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ORIGINAL PAPER

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Production of the glycopeptide antibiotic A40926 by *Nonomuraea* sp. ATCC 39727: influence of medium composition in batch fermentation

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Abstract *Nonomuraea* sp. ATCC 39727 is a novel actinomycete species and the producer of A40926, a glycopeptide antibiotic structurally similar to teichoplanin. In the present study, a defined minimal medium was designed for *Nonomuraea* fermentation. The influence of initial phosphate, glucose and ammonium concentrations on antibiotic productivity was investigated in batch fermentation and the effect of glucose limitation was studied in fed-batch fermentation. It was found that low initial concentrations of phosphate and ammonium are beneficial for A40926 production and that productivity is not enhanced during glucose limitation. Furthermore, the initiation of A40926 production was not governed by residual ammonium and phosphate concentrations, although the level of these nutrients strongly influenced A40926 production rates and final titers.

Keywords Dalbavancin · Glycopeptide antibiotics · Actinomycete fermentation · Phosphate limitation

Introduction

Nosocomial infections caused by Gram-positive bacteria such as *Staphylococcus aureus* and *Enterococus* sp. have developed into a serious problem in the United States and Europe [2, 5, 14]. This is mainly due to the lack of potent antibiotics against these pathogens, which have acquired resistance to a wide range of antibiotics. In the fight against multiresistant bacteria, the last line of defense is presently the glycopeptide antibiotic vancomycin. However, pathogens are emerging that are resistant to vancomycin, making the search for novel antibiotics a pertinent task. In 1987, Biosearch Italia SpA discovered the novel glycopeptide antibiotic A40926 by screening for new antibimicrobial compounds [6]. This antibiotic is structurally similar to teichoplanin, another glycopeptide antibiotic currently in use against infections by Gram-positive organisms. A40926 has been used as a precursor for the semi-synthetic antibiotic dalbavancin, which in clinical trials has shown considerable advantage over vancomycin and, thus, is a promising antibiotic for the treatment of infections caused by Gram-positive organisms [9, 13].

The A40926 producer, *Nonomuraea* sp. ATCC 39727, is a poorly characterized actinomycete. In the present study, the aim was to study the possible influence of different nutrients on the growth and antibiotic–producing characteristics of *Nonomuraea* in batch fermentation. The formation of secondary metabolites is normally not promoted when an organism is growing at its full potential, but is elicited only when growth is reduced, e.g. by the depletion of nutrients required for growth [10]. Generally, nutrient availability is important both for the secondary metabolite formation rate and for the initiation of secondary metabolism. In the case of actinorhodin production by *Streptomyces coelicolor*, antibiotic production was found to be triggered by phosphate depletion, nitrogen depletion or a decline in the specific growth rate [4]. Moreover, the rate of actinorhodin formation was inversely related to the residual concentration of the nitrogen source. Chloramphenicol production by *S. venezuelae* is increased by the use of poorly assimilated carbon and nitrogen sources, while changes in phosphate availability have little effect on productivity [3]. Furthermore, an elevated initial glucose concentration negatively influences chloramphenicol productivity but does not delay the initiation of antibiotic biosynthesis in *S. venezuelae* batch cultivations [1]. In the vancomycin-producer *Amycolatopsis orientalis*, phosphate limitation enhances productivity in batch and continuous fermentation [11].

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Similarly, in the case of the teichoplanin-producer *Actinoplanes teichomyceticus*, it was found that high concentrations of phosphate and ammonium reduce the production of teichoplanin in batch fermentation [7].

Obviously, a defined minimal growth medium is a preferred starting point for these types of investigations and a defined medium containing a number of amino acids has been developed, using a Plackett–Burman design for vancomycin production by *Amy. orientalis* [11]. Furthermore, adding the same cocktail of amino acids to a defined growth medium for *Act. teichomyceticus* has been reported to improve growth and teichoplanin production [15]. However, the presence of amino acids in the growth medium may make it difficult to assess the effects of starvation for nitrogen and carbon, since these elements are present in many of the medium components. In the present study, a defined minimal medium was developed that supports growth of *Nonomuraea* and production of A40926. The influence of varying phosphate, ammonium and glucose concentrations on the production of A40926 was studied in batch fermentations. Further, the influence of glucose limitation on this process was studied in fed-batch fermentation.

