



# 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
  - Mammalian cells
  - Yeast and filamentous fungi
  - Protozoa and microalgae
  - Gram-positive and Gram-negative bacteria
  - 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*, *stat* or *nat* marker genes
- Product of the resistance gene - Nourseothricin N-acetyltransferase - inactivates NTC by monoacetylation of the  $\beta$ -amino group of its  $\beta$ -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/ml)

## Table 1 Organisms suitable for NTC selection

Category	Species/cell line	MIC <sup>1)</sup>	Selection concentration
		µg/ml	µg/ml
Mammalian cells	HMEC	25 <sup>2)</sup>	50
	HEK293T	<25 <sup>2)</sup>	
	BT549	25 <sup>2)</sup>	
	U2OS	25 <sup>2)</sup>	
	A2780	75 <sup>2)</sup>	
Yeast	<i>Ashbya gossypii</i>		50-200
	<i>Candida albicans</i>	200	200-450
	<i>Candida dubliniensis</i>		100
	<i>Candida guilliermondii</i>		150
	<i>Candida glabrata</i>		100-200
	<i>Candida kefyr</i>		450
	<i>Candida lusitaniae</i>		100-450
	<i>Candida orthopsilosis</i>		200
	<i>Candida parapsilosis</i>		200
	<i>Candida tropicalis</i>		150-200
	<i>Hansenula ciferrii</i> <sup>3)</sup>		50
	<i>Hansenula polymorpha</i>		100
	<i>Kluyveromyces lactis</i>		50-100
	<i>Lipomyces starkeyi</i>		30
	<i>Pichia ciferrii</i> <sup>3)</sup>		50
	<i>Pichia pastoris</i>		50-200
	<i>Saccharomyces cerevisiae</i>	25	50-200
	<i>Schizosaccharomyces japonicus</i>		50-100
	<i>Schizosaccharomyces pombe</i>	40	90-100
	<i>Torulasporea delbrueckii</i>		50
<i>Zygosaccharomyces rouxii</i>		5	
<i>Zygosaccharomyces bailii</i>		100	



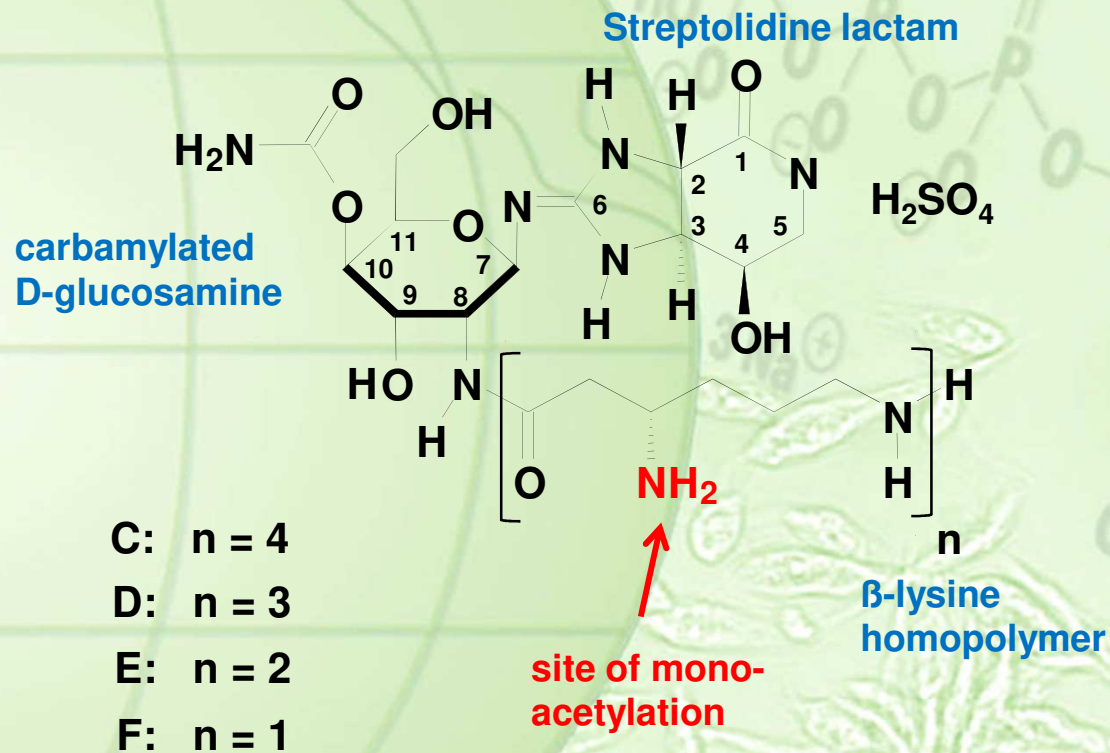
Category	Species/cell line	MIC <sup>1)</sup>	Selection concentration
		µg/ml	µg/ml
Other Ascomycota	<i>Acremonium chrysogenum</i>		25
	<i>Alternaria brassicicola</i>		200
	<i>Aspergillus nidulans</i>		120
	<i>Aspergillus tubingensis</i>		20
	<i>Botrytis cinerea</i>		50-150
	<i>Clonostachys rosea</i>		300-400
	<i>Coccidioides posadasii</i>		100
	<i>Cochliobolus heterostrophus</i>		120-300
	<i>Cochliobolus luttrellii</i>		60-100
	<i>Colletotrichum coccodes</i>		100
	<i>Colletotrichum graminicola</i>		100-400
	<i>Colletotrichum higginsianum</i>		100
	<i>Cryphonectria parasitica</i>		100
	<i>Fusarium fujikuroi</i>		100
	<i>Fusarium graminearium</i>		25-200
	<i>Fusarium oxysporum</i>		50-60
	<i>Leptosphaeria maculans</i>		50
	<i>Neurospora crassa</i>		20-200
	<i>Penicillium chrysogenum</i>		150-200
	<i>Penicillium roqueforti</i>		40
	<i>Plectosphaerella cucumerina</i>		100
	<i>Podospora anserina</i>		50-75
	<i>Rhynchosporium commune</i>	1	
	<i>Sclerotinia sclerotiorum</i>		40-200
	<i>Sordaria macrospora</i>		50
	<i>Trichoderma atroviride</i>		300-400
	<i>Trichophyton mentagrophytes</i> <sup>4)</sup>		50
	<i>Verticillium dahliae</i>		50
	<i>Yarrowia lipolytica</i>		250
	<i>Zymoseptoria tritici</i> <sup>5)</sup>		40-50

