Nourseothricin
past, present and future

Reinhard Breitling

Jena Bioscience GmbH
Löbstedter Str. 80
07749 Jena, Germany
Tel.: +49-3641-628-5000
Fax: +49-3641-628-5100
e-Mail: info@jenabioscience.com

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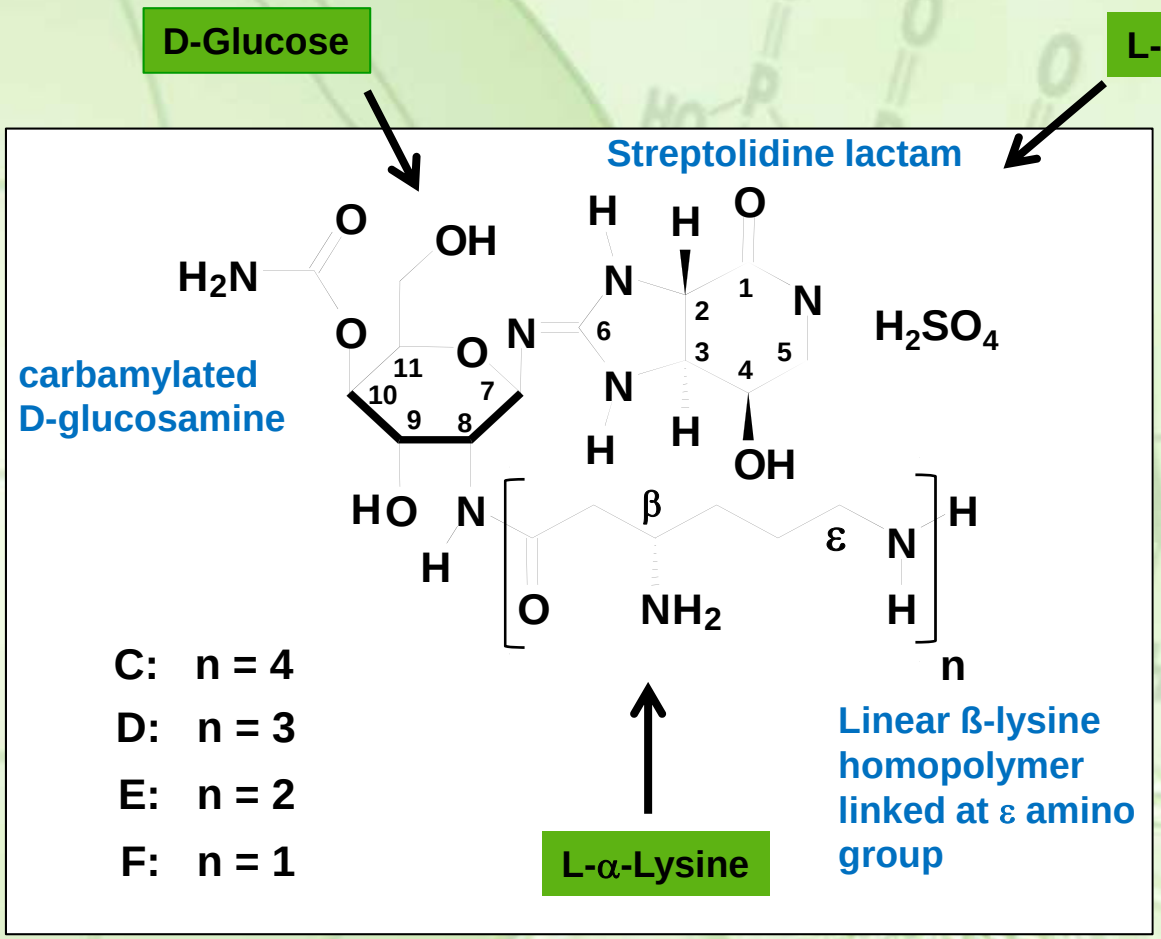
Nourseothricin is a aminoglycoside glycopeptide (nucleoside peptide) antibiotic of the Streptothricin class



Secondary metabolite of Nystatin producer *Streptomyces noursei* ZIMET JA3890 (ATCC 11455) (Bradler *et al.* 1963)

Synthesised from Glucose, Arginine and Lysine by convergent pathways (Jackson *et al.* 2002 and ref.)