Materials and methods

Strain and inoculum preparation

Nonomuraea sp. ATCC 39727 was obtained from Biosearch Italia SpA, Gerenzano, Italy. The strain was stored in 1-ml cryotubes at −80 °C in 15% glycerol and 8 g l^{-1} tryptic soy broth (Difco, Detroit, Mich.), at a biomass concentration of approximately 0.2 g dry cell weight (DCW) ml^{-1}. For seed culture preparations, a 500-ml baffled Erlenmeyer flask containing 100 ml medium was inoculated with one glycerol stock vial and incubated with shaking (150 rpm) at 30 °C. The medium contained (per liter): 45 g glucose, 8.3 g $(NH_4)_2SO_4$, 1.5 g KH_2PO_4, 1 g $MgSO_4{\cdot}7H_2O$, 5 g $CaCO_3$, 1 g yeast extract and 3 ml TMS-1 (1 l TMS-1 contained 5 g $FeSO_4{\cdot}7H_2O$, 390 mg $CuSO_4{\cdot}5H_2O$, 440 mg $ZnSO_4{\cdot}7H_2O$, 150 mg $MnSO_4{\cdot}H_2O$, 11 mg $NaMoO_4{\cdot}2H_2O$, 20 mg $CuCl_2{\cdot}2H_2O$, 50 ml 37% HCl) at pH 7.5. After 120 h of incubation, this seed culture was used for inoculating (at 5%) a new 500-ml baffled Erlenmeyer flask containing 100 ml of the same medium which was again incubated for 120 h at 30 °C and used for inoculation of the fermentors (5% v/v).

Culture conditions in shake-flask experiments

A *Nonomuraea* seed culture, grown for 120 h under the conditions described above was used for inoculating (at 5%) 100 ml aliquots of medium in 500-ml baffled Erlenmeyer flasks. The shake-flasks were incubated with shaking at 150 rpm and 30 °C. Samples for glucose and A40926 analysis were taken every 24 h. In the medium-design experiments, the media contained (per liter): 20 g glucose, 3.65 g $(NH_4)_2SO_4$, 0.45 g $MgSO_4{\cdot}7H_2O$, 0.65 g KH_2PO_4, 5 g $CaCO_3$, 3 ml TMS-1 and one of the following additives: 1.0, 0.5, 0.1, or 0.05 g yeast extract, 1 ml vitamin solution 1, 1 ml vitamin solution 2, 5 g casamino acids (Difco, Detroit, Mich.), or a mixture of amino acids as follows: 0.08 g L-asparagine, 0.4 g L-glutamate, 0.18 g L-aspartate, 0.9 g L-lysine, 0.07 g L-phenylalanine, 0.25 g L-arginine, 0.1 g L-tyrosine and 0.125 g L-leucine. Vitamin solution 1 contained (per liter): 0.05 g biotin, 1 g Ca-pantothenate, 1 g nicotinic acid, 25 g myo-inositol, 1 g thiamine-HCl, 1 g pyridoxine-HCl and 0.2 g para-aminobenzoic acid, while vitamin solution 2 contained 1 mg niacin and 1 mg thiamine-HCl. The pH of the media was 7.5.

Fermentations

The fermentations were performed in 2-l double-jacketed Applikon fermentors (Applikon, Shiedam, Netherlands) containing 1.5 l medium, with an agitation rate of 500 rpm and aeration at 1 vvm. Dissolved oxygen tension was monitored with an oxygen electrode (Mettler Toledo, Greifensee, Switzerland) and did not fall below 50% saturation during any of the fermentations. The pH was kept at 7.5 by addition of 1 M NaOH; and the temperature was controlled at 28 °C. The fermentation media contained (per liter): 0.45 g $MgSO_4{\cdot}7H_2O$, 1 ml pluronic 3 ml TMS-1 and 1 ml vitamin solution 1, with glucose, KH_2PO_4 and $(NH_4)_2SO_4$ in the concentrations presented in Table 1.

Biomass dry weight

For the dry weight measurements, 5 ml fermentation broth was filtered through a 0.45-μm pore size predried filter (Supor-450, Pall Corporation, Ann Arbor, Mich.) and dried at 105 °C for approximately 24 h before weighing the filter.