Category	Species/cell line	MIC <sup>1)</sup>	Selection concentration
		µg/ml	µg/ml
Basidiomycota	<i>Cryptococcus gattii</i>		100
	<i>Cryptococcus neoformans</i>		100-200
	<i>Physisporinus vitreus</i>		110
	<i>Rhodosporeidium kratochvilovae</i>		200
	<i>Rhodotorula graminis</i>		200
	<i>Schizophyllum commune</i>	3	8-20
	<i>Ustilago maydis</i>		75-150
	<i>Xanthophyllomyces dendrorhous</i> <sup>6)</sup>		30
	Protozoa	<i>Crithidia bombi</i>	
<i>Leptomonas seymouri</i>			250
<i>Leishmania amazonensis</i>			50
<i>Leishmania braziliensis</i>			50-100
<i>Leishmania donovani</i>		5-10 <sup>2)</sup>	50-125
<i>Leishmania infantum</i>			20-100
<i>Leishmania major</i>		32-50	50-100
<i>Leishmania mexicana</i>			25-50
<i>Leishmania tarentolae</i>		50	100
<i>Phytomonas serpens</i>			100
<i>Plasmodium falciparum</i>		75**	
<i>Toxoplasma gondii</i>			500
<i>Trypanosoma brucei</i>			150-200
<i>Trypanosoma vivax</i>			1
Microalgae		<i>Amphora coffeaeformis</i>	
	<i>Chaetoceros sp.</i>		100-500
	<i>Chaetoceros gracilis</i>	300	400
	<i>Ostreococcus tauri</i>		1500
	<i>Phaeodactylum tricorutum</i>		50-250
	<i>Thalassiosira pseudonana</i>		100-200

Category	Species/cell line	MIC <sup>1)</sup>	Selection concentration
		µg/ml	µg/ml
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
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>Mycobacterium smegmatis</i>		25
	<i>Staphylococcus aureus</i>	2-12	50
Streptomyces	<i>Streptomyces lividans</i>	6	100
Cyanobacteria	<i>Synechocystis sp. PCC 6803</i>		50

- 1) MIC: Minimal inhibitory concentration
- 2) IC50: Concentration inhibiting growth by 50%
- 3) *Hansenula ciferrii* = *Pichia ciferrii* = *Wickerhamomyces*
- 4) genotype of heterogeneous species *Arthroderma vanbreuseghemii*
- 5) synonymous *Mycosphaerella graminicola* or *Septoria tritici*
- 6) sexual (teleomorph) stage of asexual (anamorph) *Phaffia*

