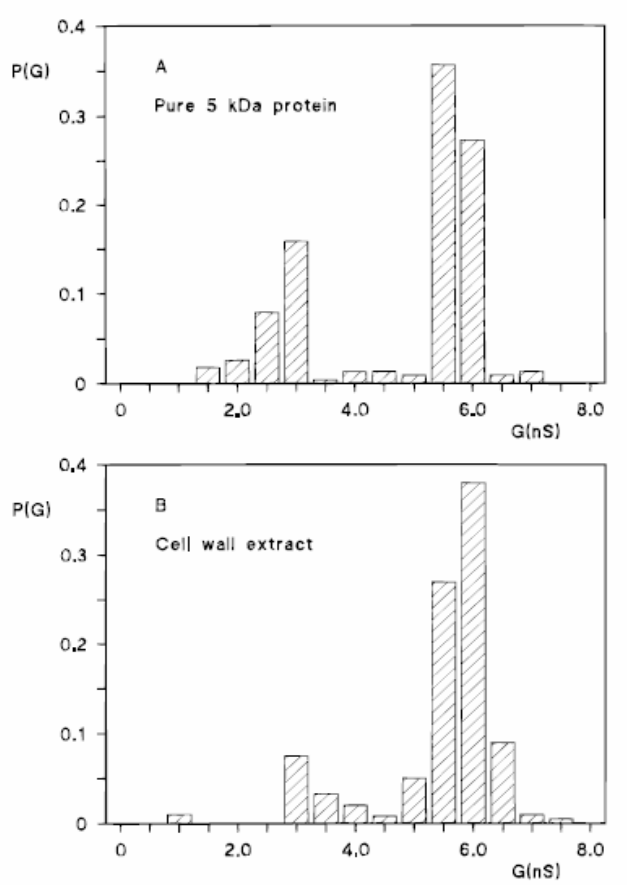
- 5kDa polypeptide with high channel-forming activity
- partial sequencing: 19aa-sequence without homology in the databases

Single-channel recording of pure 5kDa protein of the cell wall



- defined channels of 5.5nS in 1M KCl
- increase of conductance up to 30 min
- Up to 10^6 channel/cm² formed in the membrane

Histogram observed in presence of the cell wall extracts and pure 5kDa protein



- minor fraction of half conductance

→ part of an oligomer or an substate

- identical channels in whole cell extract
- conductance similar to *M. smegmatis/ chelonae*

Average single-channel conductance G of the cell wall channel in different salt solutions

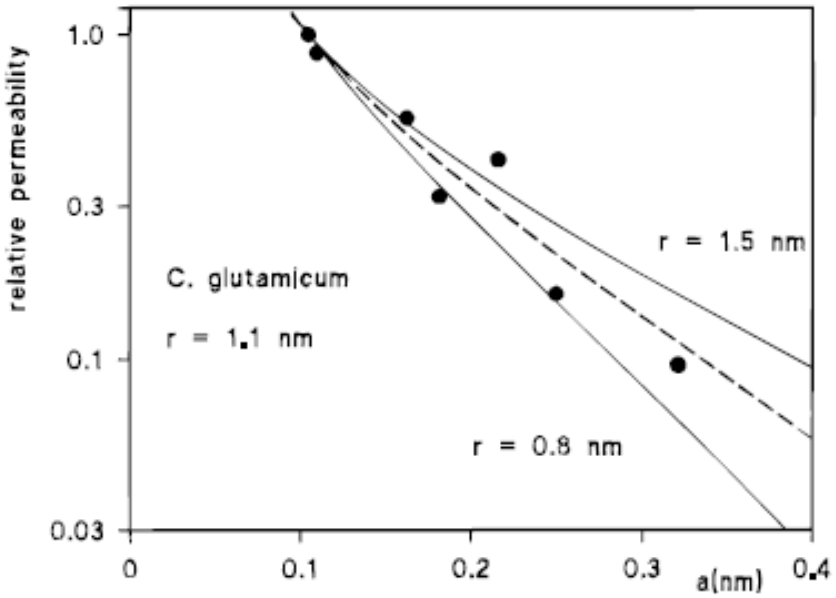
salt	concentration (M)	*G* (nS)
LiCl	1.0	2.6
NaCl	1.0	3.5
KCl	0.03	0.60
	0.10	1.1
	0.3	2.0
	1.0	5.5
	3.0	14.5
KCH_3COO (pH 7)	1.0	4.8
RbCl	1.0	6.3
CsCl	1.0	5.6
NH_4Cl	1.0	5.0
$N(CH_3)_4Cl$	1.0	2.2
$N(C_2H_5)_4Cl$	1.0	1.0
TrisCl	1.0	0.6

- cation-selectivity

- permeability follows the mobility sequence

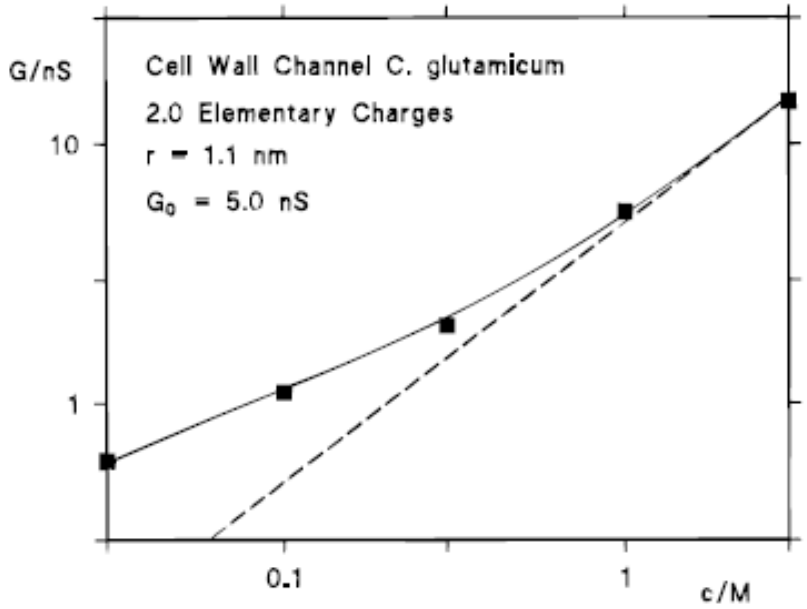
→ $Cs^+ = Rb^+ = K^+ > Na^+ > Li^+ >$ Tris$^+$ and $NH_4^+ > N(CH_3)_4^+ > N(C_2H_5)_4^+$

Fit of the single-channel conductance data by using the Renkin correction factor



→ diameter of the cell wall channel: 2.2nm

Single-channel conductance as a function of the KCl-concentration in the aqueous phase



- no linearity → influence of point net charges near the channel
- cation-selectivity not related to a binding site
- 2 negative point net charges → q = - 3,2 * 10^{-19} As

Zero-current membrane potentials V_m for a 10-fold salt-gradient

salt	V_m (mV)	P_{cation}/P_{anion}
KCl	39	8.1
LiCl	31	4.9
KCH_3COO (pH 7)	43	11.6

- more diluted site became positive → movement of cations
- anions have a certain permeability but decreased

Comparison of cell wall channel properties of *M. chelonae*, *M. smegmatis* and *C. glutamicum*

cell wall channel	*G* (nS) in 1 M KCl	selectivity P_c/P_a in KCl	negative point charges at the channel mouth	channel diameter (nm)	ref
M. chelonae	2.7	14	2.5	2.0	*17*
M. smegmatis	4.1	9.7	4	2.6, 3.0	*18*
C. glutamicum	5.5	8.1	2	2.2	This study

- higher conductance at same diameter caused by cell wall thickness (Ohms law)

summary:

1. channel-forming protein of 5kDa
2. G = 5,5 nS in 1M KCl
3. diameter: 2.2nm; 2 negative charges in or near the channel

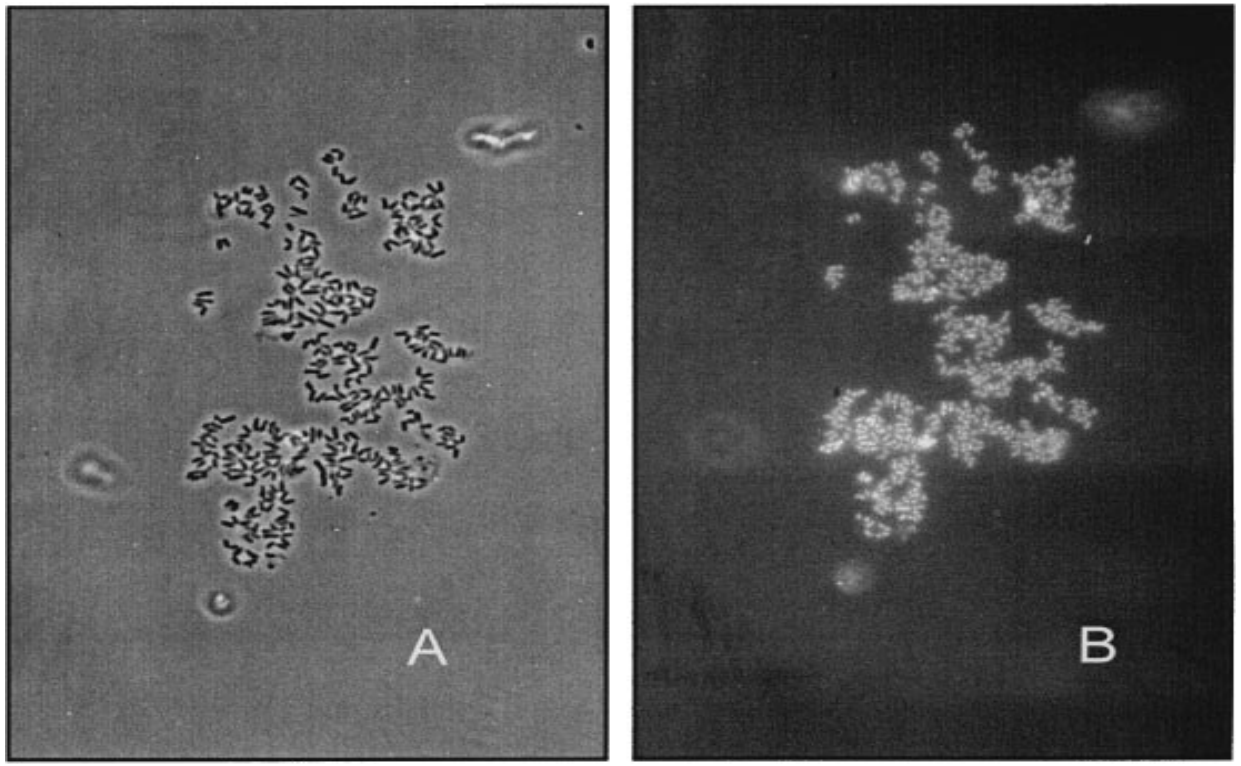
The low-molecular-mass subunit of the cell wall channel of the Gram-positive *Corynebacterium glutamicum*