Analysis of A40926

A40926 was analyzed by a HPLC method modified from Riva et al. [12]. Samples for A40926 analysis were prepared by mixing 1 ml fermentation sample with 3 ml 0.025 M KH_2PO_4, pH 11.9, vortexing, filtering the mixture through a 0.45-μm filter (Osmonics, Minnetonka, Minn.) and incubating the filtrate at 50 °C for 1 h. A40926 was analyzed by HPLC (HP-1100; Hewlett-Packard, Palo Alto, Calif.) with a reversed-phase column (Symmetry C-18, 5 μm, 4.6×150 mm; Waters, Milford, Mass.) and a UV detector (210 nm). The column temperature was 25 °C. The amount injected (standards, samples) was 20 μl and the flow rate was 2.0 ml min^{-1}. A gradient with two eluents (eluent A: 10% acetonitrile, 90% 0.025 M KH_2PO_4, pH 7.8; eluent B: 50% acetonitrile, 50% 0.025 M KH_2PO_4, pH 7.8) was applied, according to the following scheme (minutes, % eluent B): 0 min, 32%, 19 min, 60.5%, 21 min, 80%, 23 min, 80%, 25 min, 32%, 30 min, 32%. A40926 appeared as several peaks on the HPLC chromatogram, due to variations in the aliphatic side-chain of the compound [12, 16]. The area of the four main peaks (A, A1, B, B1) were used for calibration and analysis of the fermentation samples. The detection limit of A40926 was approximately 1 mg l^{-1}. A40926 standard was obtained from Biosearch Italia SpA, Gerenzano, Italy.

Table 1 Concentrations of KH_2PO_4, glucose and $(NH_4)_2SO_4$ in the fermentation media

Fermentation	KH_2PO_4 (g l^{-1})	Glucose (g l^{-1})	$(NH_4)_2SO_4$ (g l^{-1})
P300	0.65	20	3.65
P150	0.30	20	3.65
G8	0.30	8	3.65
G8LIM	0.30	8	3.65
G40	0.30	40	3.65
G40N7	0.30	40	7.3
P150p	0.30	20	3.65
P300p	0.65	20	3.65

Analysis of glucose, ammonium and phosphorus

Glucose was analyzed by HPLC (Agilent Technologies, Palo Alto, Calif.) with an Aminex HPX-87H column (Bio-Rad Laboratories, Hercules, Calif.) operating at 60 °C and a flow rate of 0.6 ml 5 mM H_2SO_4 min^{-1}, using a refractive index detector (Shimadzu, Tokyo, Japan). Ammonium was analyzed using an ion-selective ammonia electrode (Metrohm, Herisau, Switzerland). Phosphorus was analyzed using a spectrophotometric assay kit (inorganic phosphorus 80; Abx Diagnostics, Montpellier, France) in an automatic analyzer (Cobas Miras plus; Roche, Basel, Switzerland).

Carbon dioxide in exhaust gas

The partial pressure of CO_2 in the exhaust gas from the bioreactors was measured using a gas analyzer (industrial emission monitor type 1311; Brüel & Kjaer, Denmark).

Results and discussion

Design of a defined minimal medium

Defined growth media have been described for the glycopeptide-producing actinomycetes *Act. teichomyceticus* [7] and *Amy. orientalis* [11]. However, these media did not support *Nonomuraea* growth. Instead, a growth medium developed for a *S. nourseii* fermentation [8] was used as the basis for the medium design. This minimal medium could be used as a growth medium for *Nonomuraea*, when supplied with 1 g yeast extract l^{-1}. To obtain a growth medium with as low a content of complex components as possible, the yeast extract concentration was decreased or replaced with potentially vital factors, such as vitamins or amino acids in a series of shake-flask experiments. Since a buffer system containing solid particles was used, growth could not be measured by DCW and was instead monitored by glucose consumption. With 1 g yeast extract l^{-1} added to the medium, glucose was depleted after 144 h. Decreasing the yeast extract concentration to 0.5 g l^{-1} resulted in slower growth and glucose was depleted after 180 h. Further decreases of the yeast extract concentration, to 0.1 g l^{-1} and 0.05 g l^{-1}, led to even lower glucose consumption rates and glucose was not depleted during the time-course of the experiments (200 h). In the shake-flasks where yeast extract was replaced with an amino acid cocktail, casamino acids, or vitamin solution 2, slow glucose consumption was observed until approximately 100 h, whereafter the glucose concentration remained constant and no further growth occurred. The same growth pattern was also observed in the shake-flask containing the defined medium without additives; and this was presumably the result of carry-over of yeast extract from the inoculum. In contrast, the addition of 1 ml vitamin solution 1 l^{-1} supported growth and led to glucose depletion after 168 h. Importantly, this medium composition also allowed production of A40926 in as high titers as in the medium containing 1 g yeast extract l^{-1} (approximately 100 mg A40926 l^{-1}). Therefore, this medium and variations of it were used in the fermentation studies.

