



Jena Bioscience

Nourseothricin superior selection antibiotic in molecular genetics

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Nourseothricin (NTC): A superior selection antibiotic in molecular genetics

Field of use

- Streptothricin-class antibiotic for an extraordinarily broad spectrum of bacteria and eukaryotic unicellular or complex organisms (see Table 1)
- Preferred selection antibiotic for genetic modification of
 - Gram-positive and Gram-negative bacteria
 - Yeast and filamentous fungi
 - Protozoa and microalgae
 - Plants ... and many more

Mechanism of Action

- Antibiotic effect of NTC through inhibition of protein biosynthesis and induction of miscoding
- Resistance to NTC conferred by *sat1* or *nat1* marker genes
- Product of the resistance gene - Nourseothricin N-acetyltransferase - inactivates NTC by monoacetylation of β -amino group of the β -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)

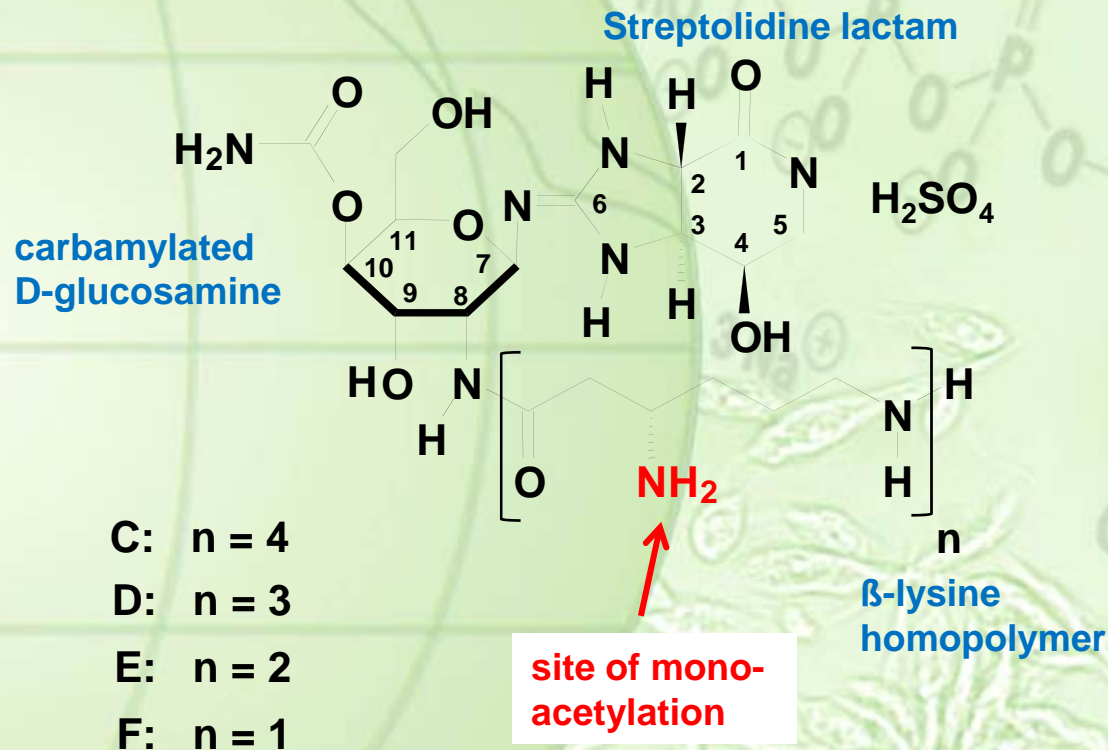
NTC is an efficient selection antibiotic for a broad range of species

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

*MIC:
Minimal inhibitory
concentration

** IC50:
Concentration inhibiting
growth by 50%

NTC is an aminoglycoside antibiotic of high stability



- Natural mixture of streptothricins C, D, E and F produced by *Streptomyces noursei*
D + F >85%
- Long-term stable without loss of activity
 - Powder can be stored for 10 years at 4°C or for 2 years at 20°C
 - Solution (100 mg/ml) is stable for >24 month at -20°C or 12 month at 4°C

NTC was successfully applied in a broad range of host systems

Selected publications 1:

Alshahni *et al.* (2010) Nourseothricin acetyltransferase: a new dominant selectable marker for the dermatophyte *Trichophyton mentagrophytes*. *Medical Mycology* **48**:665

Cox *et al.* (2003) Superoxide Dismutase Influences the Virulence of *Cryptococcus neoformans* by Affecting Growth within Macrophages. *Infect. Immunity* **71**: 173

El-Khoury *et al.* (2008) Gene deletion and allelic replacement in the filamentous fungus *Podospora anserina*. *Curr Genet* **53**: 249

Gold *et al.* (1994) Three selectable markers for transformation of *Ustilago maydis*. *Gene* **142**: 225

Goldstein *et al.* (1999) Three New Dominant Drug Resistance Cassettes for Gene Disruption in *Saccharomyces cerevisiae*. *Yeast* **15**: 1541