- Lichtinger et al, 2001 -

Microscopic analysis of *C.glutamicum* cells treated with anti-PorA IgG

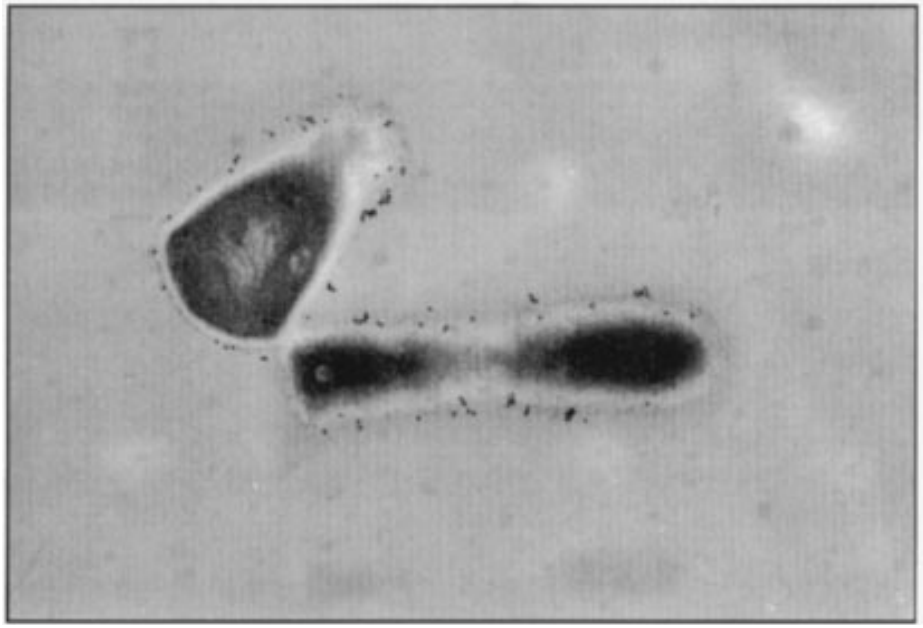
phase-contrast-image
(propidium iodide)

fluorescence-image
(anti-rabbit IgG)



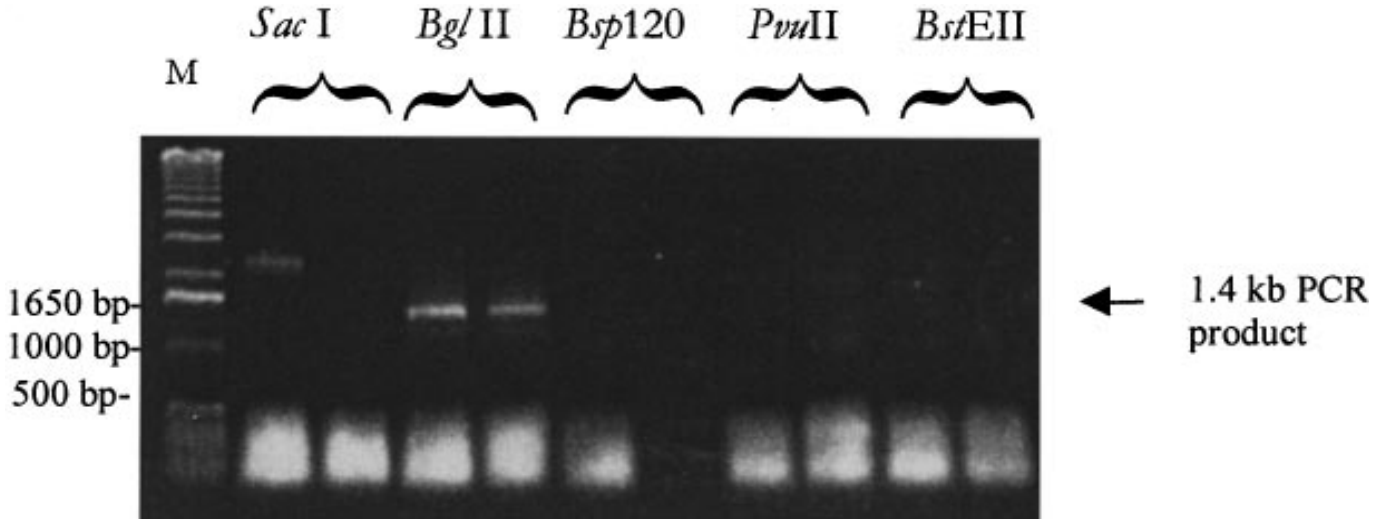
→ PorA localized in the cell envelope

Electron micrograph of *C.glutamicum* cells treated with anti-PorA IgS and anti-rabbit IgG labelled with gold particles



→ PorA localized in the cell envelope

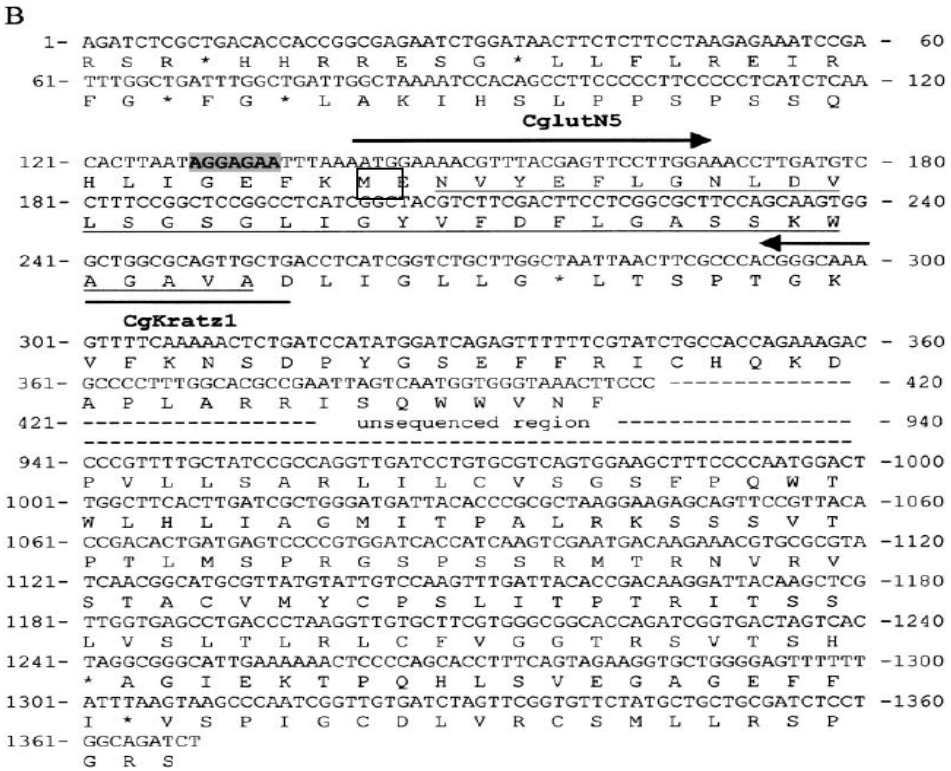
Inverse PCR of chromosomal DNA with different restriction enzymes



- after CNBr-cleavage: determination of a 43aa-polypeptide (4680,3 Da)
- PCR not completely sufficient to reconstruct whole nucleotide sequence
- restriction with Bgl II in inverse PCR: 1,4 kb PCR product

→ Reconstruction by combining the PCR and the sequences of the clones

Nucleotide sequence of the porA gene locus of *C. glutamicum* and its flanking regions



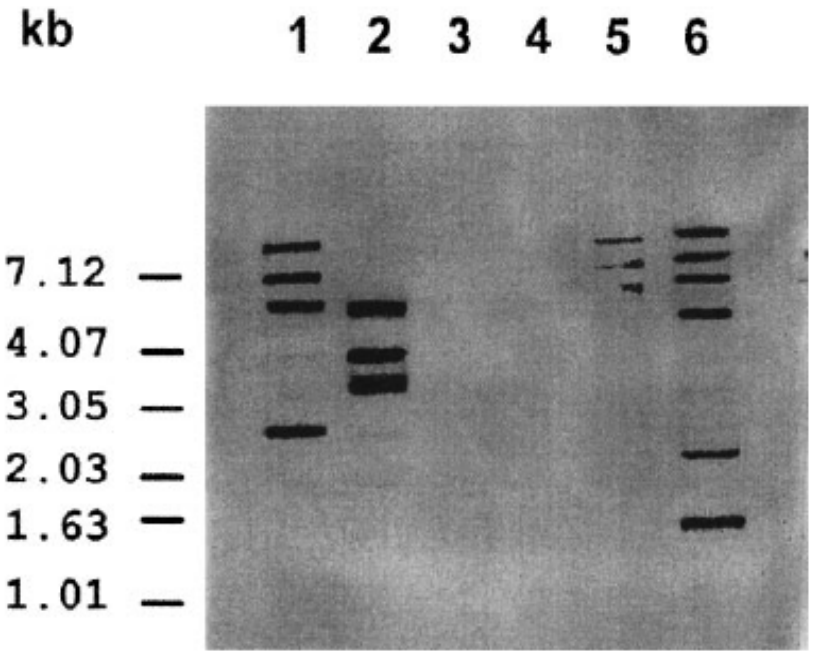
→ primers used for derivation of the DNA sequence

■ ribosome-binding site

___ the 36aa that agree with the aa-sequencing from the protein sequencing

- ORF of 138bp encoding a protein of 45aa
- No N-terminal aa-extension → no export by Sec-system

Southern blot analysis of several members of the mycolata under low stringency conditions (48°C)



1 C. callunae
2 C. glutamicum
3 Rhodococcous erythropolis
4 Nocardia corynebacteroides
5 C. pseudotuberculosis
6 chromosomal DNA

- all bands only under low stringency conditions
→ conserved sequences for porins
- more than one cell-wall channel gene
- No DNA-sequence homology to *porA* of 3 and 4