Initial phosphate concentration

Nonomuraea was cultivated in growth media containing either 300 mg or 150 mg phosphate l^{-1} (fermentations P300, P150, respectively). The specific growth rates in the two fermentations were similar (0.033 h^{-1} in P300, 0.035 h^{-1} in P150) until the depletion of phosphate in P150 at 50 h after inoculation. Interestingly, the biomass concentration ceased to increase at this point and remained approximately constant throughout the remainder of the fermentation for both P150 and P300 (Fig. 1A, B). Production of A40926 started after 46 h in both fermentations. Initially, the specific A40926 production rate was similar in the two fermentations (0.02 mg g^{-1} DCW h^{-1} in P300, 0.04 mg g^{-1} DCW h^{-1} in P150 in the period 46–59 h). However, after 59 h, the productivity increased markedly in fermentation P150 and A40926 was produced at a specific rate of 0.35 mg g^{-1}

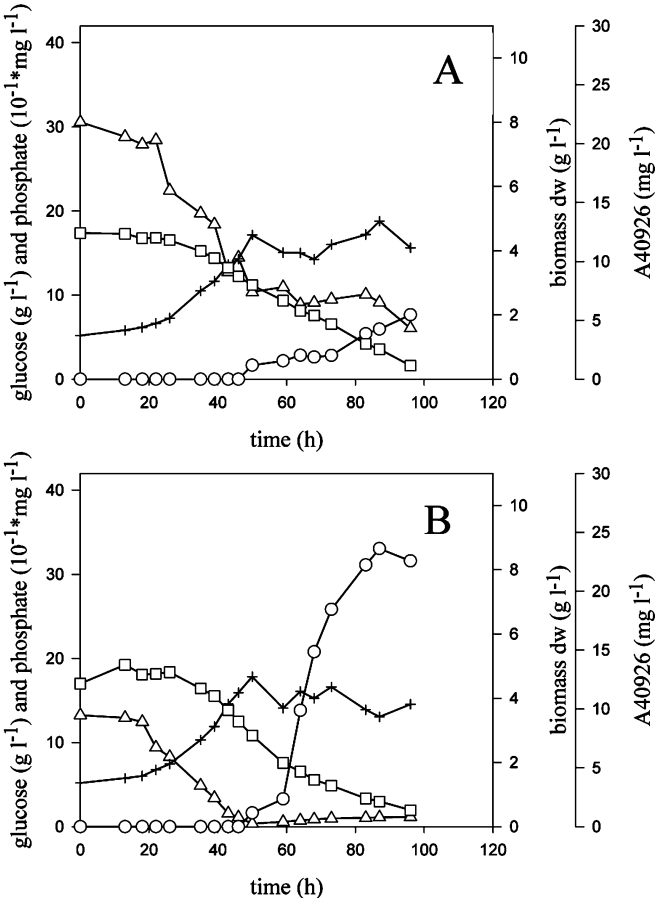

Fig. 1 *Nonomuraea* batch fermentations with initial phosphate concentrations of 300 mg l^{-1} (**A**, fermentation P300) and 150 mg l^{-1} (**B**, fermentation P150). The initial concentrations of glucose and ammonium sulfate were 20 g l^{-1} and 3.65 g l^{-1}, respectively. □ Glucose, Δ phosphate, ○ A40926, + biomass dry weight (*dw*)

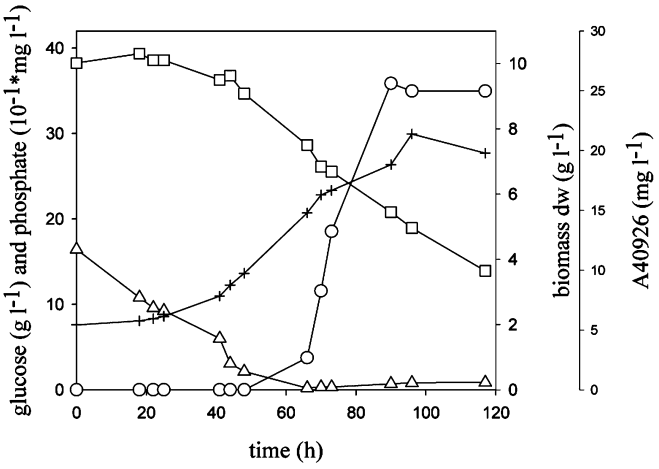

Fig. 2 Batch fermentation with an initial glucose concentration of 40 g l^{-1} and initial phosphate and ammonium sulfate concentrations of 150 mg l^{-1} and 3.65 g l^{-1}, respectively (G40). □ glucose, △ phosphate, ○ A40926, + biomass