Natural mixture of streptothricins C, D, E & F; D + F >85%

Substance produced exclusively at HKI Jena

Members of the Streptothricin class antibiotics differ by the number of ß-lysine residues (n = 1-7)

Producer	Antibiotic	Reference
Streptomyces lavendulae	Streptothricin F	Waksman *et al.* 1942
Streptomyces griseus	Streptothricin C, D, E, F (C + F > D +E)	Reynolds *et al.* 1947
Streptomyces noursei	Streptothricin C, D, E, F (D + F >85%)	Bradler *et al.* 1963
Streptomyces rochei	Streptothricin F , D, E	Singh *et al.* 1983
Streptomyces spp. SNUS 8810-111	Streptothricin D + mod	Kim *et al.* 1994
Streptomyces quinlingensis sp.nov.	Streptothricin D, F + mod	Ji *et al.* 2007

F n=1
E n=2
D n=3
C n=4
B n=5
A n=6
X n=7

- First member:Streptothricin F (n=1)
- Proposal of structural formula (van Tamelen *et al.* 1961)
- Separation of Streptothricins by ion exchange chromatography (Reshetov *et al.* 1964)
- Chemical structure and general formula (n= 1-7) (Khokhlov *et al.* 1964-1978)
- Total chemical structure (Kusumoto *et al.* 1982*)*

→Streptolin, Geomycin, Phytobacteriomycin, Racemomycin, Pleocidin, Polymycin, Yazumycin, Grisein, Nourseothricin … are mixtures of Streptothricins

Nourseothricin (NTC) was applied as an ergotropic agent

- Broad spectrum antibacterial effect
- Not used for therapeutic purposes (human or veterinary) because of nephrotoxicity
- Not resorbed by intestinum wall
- More efficient biomass production in animal farms due to inhibition of growth of (competing) microflora of digestive tract

- Produced at large scale as bentonite adsorbate of mycelium by Jenapharm in Jena
- Controlled application at selected sites in Germany in the 1980th

Streptothricin resistances were found at sites where Nourseothricin was feeded to pigs

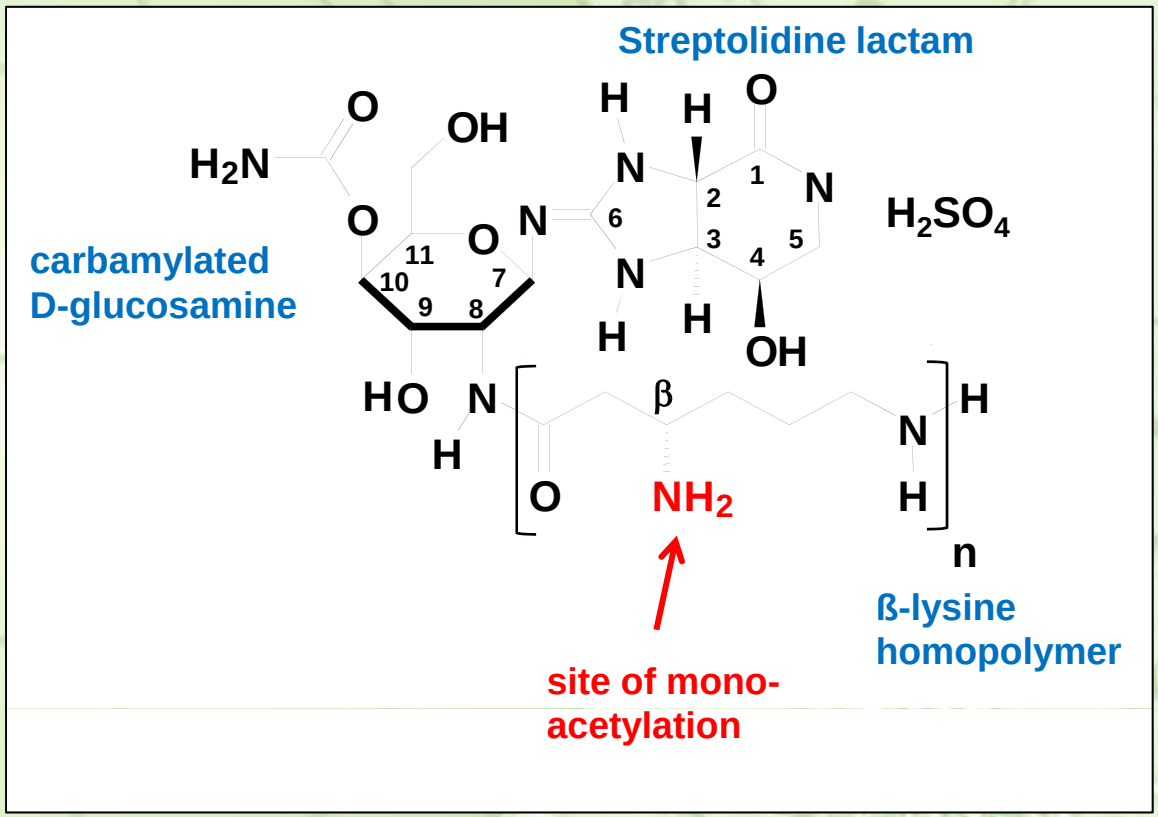
- **Resistance plasmids were found in *E. coli* from pigs and from employees in pig farms and their family members (Hummel *et al.* 1986)**
- **Resistance plasmids were also found in man without contact to animal farms but living in territories where Nourseothricin was applied as ergotropic agent (Hummel *et al.* 1986)**
- **The resistance plasmids found in *E. coli* from man were similar to the plasmids of *E. coli* from pigs and were of different incompatibility groups (Hummel *et al.* 1986)**
- **Hybridization to bacterial Streptothricin resistance gene probes was also observed with plasmids isolated a long time before the application of streptothricins (Tietze *et al.* 1990)**
- **The streptothricin resistance determinants were found to be linked to other resistance genes like streptomycin/spectinomycin- and trimethoprim-resistances on bacterial transposons (Sundström *et al.* 1991)**.