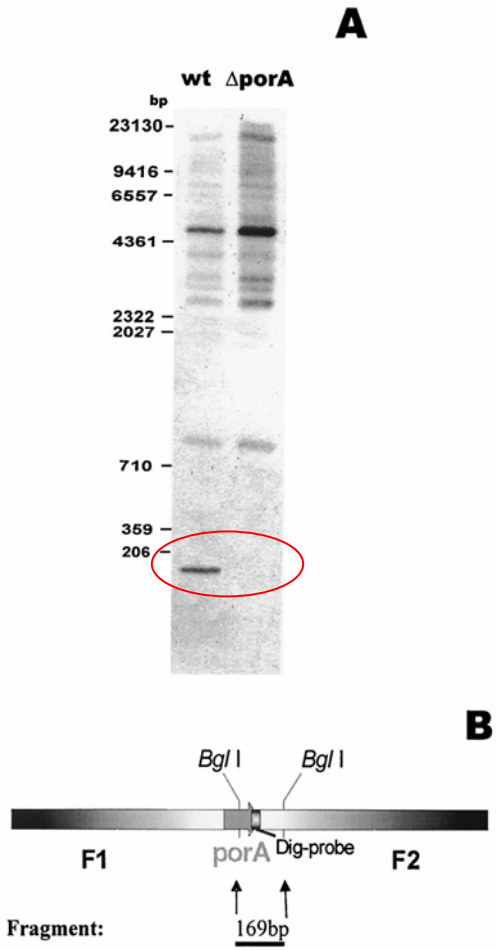
Summary

- the channel-forming protein PorA (5kDa) located in the cell envelope
- gene *porA* comprises 138bp encoding a 45aa-polypeptide
- excess of negative charges → cation-selectivity
- no N-terminal extension → no use of Sec-appartus
- α-helices and ß-strands both possible to span the mycolic acid layer (6.2nm) once as a cylinder (d=2.2nm)

Por A Represents the Major Cell Wall Channel of the Gram-Positive Bacterium *Corynebacterium glutamicum*

- Costa-Riu et al, 2003 -

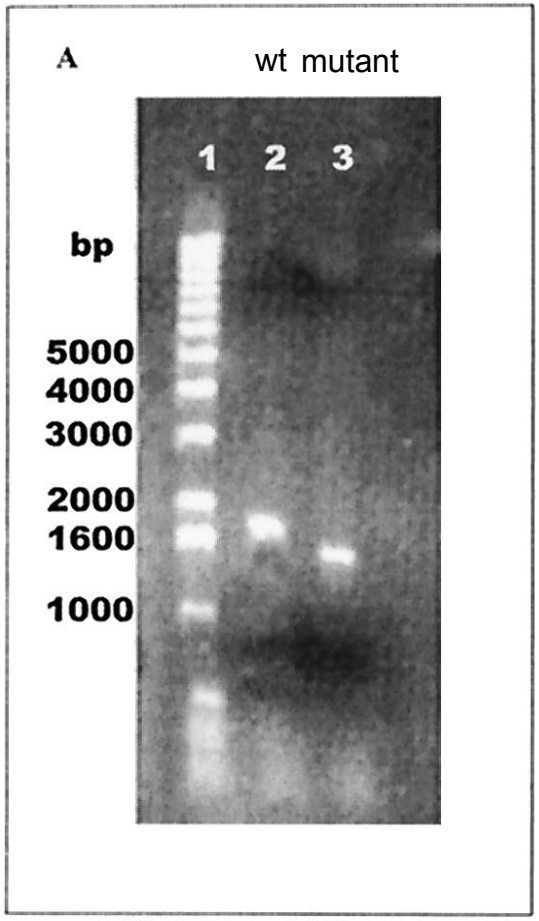
Southern blot analysis of *C. glutamicum* wild-type and ΔporA mutant cells



- release of a 169bp fragment when digested with Bgl I
- the fragment only detected in wild-type *C. glutamicum*

→ *porA* gene not present in ΔporA

0,8% agarose gel from the PCR products obtained by using wt and ΔporA mutant DNA



- deletion of a fragment of about 150bp

→ fragment contains the *porA* gene

Deletion of *porA* within the genome of *C. glutamicum*

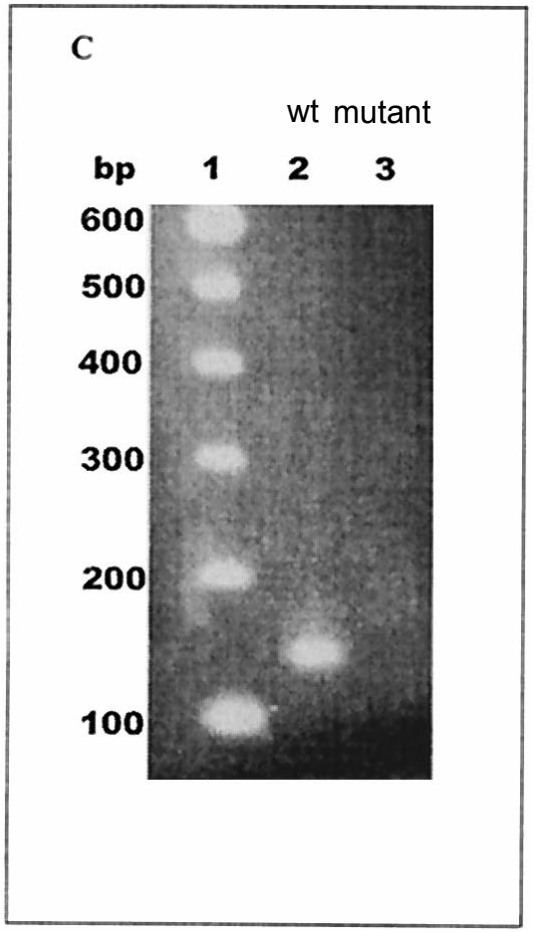
Cgl2658 (*porA*)

cctcatctcaactcttataggagaattaaa**atggaaaacgtttacgagttccttggaaac**
cttgatgtcctttccggctccggcctcatcggctacgtcttcgacttcctcggcgcttcc
agcaagtgggctggcgcagttgctgacctcatcggtctgcttggctaattaacttcgccca

- deletion of 30bp before start codon and 13bp after stop codon
- no ORF before or after *porA* was found

→ only deletion of *porA* responsible for observed phenotype

RT-PCR of total mRNA from wt *C.glutamicum* and the ΔporA mutant strain



- amplification with porA-specific primers Por1 and Por2
- only one gene coding for PorA in the C.glut-chromosome
- remember: *Mycobacterium smegmatis* contains 4 genes coding for MspA like proteins

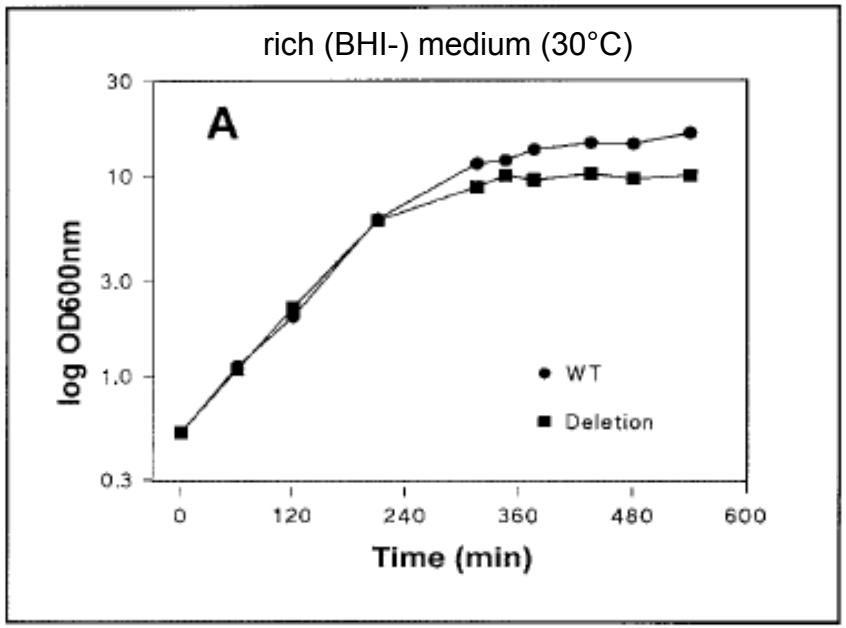
Diameter of the inhibition zones of growth of *C. glutamicum* wild-type and ΔporA mutant

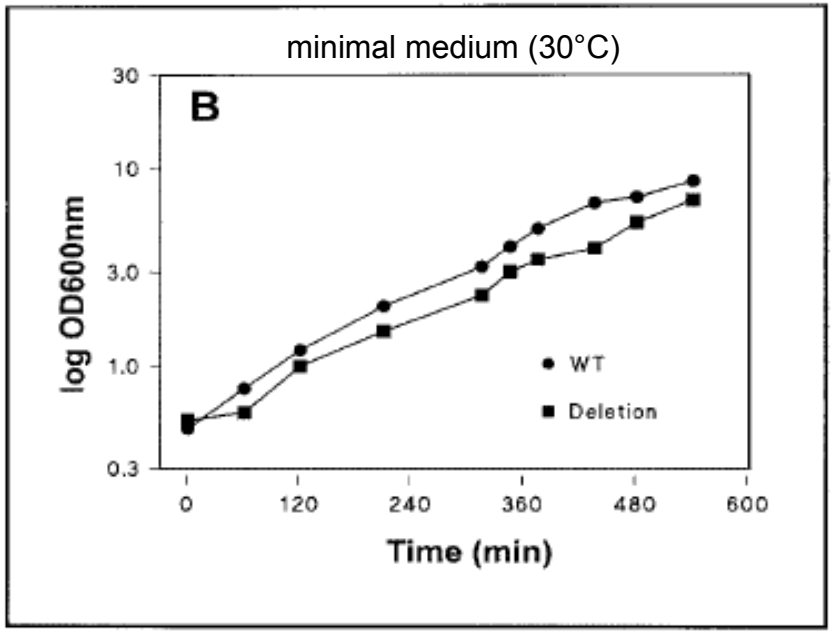
Antibiotic	Diam of inhibition zone (mm)	
	C. glutamicum wild type	*C. glutamicum* Δ*porA* mutant
Ampicillin	>25	NI
Kanamycin	>25	5
Streptomycin	>25	4
Tetracycline	>25	NI
Gentamicin	5	2