DCW h^{-1} in the period 59–68 h. The specific productivity then declined in the period 68–87 h. The maximal A40926 concentration (23.6 mg l^{-1}) was reached at 87 h, whereafter the concentration of A40926 declined during the remainder of the fermentation. In P300, the concentration of A40926 was approximately constant in the period 59–73 h and then increased at a specific rate of 0.03 mg g^{-1} DCW h^{-1} during the final 23 h of fermentation.

Thus, phosphate limitation here (P150) resulted in an approximately 11-fold increase in specific A40926 productivity. It can also be concluded that the onset of secondary metabolism is not governed by the phosphate concentration in the medium, since A40926 production started at the same time in both fermentations. Particularly interesting is the growth arrest in P300, which takes place shortly after the onset of secondary metabolism, while phosphate is still abundant in the medium. As will be further discussed, this growth arrest could not have been due to depletion of other nutrients in the medium and is not likely to be the consequence of A40926 toxicity. Table 2 summarizes the growth and A40926 production parameters in fermentations P300 and P150, presented as average data from three different fermentations.

Initial glucose concentration

To investigate the effect of a higher glucose concentration, a fermentation with 40 g initial glucose l^{-1} and 150 mg initial phosphate l^{-1} (G40) was performed (Fig. 2). At this high glucose concentration, *Nonomuraea* grew at a lower specific growth rate (0.020 h^{-1}), compared with the previous fermentation with a lower glucose start concentration, P150 (Table 2). Phosphate was depleted after 66 h of growth. However, the biomass concentration continued to increase after phosphate depletion. A40926 production started between 48 h and 66 h after inoculation and the specific production rate was 0.26 mg g^{-1} DCW h^{-1} in the period 66–73 h. The maximum concentration of A40926 (25.6 mg l^{-1}) was found in the sample taken 90 h after inoculation. The specific productivity and maximum A40926 concentration reached in G40 were similar to those obtained in P150 (Table 2). Thus, an increased initial glucose concentration resulted in a decreased specific growth rate but did not affect A40926 productivity.

Glucose limitation in fed-batch fermentation

In this experiment, the effects of glucose limitation and a decreased initial glucose concentration were studied. Two *Nonomuraea* cultivations with 8 g initial glucose l^{-1} were set up in parallel. In fermentation G8 (Fig. 3A), the glucose concentration was kept in the range 3–10 g l^{-1} by the addition of small glucose pulses. In fermentation G8LIM (Fig. 3B), glucose was allowed to be depleted, at which point a feed of glucose into the fermentor was started. The glucose feed was regulated so that the carbon dioxide evolution rate was kept at 70–80% of the parallel reference fermentation, G8, ensuring glucose limitation. Phosphate was depleted in G8 and G8LIM after 58 h of growth, coinciding with glucose depletion in G8LIM. Until this time, both cultivations were

Table 2 Growth and production parameters. *Number of generations*. The generation time between inoculation and the time of A40926 production start ($\mu t_{\text{production start}}$). See Materials and methods for parameters

Fermentation	μ before PO_4 depletion (h^{-1})	Y_{SX} before PO_4 depletion (g dry weight g^{-1} $glucose^{-1}$)	Number of generations	r_P before PO_4 depletion (mg A40926 g^{-1} cells h^{-1})	Maximum r_P after PO_4 depletion (mg A40926 g^{-1} cells h^{-1})
P300	0.029 ± 0.006	0.38 ± 0.08	1.27 ± 0.28	0.04 ± 0.01	–
P150	0.033 ± 0.004	0.44 ± 0.06	1.46 ± 0.15	0.06 ± 0.01	0.25 ± 0.09
G40	0.020	0.63	0.96[a]	0.03[a]	0.26
G8	0.037	0.36	1.70	0.04	0.11
G8LIM	0.037	0.36	1.66	0.06	0.06
G40N7	0.013	0.40	1.42	–	0.01
P150p	0.036	0.43	1.13	0.07	0.23
P300p	0.032[b]	0.47[b]	1.20	0.05	0

[a] A40926 production might have started at a later time
[b] Exponential growth until the start of A40926 production

