Jena Bioscience

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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 *sat*1 or *nat*1 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)

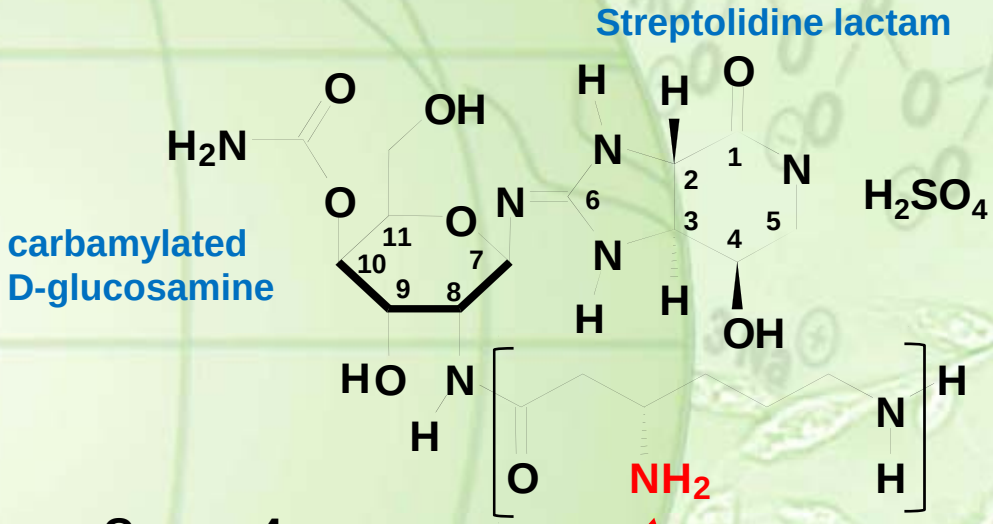
NTC is an efficient selection antibiotic for a broad range of species

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.		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

***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 ΔPcku70 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* l*actis. 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 ß-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 Acetyltransferease: 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

Zaslavskaia *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.….