- increase of antibiotical resistance in the mutant
- major role of PorA in transport mechanisms of antibiotics

→ caused by large diameter; preference for positively charged solutes

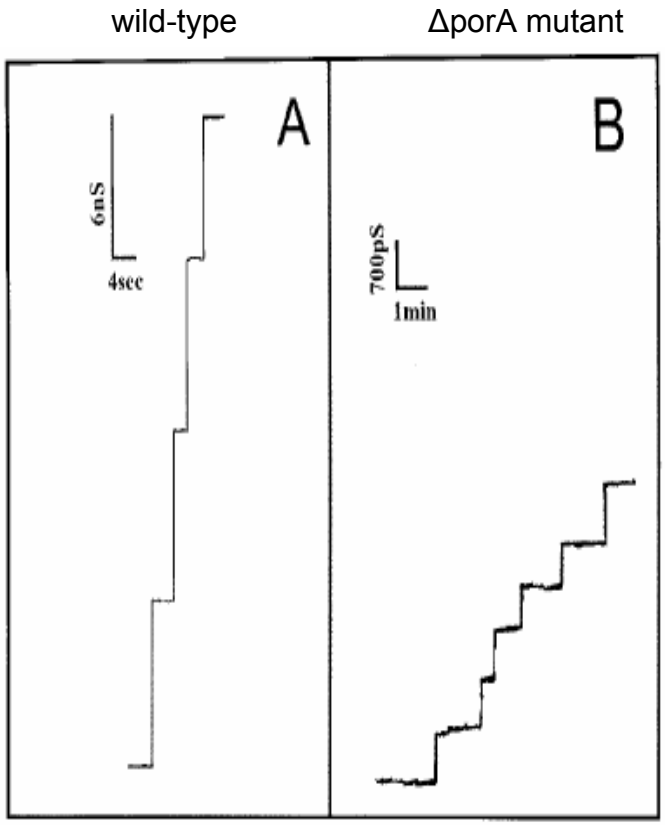
Growth curves for wild-type and ΔporA mutant of *C. glutamicum*





- high nutrient concentrations/ low cell densities: diffusion sufficient for growth
- minimal media: decreased mutant strain growth by decreased nutrient influx
- glutamate production: no (permeability) difference for export of glutamate (negative)

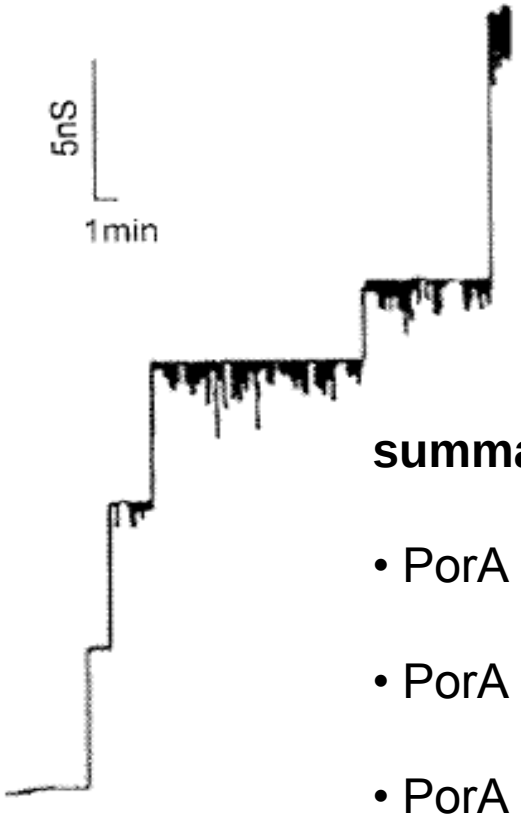
Single-channel recordings in the presence of wild-type and ΔporA mutant cells



- G (wt) = 5,5nS in 1M KCl
- G (mutant) = 0,7nS in 1M KCl
- 0,7nS-channel anion-selective

→ explains why deletion of PorA is not lethal

Single-channel recording in the presence of synthetic PorA



- same channel as in the wild-type

→ PorA alone responsible for the 5,5nS-channel in 1M KCl

summary:

- PorA is major cell wall channel, but also channels not related to PorA
- PorA responsible for transport of antibiotics across the cell wall
- PorA not important for glutamate production

Identification of an anion-specific channel in the cell wall of the Gram-positive bacterium *Corynebacterium glutamicum*

- Costa-Riu et al, 2003 -

Influences of different carbon sources of the growth parameters of wt and ΔporA mutant

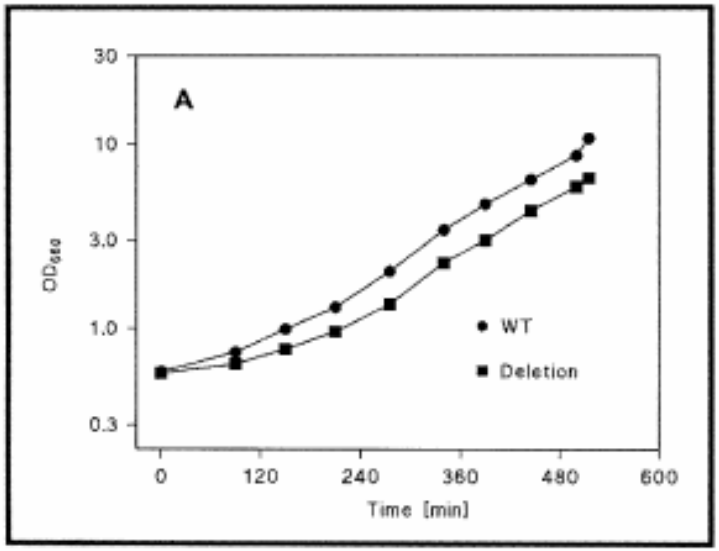
Carbon source	Doubling time (hours)		Final OD_{660}	
	Wild-type	*ΔporA* mutant	Wild-type	*ΔporA* mutant
Glucose	1.7	1.8	33	27
Maltose	2.0	1.8	31	29
Sucrose	1.7	1.6	28	27
Ribose	2.3	2.3	24	23
Pyruvate	2.5	2.3	14	11
Lactate	5.7	4.5	8	9
Citrate	3.9	>7	13	2.5

- neutral or negatively charged carbon sources: no change of growth
- only citrate points out differences

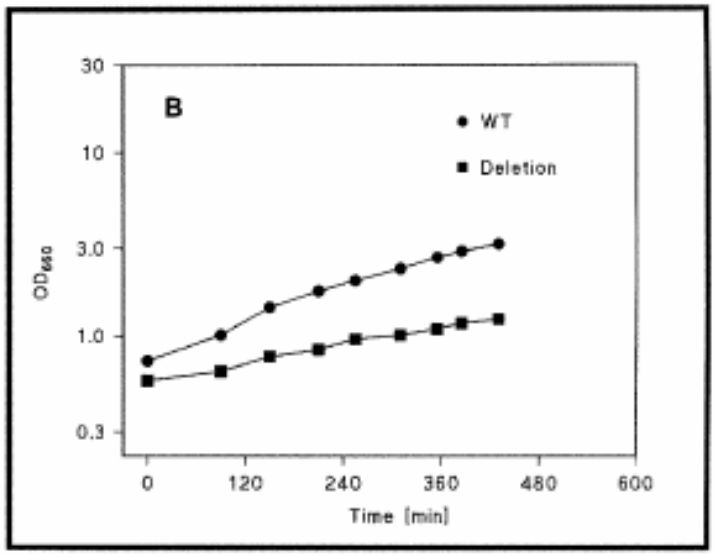
→ mutant strain shows permeabilities: existence of other channels

Growth curves of wild-type *Corynebacterium glutamicum* and the ΔporA mutant

minimal medium with 2,5% glucose



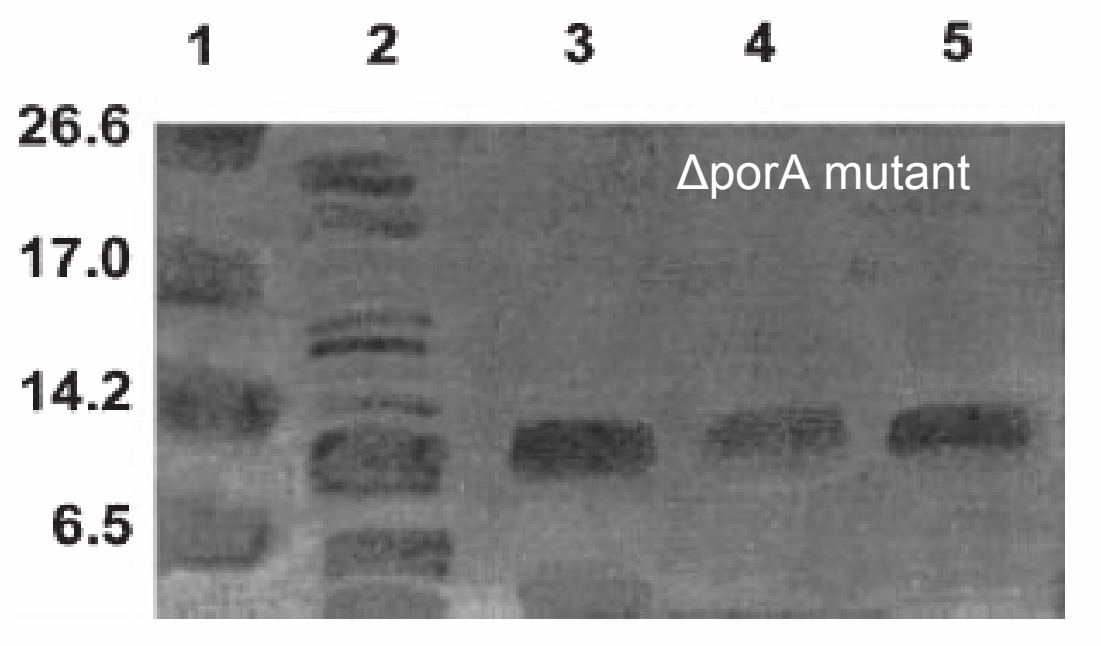
minimal medium with 2,5% citrate



- decreased growth rate with citrate as sole carbon source
- mutant strain still growth