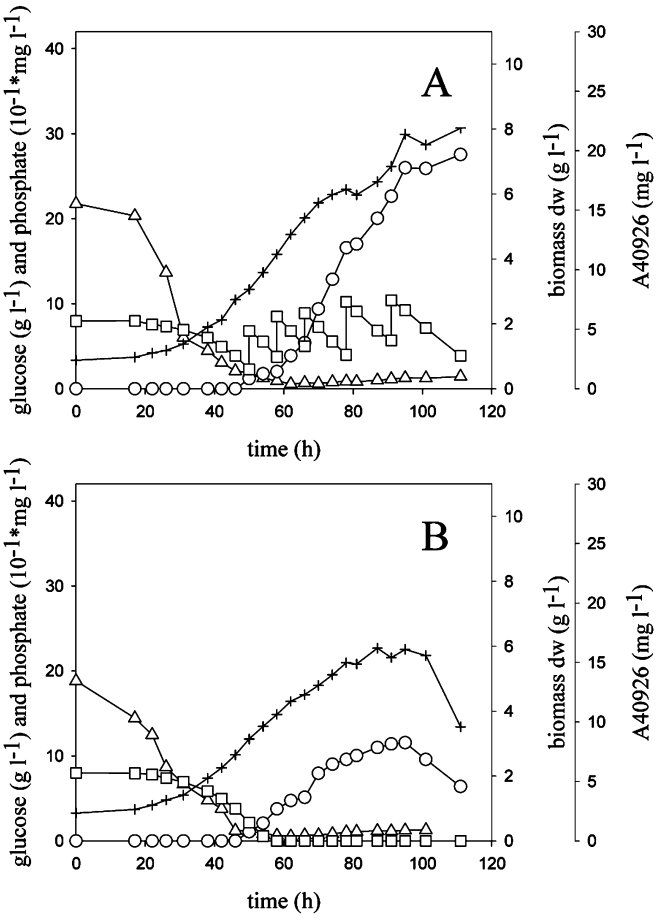

Fig. 3A, B Fermentations with an initial glucose concentration of 8 g l^{-1} and initial phosphate and ammonium sulfate concentrations of 150 mg l^{-1} and 3.65 g l^{-1}, respectively. **A** Batch fermentation with glucose supplied in small pulses to avoid depletion (G8). **B** Glucose-limited fed-batch fermentation (G8LIM). □ glucose, △ phosphate, ○ A40926, + biomass

growing at a specific growth rate of 0.037 h^{-1}. After the depletion of phosphate, the biomass concentration continued to increase in both fermentations, but at a lower rate in the glucose limited cultivation. A40926 production started after 46 h of growth in both fermentations. In G8, A40926 was produced at a specific rate of 0.04 mg g^{-1} DCW h^{-1} in the period 46–58 h, i.e. until the depletion of phosphate. The specific productivity then increased to 0.08 mg g^{-1} DCW h^{-1} in the period 58–66 h and further to 0.11 mg g^{-1} DCW h^{-1} in the period 66–78 h. The productivity then decreased to 0.06 mg g^{-1} DCW h^{-1} in the period 81–95 h. From 95 h until the end of the fermentation, the A40926 concentration increased only marginally. The maximum A40926 concentration, 19.8 mg l^{-1}, was present in the sample taken at 115 h.

In G8LIM, A40926 was produced at a specific rate of 0.06 mg g^{-1} DCW h^{-1} in the period 46–74 h, whereafter the specific production rate declined. The maximal concentration of A40926 (9.1 mg l^{-1}) was obtained after 95 h, whereafter the concentration of A40926 decreased. These results demonstrate that glucose limitation in addition to phosphate limitation does not enhance A40926 production. In contrast, glucose limitation seems to counteract the effect of phosphate limitation, since no increase in the specific A40926 productivity was observed after glucose and phosphate limitation were simultaneously imposed.