Three bacterial Streptothricin resistance genes where found at sites related to ergotropic use of streptothricins

Gene	Source	Reference	Linkage
sat1*	Bacterial transposon Tn 1825	Heim *et al.* 1989	*sat - aad*A1
sat2*	Bacterial transposon Tn 1826	Tietze *et al.* 1990a	*sat - aad*A1
sat*	Bacterial transposon Tn7	Sundström *et al.* 1991	*dhfrAI - sat - aad*A1
sat3	E. coli Plasmids pIE636, pIE637 and pIE639	Seltmann 1985 Tietze *et al.* 1990b	
sat4	*Campylobacter coli* BE/G4 *Enterococcus faecium* *S. aureus* Tn 5405	Jacob *et al.* 1994 Werner *et al.* 2001 Debrise *et al.* 1996	*aadE - sat4 – aphA-3*

sat1 = sat2 = sat Tn7 (Sundström *et al.* 1991)
aadA1 = streptomycin/spectinomycin resistance
aadE = aminoglycoside resistance
aphA-3 = kanamycin resistance
dhfrAI = trimethoprim resistance

The *sat resistance* genes encode a streptothricin N-acetyltransferase



Steptothricins are inactivated by monoacetylation of the ß-amino group of the ß-lysine residue

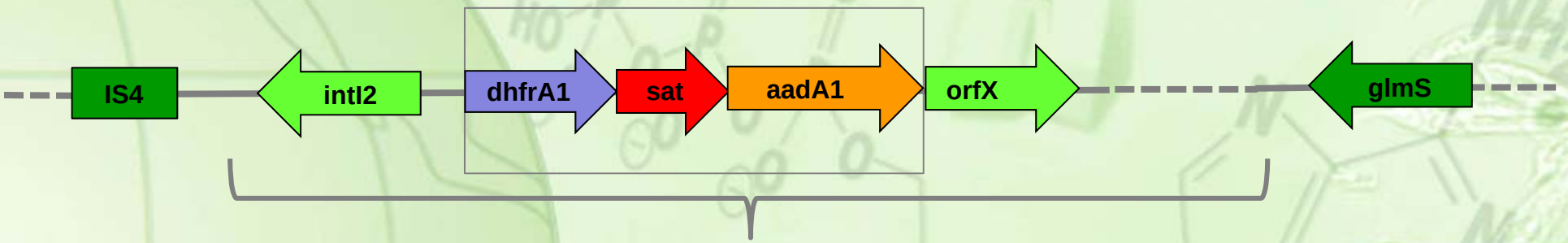
The 524 bp *sat* gene encodes a small protein of 174 aa (20 kDa)

Sat genes were found worldwide also in numerous other bacteria

Species	Source	Reference	Linkage
Aerococcus viridans	animal site	Byrne-Bailey *et al.*2008	Class1/2 integron
Acinetobacter baumannii		Ploy *et al.* 2000, Ramirez *et al*. 2005	Class 2 integron Tn7::In2-8
Burkholderia cenocepacia	clinical isolate	Ramirez *et al*. 2005	Class 2 integron Tn7::In2-1
Citrobacter freundii	beef cuttle AU	Barlow *et al*. 2006	Class 2 integron
Enterobacter cloacae	clinical isolate	Ramirez *et al*. 2005	Class2 integron with IS1 (Tn7::In2-10)
Enterococcus faecalis	clinical isolate	Xu *et al*. 2010	Class2 integron
Klebsiella oxytoca & pneumonia	clinical isolate	Xu *et al*. 2007	Class2 integron
Morganella morganii	clinical isolate	Power *et al.* 2005	Class 2 integron
Proteus mirabilis	meat products	Kim *et al.* 2004	
Pseudomonas aeroginosa Tn7		Ramirez *et al*. 2005	MDR
Psychrobacter maritimus & sp.	animal site	Byrne-Bailey *et al.* 2008	Class1/2 integron
Raoultella terrigena	clinical isolate	Ramirez *et al*. 2005	Class2 integron-Tn7
Salmonella enterica SV enteritidis	clinical isolate	Ahmed *et al.* 2005	Class 2 integron
Serratia marcescens	clinical isolate	Crowley *et al.* 2006	Class2 integron
Shigella flexneri & sonnei	clinical isolate	Halloran *et al.* 2002, Pan *et al.* 2006	Class1/2 integron MDR
Vibrio cholerae	clinical isolate	Coelho *et al.* 1995, Ahmed *et al*. 2006	Class1/2 integron