→ existence of other cell wall channels

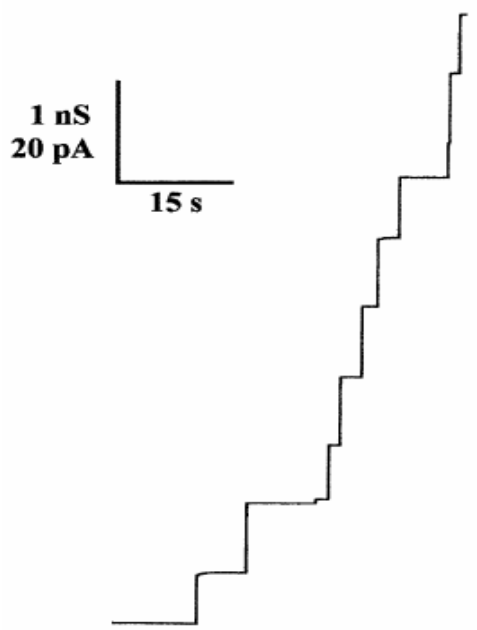
10% Tricine SDS-PAGE of the purification procedure of PorB

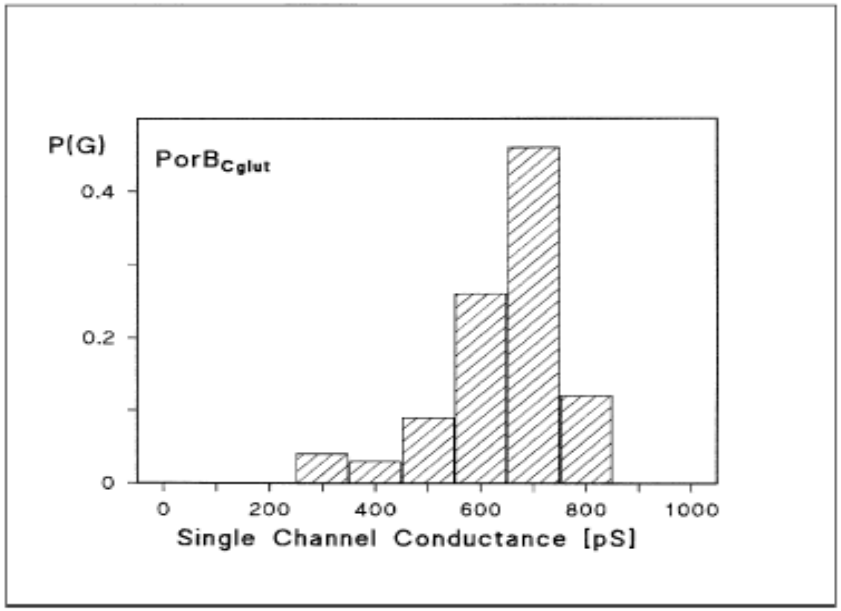


1 marker
2 organic-solvent-precipitate
3 FPLC-fraction 23
4 FPLC-fraction 21
5 FPLC-fraction 25

→pure highly-active 10kDa-protein in fraction 23 after extraction, precipitation and purification of the ΔporA mutant

Single-channel recording and histogram in thes presence of the pure 10kDa protein ($PorB_{Cglut}$)





- defined channels with G = 700pS in 1M KCl
- increase of conductance up to 20min
- long lifetime of channels (similar to *Mycobacterium chelonae* and *M. smegmatis*)

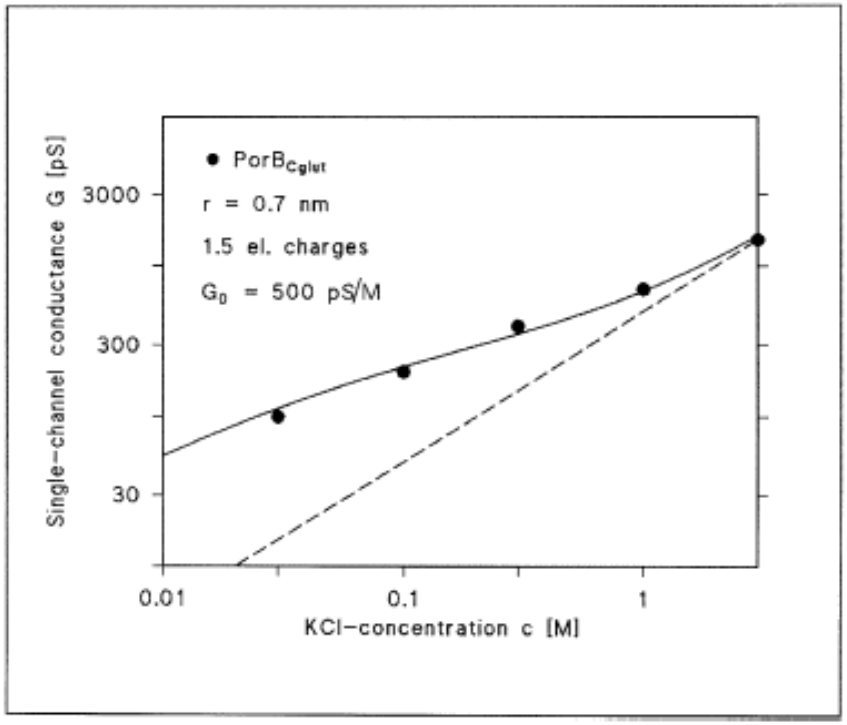
Average single-channel conductance G of $PorB_{Cglut}$ in different salt solutions

Salt	Concentration (M)	Single-channel conductance G (pS)
LiCl	0.1	200
	1.0	700
KCl	0.03	100
	0.1	200
	0.3	400
	1.0	700
	3.0	1500
KCH_3COO (pH 7)	0.1	100
	1.0	250

more influence of anions if exchanged

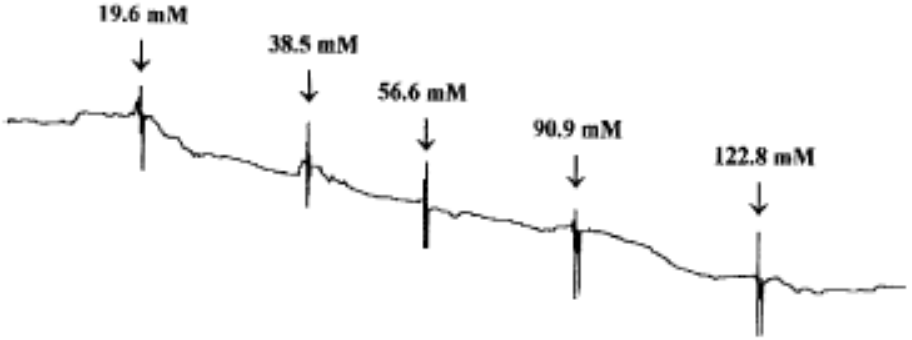
→ anion-selectivity

Single-channel conductance of $PorB_{Cglut}$ vs. the KCl concentration in the aqueous phase

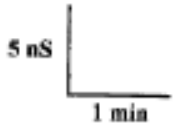


- no linearity → 1,5 positive charges near or in the channel
- diameter of about 1,4nm

Titration of membran conductance induced by $PorB_{Cglut}$ with sodium citrate (pH 6)



→ addition of sodium citrate solutions



- dose-dependent block of conductance

→ binding-site for citrate possible

→ conductance of the 700ps-channel depends on presence of citrate

Zero-current membrane potentials V_m for a 10-fold salt-gradient

Salt	Zero-current membrane potential V_m [mV]	P_{cation}/P_{anion}
KCl	–30	0.12
LiCl	–28	0.14
KCH_3COO (pH 7)	–29	0.13

- more diluted site becomes negativ → pass of anions → anion-selectivity
- V_m: about -29mV
- P_{cation}/P_{anion}: cations have still certain permeability

Aa-sequence of $PorB_{Cglut}$ compared with $PorC_{Cglut}$ and 2 homologues from *C.efficiens*

```
                signal sequence                 -       +-          *   -   +     --    +
PorB_Cg   MKLSHRIAAMAATAGITVAAFAAP-ASASDFANLSSTNKELSPQYNWVACGILEGGLKAAGVLEEGQYNR
PorB_Ce   MKISTRVAAIGAAAALGLTAFAGP-ASA------VSSSDELSDRFDWVGCPIVEASLAFYGLPEEGMRNN
PorC_Cg    MKKLRFATIAAATV-ALTASLTPSASA-------QDFNQIIDNFD---CGILQTAIYTTGLAHENSTRS
PorC_Ce    MNLRRTLAVAAASVMALTATIAP-AQA-QNADIVSGINNLIDTFD---CDLLRTGLTQTGLVTPETTRS

                -   -     + -             -   --       -+    *    + -           -
PorB_Cg   ELAEAIAAKGEGFWTTQFPQIGDWNEDQAAALADRAQTCGLVKAD------TYLSELSSNFSS
PorB_Ce   QLAAALEEKNANF-AAYFEGGGDWNAQASADYADRAQKCGIVEPN------TAIENASSNLNDFFAGLSS
PorC_Cg   ELAANL--RNSAAVGQLDFPLNIAATGYSERIANRALTCGIVKEDP-QDFLSQLQLLSSNLSSSFFTA
PorC_Ce   ELAATL--RTTANLGEIDVAFAFVGSAYAGRIADRAQTCGIVQPDPEQDILTQLQNLSSNLSS
```

In bold: conserved in at least 3 of the 4 homologues

Identity

PorB_Cg : PorC_Cg: 30,9%
PorB_Ce : PorC_Ce: 31,3%
PorB_Cg : PorB_Ce: 42,1%
PorC_Cg : PorC_Ce: 48,1%

Charges in the mature protein

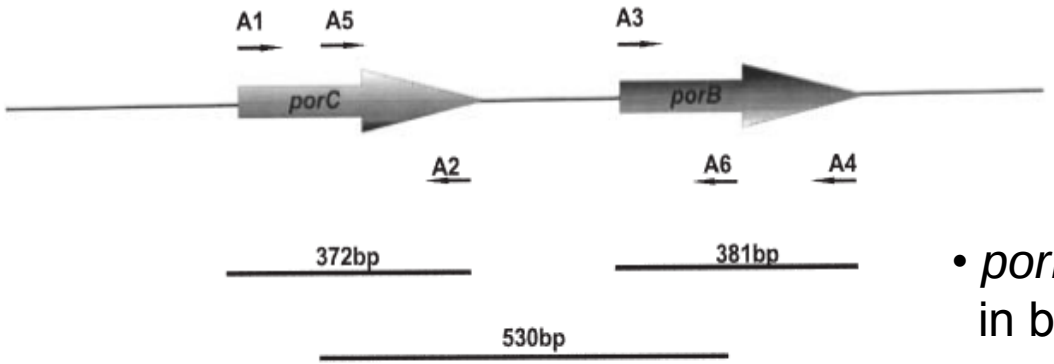
PorB_Cg: 6pos 14neg
PorB_Ce: 5pos 16neg
PorC_Cg: 5pos 14neg
PorC_Ce: 5pos 12neg