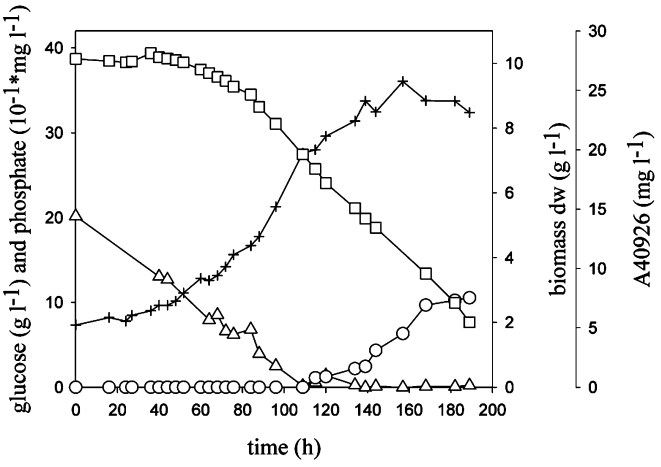

Fig. 4 Batch fermentation with an initial ammonium sulfate concentration of 7.3 g l^{-1} (G40N7). The initial concentrations of glucose and phosphate were 40 g l^{-1} and 150 mg l^{-1}, respectively. □ glucose, △ phosphate, ○ A40926, + biomass

Initial ammonium concentration

To assess the influence of ammonium on *Nonomuraea* growth and A40926 production, a batch fermentation was performed with a higher initial concentration of ammonium (G40N7). The increased initial ammonium concentration resulted in a substantially decreased specific growth rate (0.013 h^{-1}), a markedly decreased specific A40926 productivity and a later onset of the secondary metabolism, compared with fermentations with lower initial ammonium concentrations (Fig. 4, Table 2). Even though antibiotic production started late in this fermentation, the onset of secondary metabolism was in fact not delayed in relation to growth. Production of A40926 started after 1.42 generation times in G40N7, which is essentially in the same range as in the previously discussed fermentations (Table 2). The residual ammonium concentration (data not shown) at the time of production start was higher in fermentation G40N7 than in the fermentations with lower initial ammonium concentration (P150, P300, G8, G40, G8LIM). Thus, the onset of A40926 production was not controlled by the ammonium concentration in the medium.

In order to investigate a possible effect of ammonium limitation, fermentations were also performed where ammonium was the first nutrient to be depleted (data not shown). These experiments showed that ammonium depletion did not boost antibiotic production, but in fact caused the production of A40926 to be interrupted. This effect can be explained by the requirement for nitrogen in biosynthesis of A40926 precursors.

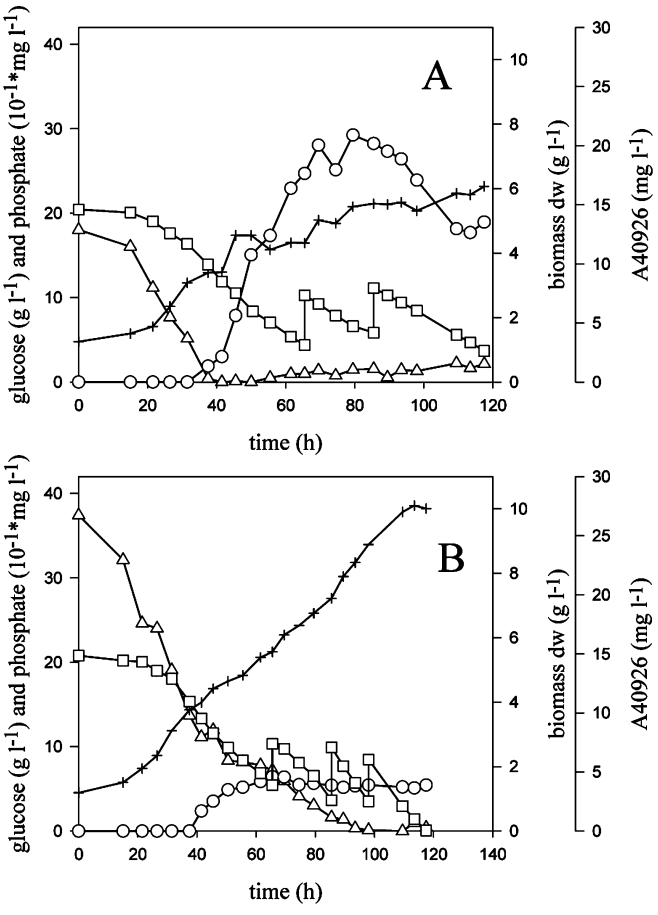

Fig. 5A, B Batch fermentations with pulses of glucose and ammonium added in the late part of the fermentation. **A** Initial phosphate concentration of 150 mg l^{-1} (P150p). **B** Initial phosphate concentration of 300 mg l^{-1} (P300p). □ glucose, △ phosphate, ○ A40926, + biomass

Pulse experiments

In P150, G8 and G40, ammonium was depleted in the medium approximately at the same time as the concentration of A40926 ceased to increase. In an attempt to prolong the production phase, ammonium and glucose were added in pulses towards the end of two batch cultivations. The starting media contained 20 g glucose l^{-1} and 300 mg or 150 mg phosphate l^{-1} (fermentations P300p, P150p, respectively).