Protein Blast *"sat":* 99-100% identity

The integrons contained multiple antibiotic resistance markers



Tn7 containing a class 2 integron (14 kbp) isolated from *Shigella sonnei* after Pan *et al.* 2006

→ **Co-selection of resistances to therapeutic antibiotics (Streptomycin)**

→ **Ergotropic use of Nourseothricin was stopped after 1989 in Germany**

Streptothricin producers harbour self-resistance genes encoding a streptothricin N-acetyltransferase

Gene	Species	Reference
stat	*Streptomyces lavendulae*	Horinouchi et al. 1987
nat1 & 2	*Streptomyces noursei*	Krügel et al. 1993
gsr	*Streptomyces griseus*	Sezonov *et al.* 1990
sttR	*Streptomyces rochei*	Anukool *et al.* 2004
NAT_SF	*Streptomyces ambofaciens*	Choulet et al. 2006
NAT_SF	*Streptomyces pactum*	Ito *et al.* 2008
NAT_SF	*Streptomyces sp. C*	Fischbach et al. 2009
NAT_SF	*Streptomyces sp. e14*	Fischbach *et al.* 2010
NAT_SF	*Streptomyces roseosporus*	genome shotgun sequences 2010
NAT_SF	*Streptomyces cattleya*	Centre National de Sequencage 2011

Protein Blast *"nat1":* 66-78% similarity

→ opens the way for genetic manipulation of sensitive organisms in combination with streptothricin antibiotics

Nourseothricin: A superior selection antibiotic in molecular genetics

Field of use

- Extraordinarily broad spectrum of sensitive bacteria and eukaryotic organisms
- Excellent selection antibiotic for genetic modification of
  - Gram-positive and Gram-negative bacteria
  - Yeast and filamentous fungi
  - Protozoa and microalgae
  - Plants … and more

Mechanism of Action

- Antibiotic effect of Nourseothricin through inhibition of protein biosynthesis and induction of miscoding
- Resistance to Nourseothricin conferred by *sat* or *nat* marker genes
- Product of the resistance gene - Nouresothricin N-acetyltransferase - inactivates NTC by monoacetylation of ß-amino group of the ß-lysine residue

Advantages

- Low or no background: Resistance protein is localized intracellularly and cannot be degraded in the cell culture medium
- Not used in human or veterinary medicine, therefore, no conflict with regulatory requirements
- No cross-reactivity with other aminoglycosid antibiotics such as Hygromycin or Geneticin
- No cross-resistance with therapeutic antibiotics
- Long-term stable as powder or solution
- Highly soluble in water (1 g/L)

Group	Species	MIC* µg/ml	Selection conc. µg/ml
Gram-negative bacteria	*Agrobacterium tumefaciens*		100
	Escherichia coli	2-12	50
	Francisella tularensis		50
	Pseudomonas aeruginosa	50	100
Gram-positive bacteria	*Bacillus subtilis*	5	50
	Enterococcus faecium	8-256	500
	Staphylococcus aureus	2-12	50
Streptomycetes	*Streptomyces lividans*	6	100
Yeast	*Candida albicans*	200	250-450
	Hansenula polymorpha		100
	Kluyveromyces lactis		50
	Pichia pastoris		100
	Saccharomyces cerevisiae	25	75-100
	Schizosaccharomyces pombe	40	100
Other Ascomycota	*Acremonium chrysogenum*		25
	Aspergillus nidulans		120
	Cryphonectria parasitica		100
	Neurospora crassa		200
	Penicillium chrysogenum		150-200
	Podospora anserina		50
	Sordaria macrospora		50
	Trichophyton mentagrophytes		50
Basidiomycota	*Cryptococcus neoformans*		100
	Schizophyllum commune	3	8
	Ustilago maydis		75-100
Protozoa	*Leishmania tarentolae, major* etc.	30-50	100
	Phytomonas serpens		100
	Plasmodium falciparum	75**	
	Toxoplasma gondii		500
Microalgae	*Phaeodactylum tricornutum*		50-250
	Thalassiosira pseudonana		100
Plants	*Arabidopsis thaliana*	20	50-200
	Daucus carota		100
	Lotus corniculatus		50
	Nicotiana tabacum		100
	Oryza sativa	20	200

Nourseothricin is used in about 50 recombinant host-vector systems

permanently increasing number of species for genetic engineering will further extend its application

***MIC:**
Minimal inhibitory concentration

****IC50:**
Concentration inhibiting growth by 50%

Acknowledgments

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Thank you for your attention!