- partial sequencing and blast: 126aa long *porB*$_{Cglut}$ and PorB-like protein named $PorC_{Cglut}$
- *porB*$_{Cglut}$ and *porC*$_{Cglut}$ contain N-terminal extension → Sec-apparatus

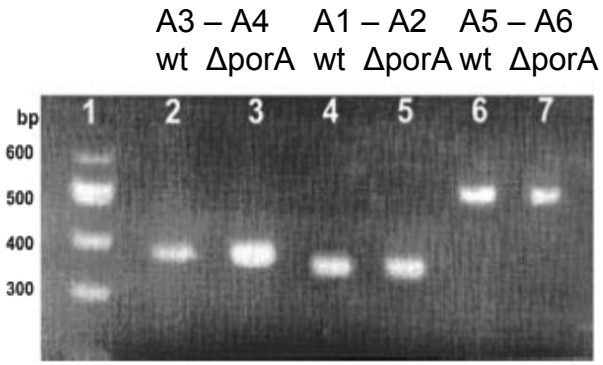
→both genes could be cotranscribed (no transcription terminator between)

porB-porC locus and reverse transcription of total wild-type and ΔporA mutant mRNA

A



B



- $porB_{Cglut}$ and $PorC_{Cglut}$ are transcribed in both strains

→ both form a cotranscriptional unit

Properties of cell wall channels from the mycolata

Cell wall porin	G [nS] in 1 M KCl	Selectivity P_c/P_a in KCl	Charges at the channel mouth	Channel diameter (nm)	Reference
C. glutamicum $PorB_{Cglut}$	0.7	0.12	+1.5	1.4	This work
C. glutamicum $PorA_{Cglut}$	5.50	8.10	−2.0	2.2 nm[1,2]	Lichtinger *et al.* (1998)
M. chelonae	2.7	6.3	−2.5	2.0 nm 2.2 nm	Trias and Benz (1992); (1993)
M. phlei	4.5	14.9	−2.2	1.8 nm[1]; 2.0 nm[2]	Rieß *et al.* (2001)
M. smegmatis	4.1	9.7	−4.0	1.8 nm[1]3.0 nm[2]	Trias and Benz (1994)
N. corynebacteroides (*R. corynebacteroides*)	5.50	3.80	−2.7	2.0 nm[1], 2.2 nm[2]	Rieß and Benz (2000)
N. farcinica	3.0	8.2	−1.3	1.4 nm[1], 1.6 nm[2]	Rieß *et al.* (1998)
R. erythropolis	6.00	11.80	−2.7	2.0 nm[1,2]	Lichtinger *et al.* (2000)
R. equi					
$PorA_{Req}$	4.00	9.0	−1.5	1.8 nm[1], 2.0 nm[2]	Rieß *et al.* (2003)
$PorB_{Req}$	0.30	0.16	+1.5	1.4 nm[1,2]	
M. bovis BCG					
$PorA_{Mbo}$	4.30	>1	ND	ND	Lichtinger *et al.* (1999)
$PorB_{Mbo}$	0.78	<1	ND	ND	

Summary:

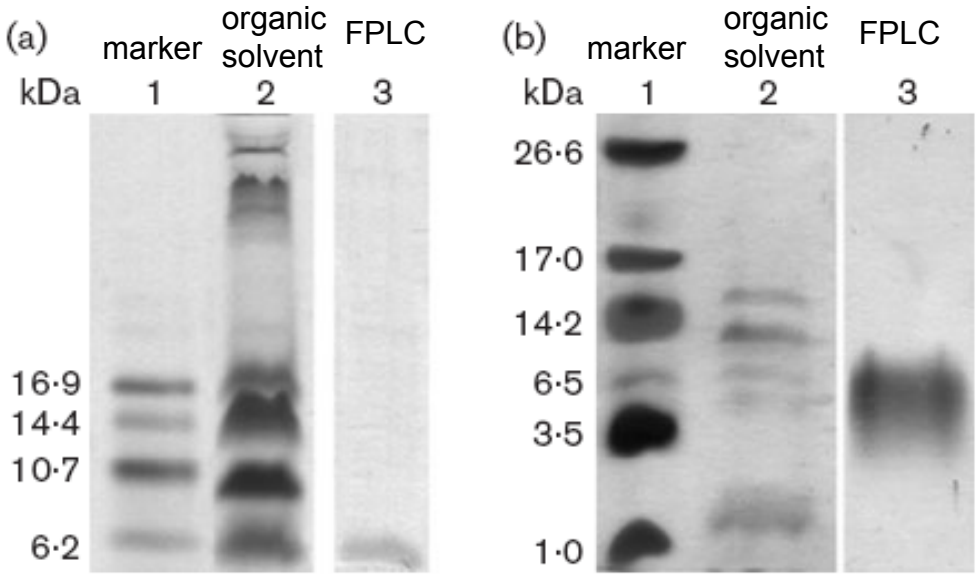
- $PorB_{Cglut}$ and $PorC_{Cglut}$ cotransribed → use of Sec-system

- small proteins (10kDa) of *C. glutamicum* in contrast to MspA of M. smegmatis (20kDA) results of the cell wall thickness and the length of the mycolic acids

PorH, a new channel-forming protein present in the cell wall of *Corynebacterium efficiens* and *Corynebacterium callunae*

- Hünten et al, 2005 -

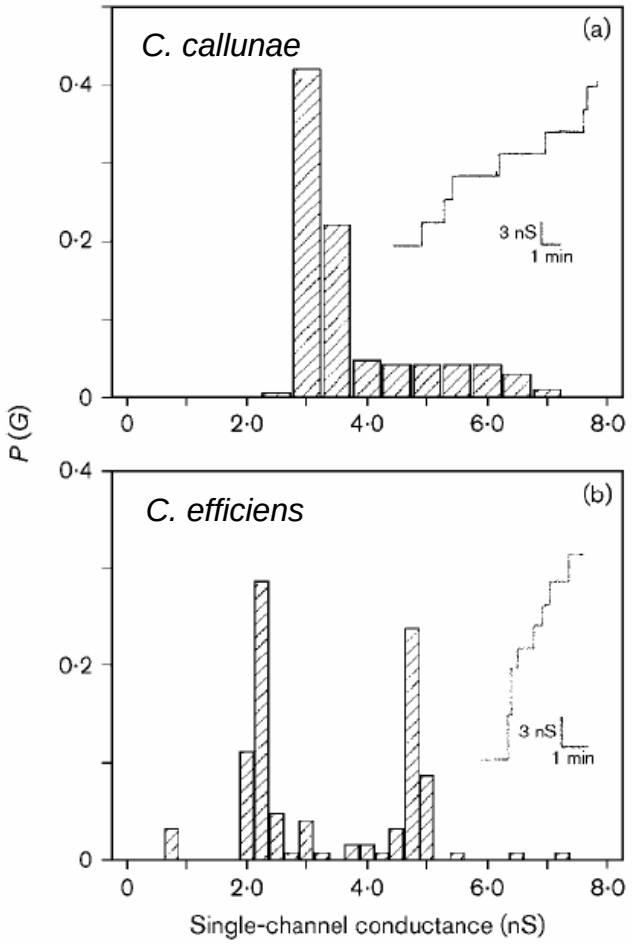
Tricine (10%) SDS-PAGE of the *C. callunae* and *C. efficiens* PorH purification procedure



C. callunae *C. efficiens*

→ Existence of a 6kDa protein after purification by FPLC

Histograms observed in the presence of pure cell-wall proteins of *C. callunae* and *C. efficiens*



G ($PorH_{Ccall}$) = 3nS in 1M KCl

→ voltage-dependent closure for voltages higher than 30-40mV

G ($PorH_{Ceff}$) = 2,3nS or 4,7nS in 1M KCl

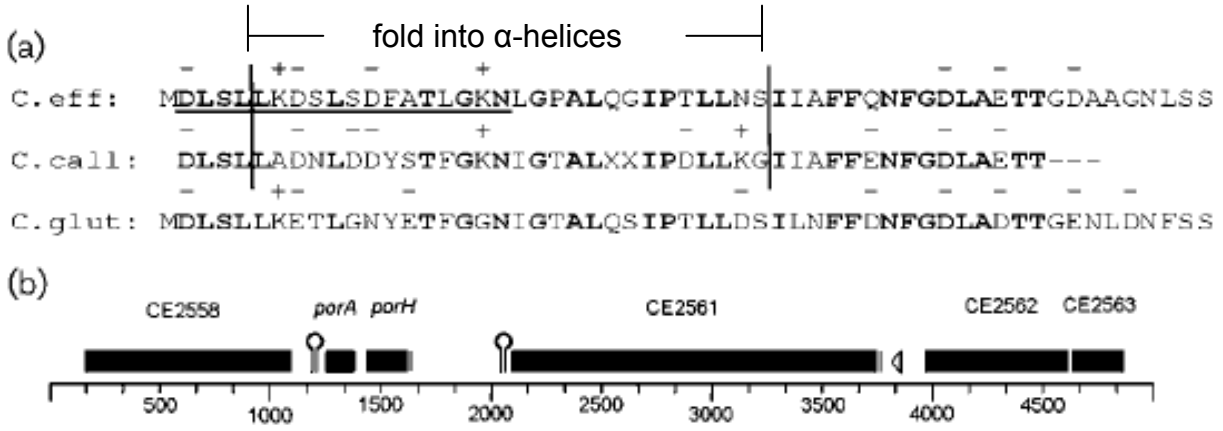
→ reconstitution of 2 channels at once

Average single-channel conductance G of $PorH_{C.call}$ and $PorH_{C.eff}$ in different salt solutions

Salt	Concentration (M)	$PorH_{C.call}$ G (nS)	$PorH_{C.eff}$ G (nS)
LiCl	1·0	1·25	1·50
NaCl	1·0	1·75	NM
KCl	0·01	NM	0·025
KCl	0·03	0·35	0·075
KCl	0·1	0·55	0·45
KCl	0·3	1·10	0·70
KCl	1·0	3·0	2·3
KCl	3·0	7·0	6·5
RbCl	1·0	3·0	NM
$N(CH_3)_4Cl$	1·0	1·0	1·8
$N(C_2H_5)_4Cl$	1·0	0·70	1·7
KCH_3COO (pH 7)	1·0	2·0	1·0