In the fermentation with the low initial phosphate concentration (Fig. 5A), *Nonomuraea* grew at a specific growth rate of 0.036 h^{-1}, and phosphate was depleted after 41.5 h. After phosphate depletion, the biomass concentration was approximately constant for 20 h, whereafter it slowly increased throughout the fermentation. Pulses of ammonium and glucose were added to the medium after 65.5 h and 85.5 h, keeping these nutrients from being depleted. A40926 production started after 31.5 h and was produced at a specific rate of 0.07 mg g^{-1} DCW h^{-1} from 31.5 h to 41.5 h. After this period, the specific productivity increased to 0.23 mg g^{-1} DCW h^{-1} between 41.5 h and 50.5 h. The specific productivity then decreased somewhat and the maximum A40926 concentration, 20.9 mg l^{-1}, was obtained 79.5 h after inoculation. After this time, the concentration of A40926 decreased. Thus, the production phase was not prolonged by the additional supply of ammonium and glucose, indicating that either the long-lasting phosphate limitation negatively influenced the ability to synthesize glycopeptide or a different medium component, for example a vitamin or trace metal, limited the biosynthesis.

In the fermentation with the high phosphate start concentration, ammonium and glucose were added in pulses at 65.5, 85.5 and 98 h and were not depleted during the time-course of the fermentation. Here, *Nonomuraea* grew at a specific growth rate of 0.032 h^{-1} until 37.5 h after inoculation, whereafter growth continued at a substantially decreased specific growth rate (0.014 h^{-1}). This shift in specific growth rate occurred when A40926 production started. A40926 was produced at a specific rate of 0.05 mg g^{-1} DCW h^{-1} at 37.5–55.5 h, whereafter the A40926 concentration remained constant until the end of the fermentation. Interestingly, A40926 production did not resume after the depletion of phosphate at 98 h. This observation supports the suggestion that a medium component other than ammonium might limit A40926 production at this late stage of the fermentation.

In P300p, a marked decrease in specific growth rate occurred at the start of A40926 production. Similarly, the biomass concentration ceased to increase shortly after the start of A40926 production in the previously discussed fermentation, P300. Thus, it appears the onset of secondary metabolism in *Nonomuraea* results in growth impairment. However, this effect was not observed in G8, where exponential growth continued unaltered after the A40926 production start. It is therefore not likely that the growth arrest, sometimes observed in *Nonomuraea* fermentation, is due to autotoxicity of A40926. Nor can it be explained by possible depletion of vital medium components, since measurable components were still abundant at the time of growth arrest/growth impairment and the remaining components (i.e. magnesium sulfate, trace metals, vitamins) supported exponential growth to (in this context) high biomass concentrations (G8, G40N7). We have no mechanistic explanations for these variations in *Nonomuraea* fermentations but it is known that the previous growth conditions of the cells have a significant impact on their metabolism and even small variations in the inoculum preparation may therefore affect the fermentation profile substantially.

Conclusions

A defined minimal medium was developed that supports *Nonomuraea* growth and production of the glycopeptide antibiotic A40926. Using this medium as a basis, the influence of phosphate, glucose and ammonium concentrations on growth and glycopeptide production by *Nonomuraea* was investigated. It was shown that phos-

phate limitation substantially improved A40926 productivity, while glucose limitation had no such effect. An increased initial glucose concentration led to a slightly decreased specific growth rate but had no effect on glycopeptide productivity. Furthermore, high initial ammonium concentrations resulted in impaired growth and decreased productivity of the antibiotic. Initiation of A40926 production was not controlled by the concentrations of phosphate, glucose or ammonium, since antibiotic production started at different residual concentrations of these nutrients in the different fermentations. Rather, A40926 synthesis appeared to start at a certain stage of growth, i.e. after 1.1–1.7 generation times. However, it is possible that the onset of production is governed by the residual concentration of one of the medium components that were not varied in this study.

The antibiotic production phase in phosphate-limited medium could not be prolonged by supplying additional ammonium and glucose in pulses towards the end of the fermentation. From this, it was deduced that depletion of another medium component, such as a trace metal or vitamin, might be limiting the glycopeptide biosynthesis in the medium used in this study. Therefore, a phosphate-limited fed-batch or continuous process might be suitable for A40926 production.

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