# NTC is a aminoglycoside antibiotic of high stability

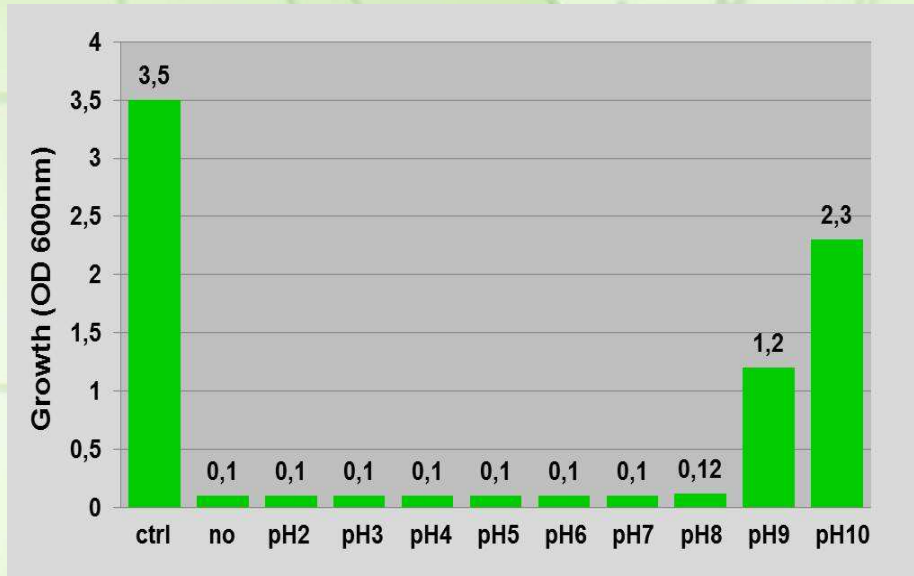


- Natural mixture of streptothricins C, D, E and F produced by *Streptomyces noursei*  
D + F >85 %
- Long-term stable without loss of activity
  - Powder can be stored for 10 years at 4 °C or for two years at 20°C
  - Solution (100 mg/ml) is stable for >24 months at -20 °C or 12 months at 4 °C



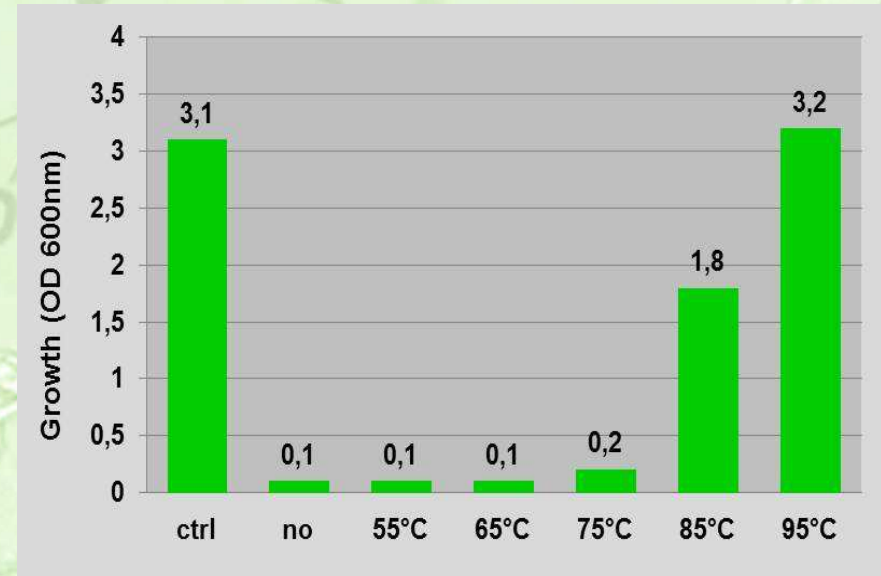
# Nourseothricin is resistant to heat treatment and pH shift

## Nourseothricin solutions are stable at pH 2-8



Nourseothricin solutions are stable at pH 2-8 for > 7 days at 26 °C. Stock solutions were incubated at indicated pH for 4 days and 26 °C before its addition at 100 µg/ml concentration to a 1:50 inoculated LEXSY culture (organism: *Leishmania tarentolae*). Growth was monitored daily; shown is growth after 3 days. Control = no antibiotic added; no = no pH treatment of antibiotic. The pH of the culturing medium was in the range of 7.3-7.6 and not influenced by the antibiotic addition.

## Nourseothricin solutions are stable at 75°C



Nourseothricin solutions are stable at temperatures up to 75 °C even after 24 h of heat treatment. Stock solutions were heated for 24 h at the indicated temperatures and then added at 100 µg/ml concentration to a 1:50 inoculated LEXSY culture (organism: *Leishmania tarentolae*). Growth was monitored daily; shown is growth after 3 days. Control = no NTC addition; no = no heat-treatment.



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