- no linearity between conductance and salt-concentrations → point net charges
- $PorH_{Ccall}$: higher cation-influence, V_m = 28mV, P_{cat}/P_{an} = 7 → highly cation-selective
- $PorH_{Ceff}$: higher anion-influence, V_m = -6mV, P_{cat}/P_{an} = 0,7 → slightly anion-selective

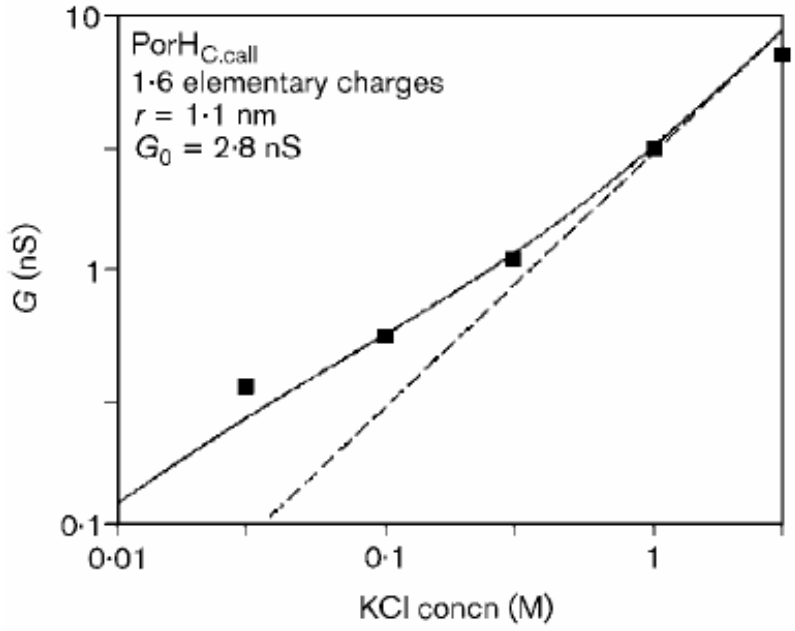
Comparison of aa-sequences/ overview of the *porH* gene locus within the *C.efficiens* genome



- *porH*$_{Ceff}$: 174bp encoding a 57aa long acidic polypeptide (6-/2+)
  no leader sequence → no Sec-system
  slightly anion-selective
- *porH*$_{Ccall}$: high homology to *porH*$_{Ceff}$, also acidic (8-/2+)
  only separated to *porA*$_{Ccall}$ by 77bp without a transcription terminator

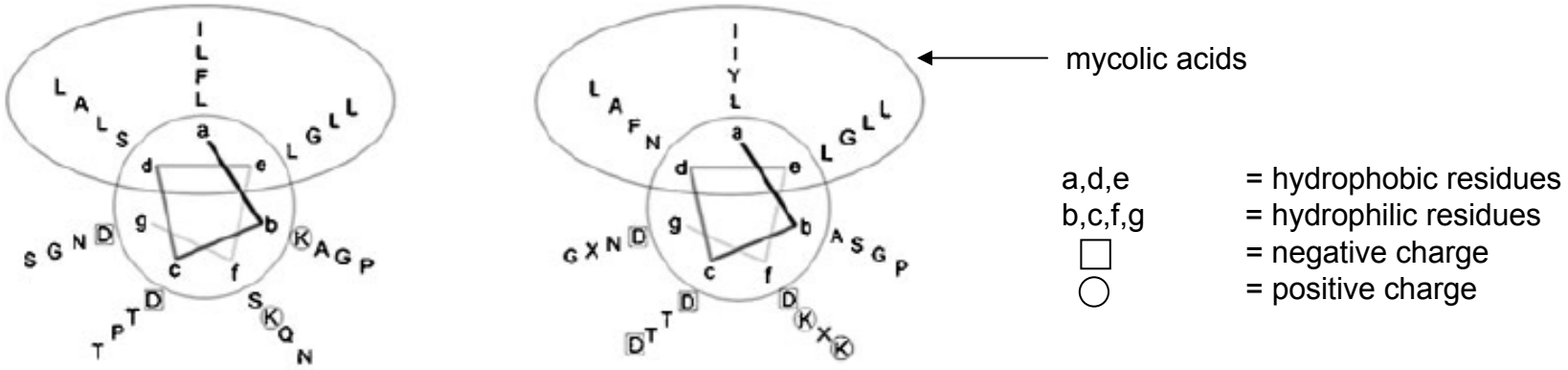
→ different ion-selectivity caused by arrangement in the channel-forming unit?

Single-channel conductance of $PorH_{C.call}$ as a function of the KCl concentration



- best fit for d = 2,2nm and 1,6 negative charges (q = -2,4*10^{-19}As)
- remember: $PorA_{Cglut}$ controlled by 2 negative charges (q = -3,2*10^{-19}As)

Schematic prediction of the secondary structures of PorH



C. efficiens

→ anion-selective

C. calunae

→ cation-selective

- heptamers of amphipathic α-helices with about 8 windings and a length of 4,2nm
- remember: ß-strands in MspA of *Mycobacterium smegmatis*
- charges in agreement with the ion-selectivity

→ smaller peptides arranged as α-helices sufficient to span the mycolic acid layer of *C.g.*

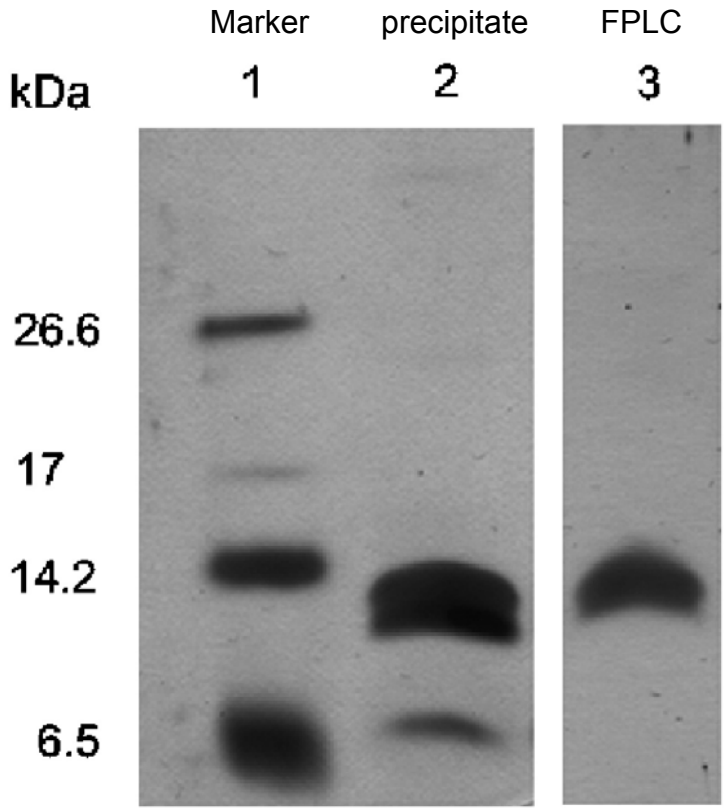
Summary

- $PorH_{Ccall}$ (highly cation-selective) and $PorH_{Ceff}$ (slightly anion-selective) show different ion-selectivity caused by different arrangements
- defined channels of 2nS to 3nS in 1M KCl
- no Sec-system for transport out of the cell wall
- $porH_{Ccall}$ and $porH_{Ceff}$ highly homologous
- genes coding for PorA and PorH only separated by some bp without transcription terminator between them

Identification and characterization of PorH, a new cell wall channel of *Corynebacterium glutamicum*

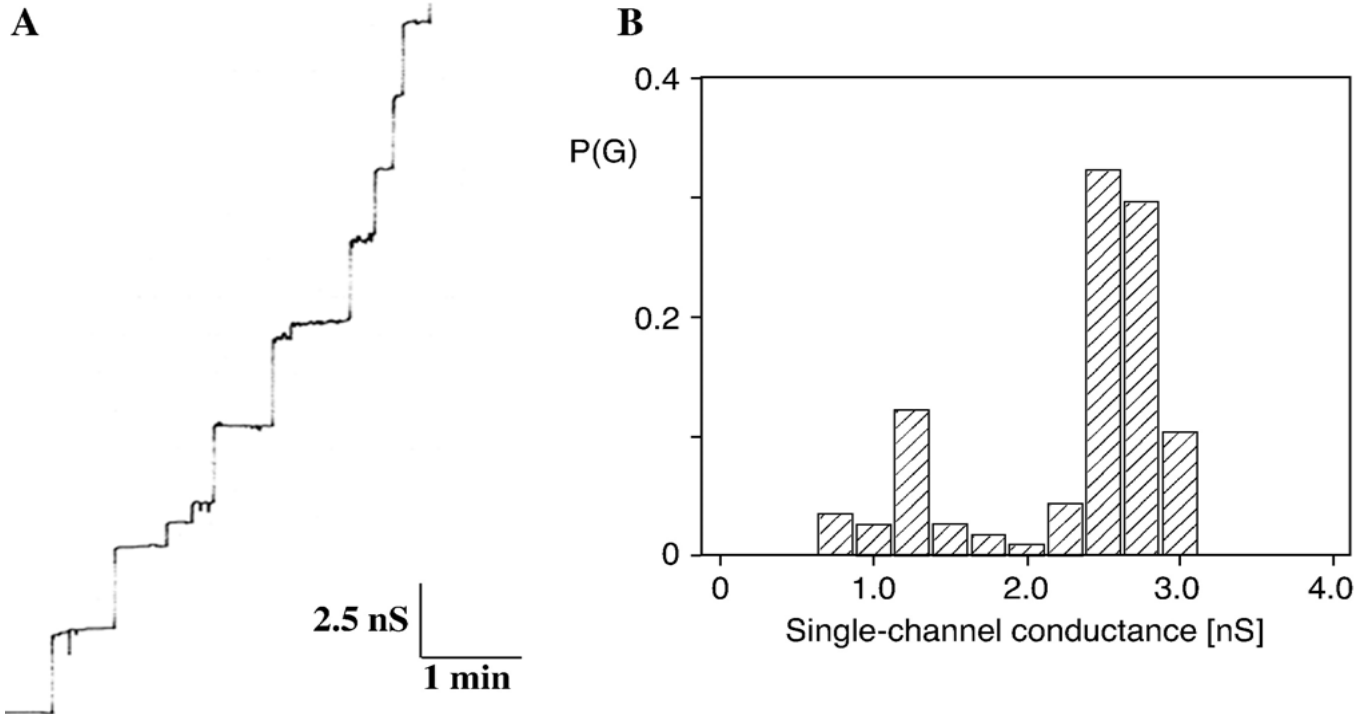
- Hünten et al, 2005 -

12% tricine SDS-PAGE of the purification procedure of $PorH_{C.glut}$



→ pure 12kDa-protein named $PorH_{Cglut}$

Single-channel recording and histogram in the presence of pure 12kDA protein ($PorH_{Cglut}$)