Hamano *et al.* (2006) A Novel Enzyme Conferring Streptothricin Resistance Alters the Toxicity of Streptothricin D from Broad-spectrum to Bacteria-specific. *J. Biol. Chem.* **281**: 16842

Hentges *et al.* (2005) Three novel antibiotic marker cassettes for gene disruption and marker switching in *Schizosaccharomyces pombe*. *Yeast* **22**: 1013

Hoff *et al.* (2009) Homologous recombination in the antibiotic producer *Penicillium chrysogenum*: strain Δ Pcku70 shows up-regulation of genes from the HOG pathway. *Appl Microbiol Biotechnol* **85**:1081

Jacobs *et al.* (2009) Engineering complex-type N-glycosylation in *Pichia pastoris* using GlycoSwitch technology. *Nature Protocols* **4**: 58

Jelenska *et al.* (2000) Streptothricin resistance as a novel selectable marker for transgenic plant cells. *Plant Cell Reports* **19**: 298

Ji *et al.* (2007) Two New Members of Streptothricin Class Antibiotics from *Streptomyces qinlingensis* sp. Nov. *J. Antibiot.* **60**: 739

Joshi *et al.* (1995) The gene encoding streptothricin acetyltransferase (*sat*) as a selectable marker for *Leishmania* expression vectors. *Gene* **156**: 145

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Selected publications 2:

Kloti *et al.* (2004) Selectable marker in plants. *United States Patent* 6696621

Kojic *et al.* (2000) Shuttle vectors for genetic manipulations in *Ustilago maydis*. *Can. J. Microbiology* **46**: 333

Kooistra *et al.* (2004) Efficient gene targeting in *Kluyveromyces lactis*. *Yeast* **21**: 781

Kück *et al.* (2006) Application of the nourseothricin acetyltransferase gene (*nat1*) as dominant marker for the transformation of filamentous fungi. *Fungal Genetics Newsletter* **53**: 9

Laubinger (2008) Analysis of Neuronal Diseases in the Model Organism *Aspergillus nidulans*. *Dissertation Univ. Göttingen*

Lukeš *et al.* (2006) Translational initiation in *Leishmania tarentolae* and *Phytomonas serpens* (Kinetoplastida) is strongly influenced by pre-ATG triplet and its 5' sequence context. *Molecular & Biochemical Parasitology* **148**: 125

Maier *et al.* (2006) In Vivo *Himar1*-Based Transposon Mutagenesis of *Francisella tularensis*. *Appl. Environment. Microbiol.* **72**: 1878

McDade *et al.* (2001) A new dominant selectable marker for use in *Cryptococcus neoformans*. *Med. Mycol.* **39**: 151

Mamoun *et al.* (1999) A set of independent selectable markers for transfection of the human malaria parasite *Plasmodium falciparum*. *PNAS* **96**: 8716

Mandal *et al.* (2009) High-throughput screening of amastigotes of *Leishmania donovani* clinical isolates against drugs using colorimetric β -lactamase assay. *Ind. J. Exptl. Biol.* **47**: 475

Nagotu *et al.* (2008) Peroxisome Fission in *Hansenula polymorpha* Requires Mdv1 and Fis1, Two Proteins Also Involved in Mitochondrial Fission. *Traffic* **9**: 1471

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Selected publications 3:

- Ohm *et al.* (2010) An efficient gene deletion procedure for the mushroom-forming basidiomycete *Schizophyllum commune*. *World J Microbiol Biotechnol* **26**:1919
- Poulsen *et al.* (2006) Molecular genetic manipulation of the diatom *Thalassiosira pseudonana* (bacillariophyceae) *Phycology* **42**: 1059
- Roemer *et al.* (2003) Large-scale essential gene identification in *Candida albicans* and application to antifungal drug discovery. *Molec. Microbiol.* **50**: 167
- Shen *et al.* (2005) CaNAT1, a heterologous dominant selectable marker for transformation of *Candida albicans* and other pathogenic *Candida species*. *Infection and Immunity*, **73**: 1239
- Smith *et al.* (2007) Two yeast plasmids that confer nourseothricin-dihydrogen sulfate and hygromycin B resistance in *Neurospora crassa* and *Cryphonectria parasitica*. *Fungal Genetics Newsletter* **54**: 12
- Van *et al.* (2006) Nourseothricin Acetyltransferase: A Positive Selectable Marker for *Toxoplasma gondii*. *J. Parasitol.* **92**: 668
- van Peer *et al.* (2009) Phleomycin Increases transformation efficiency and promotes single integrations in *Schizophyllum commune*. *Appl. Environ. Microbiol.* **75**, 1243-1247
- Werner *et al.* (2001) Aminoglycoside-Streptothricin Resistance Gene Cluster *aadE-sat4-aphA-3* Disseminated among Multiresistant Isolates of *Enterococcus faecium*. *Antimicrob. Agents Chemotherapy* **45**: 3267
- Zaslavskaja *et al.* 2000 Transformation of the diatom *Phaeo-dactylum tricornutum* (bacillariophyceae) with a variety of selectable marker and reporter genes. *J. Phycol.* **36**: 379
- etc.....