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Effects of constant and shifting dissolved oxygen concentration on the growth and antibiotic activity of *Xenorhabdus nematophila*

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ABSTRACT

To evaluate the effects of dissolved oxygen (DO) control strategy on cell growth and the production of antibiotic (cyclo(2-Me-BABA-Gly)) by *Xenorhabdus nematophila*. The effects of different agitation speeds and DO concentrations on cell growth and antibiotic activity of *X. nematophila* YL001 were examined. Experiments showed that higher agitation speeds and DO concentrations at earlier fermentation stage were favorable for cell growth and antibiotic production. At mid- and later-stage, properly decreasing DO concentration can strengthen cell growth and antibiotic production. Based on the kinetic information about the effects of agitation speeds and DO concentrations on the fermentation, the two-stage DO control strategy in which DO concentration was controlled to 70% in the first 18 h, and then switched to 50% after 18 h, was established to improve the biomass and antibiotic activity. By applying this DO-shift strategy in *X. nematophila* YL001 fermentation, maximal antibiotic activity and biomass reached 252.0 ± 6.10 U/mL and 30.04 ± 2.50 g/L, respectively, thus was 18.99% and 15.36% more than in the cultures at constantly 50% DO.

1. Introduction

Xenorhabdus nematophila is a Gram-negative bacterium, belonging to the family Enterobacteriaceae which is symbiotically associated with the entomopathogenic nematode of the genus *Steinernema* (Steinernematidae) (Thomas and Poinar, 1979). The production of metabolites with antibiotic properties is a characteristic common to the bacteria. *X. nematophila* has been known to produce xenocoumacins (benzopyranone derivatives) (McInerney et al., 1991), nematophin (indoles derivatives) (Li et al., 1997), benzylineacetone (monoterpenoid) (Ji et al., 2004), and xenematide (peptides) (Lang et al., 2008). These metabolites not only have diverse chemical structures, but also have a wide range of bioactivities of medicinal and agricultural interest, such as antibiotic, antimycotic, insecticidal, nematicidal, antiulcer, antineoplastic and antiviral properties (Webster et al., 2002). These naturally occurring antibiotics provide useful leads in the research and development of drugs and agrochemicals (Bode, 2009).

We isolated a new strain of *X. nematophila* YL001 from the symbiotic nematode, *Steinernema* sp. YL001, collected in China, the strain has unique genetic (e.g., 16S ribosomal DNA sequence) and biochemical characteristics compared to other isolates of this genus (Wang and Zhang, 2006; Fang et al., 2008a). *In vitro* and *In vivo*, the bacterium showed high antibiotic activity against some bacteria and fungi such as *Phytophthora capsici*, *Blumeria graminis* and *Pseudoperonospora cubensis*, which have not been thoroughly evaluated as antifungal targets of *X. nematophila* (Fang et al., 2008b). The study showed for the first time the potential of these products to control diseases of living plants although such crude mixtures were unlikely to be registered for use in commercial agriculture. Moreover, a new antibiotic, cyclo(2-Me-BABA-Gly), was isolated from the strain (Li, 2006). The results suggest that *X. nematophila* YL001 is a unique, potential resource of new agrochemicals, and antimicrobial compounds.

Antibiotic production by *X. nematophila* differs qualitatively and quantitatively depending on the strains of bacteria and their culture conditions (Webster et al., 2002). *Xenorhabdus* spp. grows facultatively anaerobic, but oxygen supply is identified as one of the most important factors affecting the cell growth and antibiotic production (Akhurst, 1982; Chen et al., 1996; Yang et al., 2001, 2006; Wang et al., 2008). However, most of the studies on the antibiotic production are confined to shake flasks, where the quantification of dissolved oxygen (DO) level is rather difficult. There are some reports on the effect of agitation and aeration rates on the production of antibiotic in a bioreactor (Wang and Zhang, 2007). However, these results did not include the effect of quantitative dissolved

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Nomenclature

DCW	dry cell weight (g/l)
X_{max}	maximal cell density (g/l)
P_{max}	maximal antibiotic activity (U/mL)
P_X	cell production rate (g/L/h)
P_P	antibiotic productivity (U/mL/h)
q_p	specific antibiotic production rate (U/g/h)
$\overline{q_p}$	average specific antibiotic production rate (U/g/h)
$Y_{X/S}$	cell yield on glucose (g/g)
$Y_{P/S}$	antibiotic yield on glucose (U/g)
$Y_{P/X}$	antibiotic yield on cell (U/g)

Greek letter

μ	specific cell growth rate (h^{-1})
$\overline{\mu}$	average specific cell growth rate (h^{-1})
μ_{max}	maximal specific cell growth rate (h^{-1})

oxygen concentration (constant DO level) on the cell growth and antibiotic production. Also, in general, the cell growth and product accumulation vary with medium composition and process parameters, including dissolved oxygen concentration. In order to obtain high biomass and metabolites, it is important to optimize the conditions for cell growth and metabolite biosynthesis during *X. nematophila* fermentation. Well-directed process parameters shift representing a valuable control strategy will be beneficial to the cell growth and metabolite biosynthesis. Shift in concentration of dissolved oxygen (DO-shift) has been proven to be an efficient strategy for the production of bioactive metabolites in other microorganism (Tang et al., 2009; Wang et al., 2005; Mao and Zhong, 2004; Li et al., 2002).