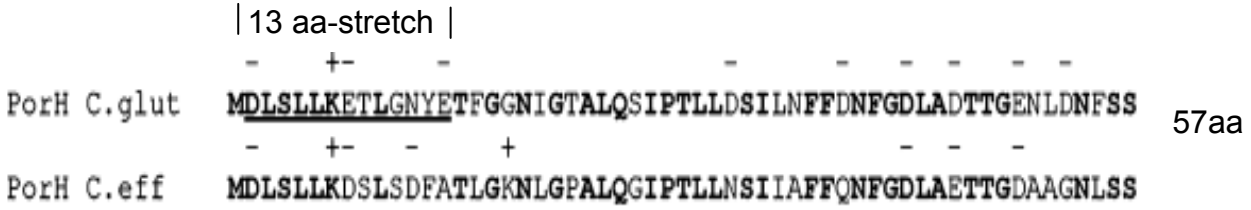
- main conductance of G = 2,5nS in 1M KCl
- minor fraction with lower conductance

Average single-channel conductance G of $PorH_{C.glut}$ in different salt solutions

Salt	Concentration c (M)	Single-channel conductance G (nS)
LiCl	1.0	1.0
KCl	0.01	0.15
	0.03	0.35
	0.1	0.4
	0.3	0.9
	1.0	2.5
	3.0	7.0
KCH_3COO (pH 7)	1.0	1.5

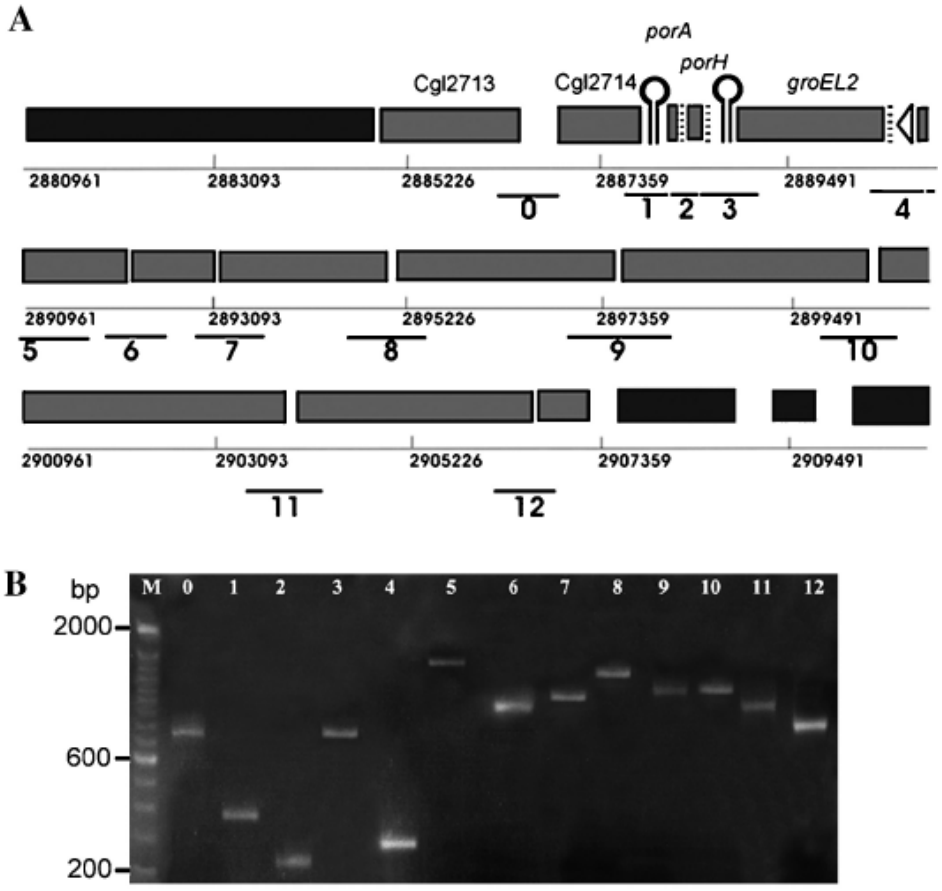
- higher cation-influence → cation-selectivity
- no linearity → point net charges
- zero current membrane potential V_m of +25mV → cation-selectivity
- P_{cat}/P_{an} = 5,1 → anions have certain permeability

Comparison of the amino acid sequences of $PorH_{C.glut}$ and $PorH_{C.eff}$



- partial sequencing: 13aa stretch as a part of a 57aa long hypothetical protein encoded by *porH*$_{Cglut}$ (174bp); highly homologous
- total mass: 6,1kDa → formation of dimers
- negative charges in agreement with cation-selectivity

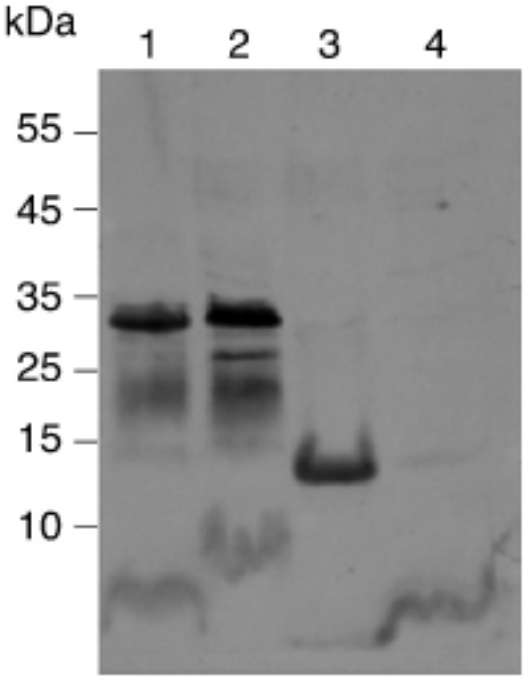
Overview of the *porH*$_{C.glut}$ gene locus and results of RT-PCR



- *porA* and *porH* only separated by 83bp without transcription terminator
- amplification with the primers

→ *porA* and *porH* part of transcriptional unit of 13 genes

Western-Blot analysis of $PorH_{C.glut}$ using anti-$PorH_{C.glut}$ antibodies

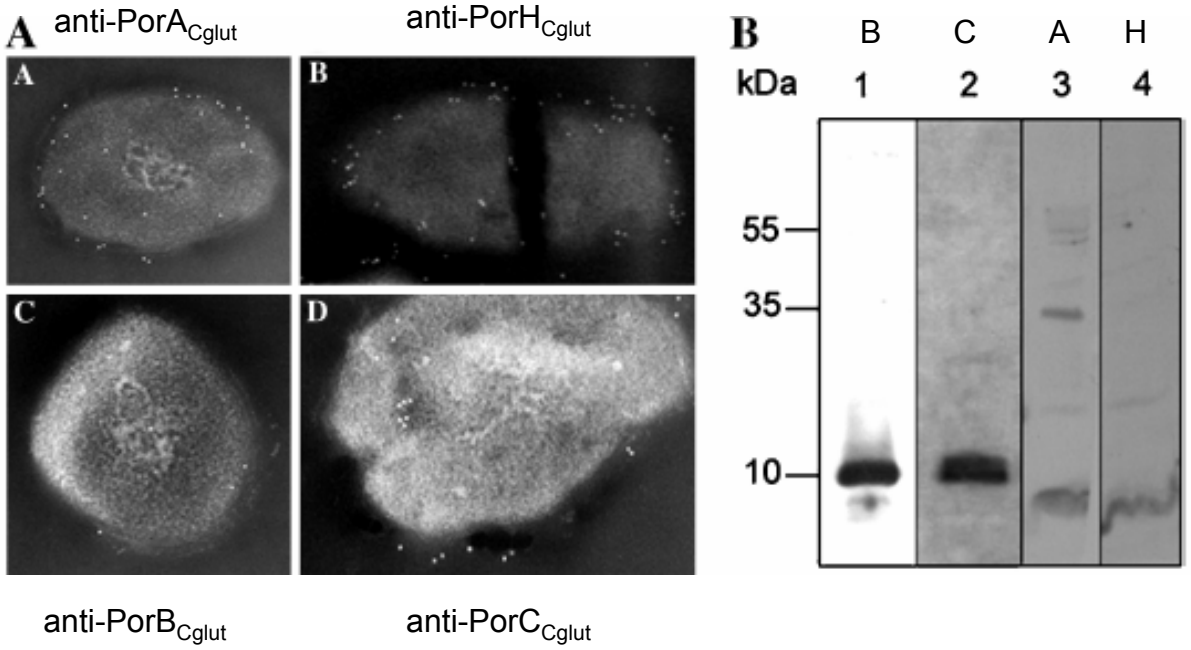


1 supernatant of 2% LDAO
2 supernatant of 2% LDAO (boiled)
3 supernatant of 8M Urea (boiled)
4 precipitated pellet (boiled)

→ search for oligomers

- LDAO-extraction: formation of oligomers (hexamers) resistant to 5min boiling
- Urea/organic-solvent: oligomers destroyed (mono-/dimers)

Electron micrograph of *C. glutamicum* cells, treated with several antibodies



- all channels are present in the channel at the same time

→ coexistence of all 4 channel PorA, PorB, PorC and PorH in *C. glutamicum*

Summary

- coexistence of 4 channel-forming proteins in *Corynebacterium glutamicum*

1) PorA: 45aa long polypeptide
cation-selective channel formed by an oligomer; G = 5,5nS in 1M KCl

2) PorB: 99aa long polypeptide
anion-selective channel; G = 700pS in 1M KCl
channel can be blocked by citrate

3) PorC: PorB-like protein located 138bp downstream from *porB*
porB and *porC* belong to same cluster and are cotranscribed

4) PorH: 57aa long polypeptide
cation-selective channel; G = 2,5nS in 1M KCl
porH located next to *porA*; both are cotranscribed