To our knowledge, until now there is no report about the effect of dissolved oxygen concentration control strategy based on the kinetic information about the constant DO concentrations process on cell growth and antibiotic activity of *X. nematophila*. Therefore, it would be interesting to investigate whether the DO control strategy will lead to a quantum improvement in its biomass and antibiotic activity. The objective of this work was to evaluate the effects of DO control strategy on cell growth and antibiotic activity of *X. nematophila* YL001 in batch fermentation. An optimum DO control strategy was proposed to optimize its cell growth and metabolites biosynthesis, and to optimize fermentation efficiency.

2. Methods

2.1. Microorganism

X. nematophila YL001 was isolated from its nematode symbiont, *Steinernema* sp. YL001 obtained from the soil of Yangling, China. Phase I variant of the bacteria was used throughout the study.

X. nematophila YL001 was maintained on nutrient agar (NA) slants and subcultured monthly. Due to the instability of the phase I under normal culture conditions, glycerinated stocks of the bacteria frozen at −70 °C were used as a starter inoculum for cultures. NBTA medium, NA supplemented with triphenyltetrazolium chloride 0.040 g/L and bromothymol blue 0.025 g/L, was used to test the phase variant of the bacteria. Phase I is distinguished from phase II by its adsorption of bromothymol blue to produce a red core colony overlaid by dark blue and surrounded by a clear zone after 2–3 days of incubation in darkness at 28 °C.

2.2. Inoculum preparation

A loopful of the phase I of *X. nematophila* YL001 growing on an NBTA plate was inoculated into a 250 mL flask containing 100 mL fresh NB (NA without agar) medium, which was adjusted to a final pH of 7.20, and then cultivated in darkness at 28 °C on an Eberbach rotary shaker at 150 rpm for 16–24 h, during which time the optical density (600 nm) was approximately between 1.50 and 2.00.

2.3. Fermentation process in shake flasks

The effect of aeration on the cell growth and antibiotic activity was studied in shake flask containing various volumes of fermentation media to give different levels of dissolved oxygen (DO). 500 ml Erlenmeyer flask contained 50, 100, 150, 200 and 250 ml medium consisting of the following components (g/L): glucose 6.13, peptone 21.29, $MgSO_4{\cdot}7H_2O$ 1.50, $(NH_4)_2SO_4$ 2.46, KH_2PO_4 0.86, K_2HPO_4 1.11 and Na_2SO_4 1.72 (Wang et al., 2008a,b). The medium pH was adjusted to 7.0 by adding 1 mol/L NaOH or 1 mol/L HCl. Ten percent (v/v) of the seed culture was used to inoculate the flasks. The culture was incubated on a rotary shaker at 28 °C and 150 rpm for 72 h. Three batches were repeated for each experiment.

2.4. Batch fermentation process in 5-L laboratory-scale fermentor

The effect of DO on the strain culture was also studied by batch fermentation in a 5-L bioreactor (Eastbio, China) with a working volume of 3.5 L. The fermenter was equipped with one six-blade disk turbine impeller, the probes of pH (Mettler-Toledo GmbH, Switzerland), DO (Mettler-Toledo GmbH, Switzerland), temperature and foam. Temperature, pH, dissolved oxygen and agitation speed were measured on-line. The medium, inoculum ratio, pH and temperature used in this study were the same as that of the shake flask culture. The aeration ratio was 2.5 L/min. A pH profile was adjusted to a set pH with 2.0 mol/L NaOH and 2.0 mol/L HCl. Temperature was controlled automatically. The fermenters were incubated according to the culture condition for 72 h.

Four cultures were carried out simultaneously in the fermenter with homogeneous cell source under well-controlled process conditions but at different test culture conditions.

2.4.1. Effect of agitation speed

During the entire fermentation process, the agitation speed was constantly controlled at 100, 200, 300 and 400 rpm, respectively, to investigate the effects of different agitation speeds on cell growth and antibiotic activity of *X. nematophila* YL001 with aeration rate fixed at 2.5 L/min.

2.4.2. Effect of DO

In the fermentation at different DO level, the DO was controlled at 10%, 30%, 50% and 70% by adjusting agitation speeds at 100–800 rpm. Aeration rate was fixed at 2.5 L/min.

2.4.3. DO-shift culture

Based on the impact of DO, a DO-shift culture was proposed by combining the first-stage (0–18 h) at a DO concentration of 70% with the following culture at 50% DO level (i.e., the second-stage) (18–72 h). The control experiment was conducted without the DO control in the second-stage culture for comparison. The other culture conditions were the same as in the experiments above.