Microbiological Research **165** (2010) 221—231

Available online at www.sciencedirect.com

ScienceDirect

Microbiological Research

www.elsevier.de/micres

SigN is responsible for differentiation and stress responses based on comparative proteomic analyses of *Streptomyces coelicolor* wild-type and *sigN* deletion strains

Chunxia Wang, Xiaohui Long, Xuming Mao, Huijun Dong, Linxi Xu, Yongquan Li*

Zhejiang University, Institute of Biochemistry, Hangzhou 310058, China

Received 26 March 2009; received in revised form 12 May 2009; accepted 16 May 2009

KEYWORDS
SigN;
Streptomyces coelicolor;
Differentiation;
Stress response;
2DE

Summary

To address the functions of SigN (SCO4034) in *Streptomyces coelicolor*, we constructed a *sigN* null mutant M145Z, which showed a defect in sporulation. The differential proteomic profiles of wild-type *S. coelicolors* M145 and M145Z were demonstrated by two-dimensional polyacrylamide gel electrophoresis (2D PAGE), which identified 24 different spots that were up- or down-regulated due to *sigN* disruption. Among them, 22 proteins were identified by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS). The up-regulated proteins were involved in energy metabolism, stress responses, ATP binding, ppGpp stringent stress response and transcriptional anti-termination, etc. The down-regulated proteins were related to antibiotic synthesis and differentiation. The results gave a new insight into the regulatory mechanism of SigN in *S. coelicolor*. Furthermore, deletion of *sigN* caused growth retardation under all stress conditions examined including heat, cold, acid, oxidation, salt and ethanol. These results indicated that SigN was involved in morphological development, secondary metabolism and stress responses in *S. coelicolor*.

Introduction

The sigma factor plays a pivotal role in the bacterial survival strategies such as stress responses, differentiation, social behavior and pathogenesis (Gruber and

*Corresponding author. Tel.: +86 571 8820 6632; fax: +86 571 8820 8569.
E-mail address: lyq@zju.edu.cn (Y. Li).

0944-5013/$ - see front matter © 2009 Elsevier GmbH. All rights reserved.
doi:10.1016/j.micres.2009.05.003

Gross 2003). Usually, bacteria have one housekeeping factor and a variable number of alternative sigma factors that possess different promoter-recognition properties to alter its transcriptional program in response to stress. Furthermore, alternative sigma factors can regulate plentiful genes to control the biological process under stress condition. σ^B, as an alternative sigma factor, is a master general stress regulator in the expression of more than 200 genes under heat, acid, ethanol, salt and oxidative stress in *Bacillus subtilis* (Hecker and Volker 1998; Petersohn et al. 2001).

Streptomyces bacteria live in the soil and are often challenged with diverse environmental stresses such as nutritional starvation, high osmolarity and non-optimal pH or temperature in its environment and development processes. *Streptomyces coelicolor*, as a model representative of *Streptomyces*, is a Gram-positive bacterium with a complex lifecycle and a unique capacity for the production of a multitude of varied and complex secondary metabolites including red-pigmented tripyrrole undecylprodigiosin (Red), blue-pigmented polyketide actinorhodin (Act), Mmy and lipopeptide calcium-dependent antibiotic (CDA) (Huang et al. 2001). The genome sequence of *S. coelicolor* revealed 10 group 3 sigma factors (Bentley et al. 2002; Hahn et al. 2003) including σ^B(SCO0600), σ^L(SCO7278), σ^I(SCO3068), σ^N(SCO4034), σ^F(SCO4035), σ^H(SCO524), σ^K(SCO6520), σ^M(SCO7314), σ^G(SCO7341) and σ^{WhiG}(SCO5621) in the order of similarity to σ^B of *B. subtilis*. In *S. coelicolor*, the environmental stresses always trigger cell morphological differentiation associated with secondary metabolism (Viollier et al. 2003a). Among these sigma factors, σ^H is involved in sporulation and salt-stress response (Sevcikova et al. 2001; Viollier et al. 2003b), and σ^B plays a key role in osmotic and oxidative responses, which are required for both aerial hyphal formation and antibiotic biosynthesis (Cho et al. 2001; Lee et al. 2005; Viollier et al. 2003a). σ^F and σ^{WhiG} are the sporulation-specific sigma factors (Homerova et al. 2000; Tan et al. 1998). Moreover, microarray analysis revealed σ^B-dependent induction of more than 280 genes by 0.2 M KCl (Lee et al. 2005). The sigma factor shows a strong emphasis on the regulation of stress response and differentiation in *S. coelicolor*.

Two-dimensional electrophoresis (2DE) combined with mass spectrometry (MS) has been considered as a widely used tool for investigating protein expression profiles, which can provide insight into biological processes (Hamdan and Righetti 2005.). In this report, σ^N (SCO4034) from *S. coelicolor* was identified to be involved in differentiation and stress responses in *S. coelicolor*. Using the 2DE-MS analysis, the molecular regulation network of SigN was also established.

Materials and methods

Bacterial strains, growth conditions and chemicals

Growth and maintenance of *S. coelicolor* M145 and its derivative were done as described by Kieser et al. (2000). For liquid culture, the spores were germinated in the $2\times$YT culture (about 10^8 to $\sim10^9$ spores/100 ml of broth). To apply stresses in liquid cultures, $2\times$YT culture with 4% NaCl, 4% ethanol, 1 mM H_2O_2, pH to 5.3 and temperature at 37 or 25 °C were used to culture the spores of M145 and its derivative for various lengths of time before harvesting. And antibiotic synthesis was determined in R_5^- liquid medium. For plate culture, the morphological study was conducted on the R_5 plate.

For 2DE analysis, IPG buffer (pH3–10 NL), Immobiline drystrip (pH3–10 NL), drystip cover fluid, urea, bromophenol blue, CHAPS, agarose, acrylamide, Bis, Tris, SDS and TEMED were from Amersham Biosciences (AB, Uppsala, Sweden). DTT and iodoacetamide were from Sigma (St. Louis, MO, USA). DC deionized water was obtained in the laboratory using Milli-Q equipment.

DNA manipulations

Escheriachia coli TG1, methylation-negative *E. coli* ET12567 and *S. coelicolor* M145 cells were used as hosts for various recombinant DNAs. *E. coli* were cultured as described previously (Sambrook et al. 1989). LB broth or plate was supplemented with ampicillin (*Amp*, 100 μg ml^{-1}), kanamycin (*Km*, 50 μg ml^{-1}) or apramycin (*Apra*, 50 μg ml^{-1}). For selection of mutant, R_5 plate with kanamycin (50 μg ml^{-1}) or apramycin (50 μg ml^{-1}) was used. All antibiotics were purchased from Sigma. Restriction enzymes were used according to the manufacturer's recommendations (TOYOBO, TAKARA). Standard recombinant DNA methods were used.

Gene disruption and complementation

The *sigN* disruptive mutant was constructed via double crossover homologous recombination as described (Figure 1A) (Wang et al. 2007). Briefly, the upstream (*sl*) and downstream (*sr*) gene fragments of *sigN* were cloned with primers of *sl*5, *sl*3 and *sr*5, *sr*3 and genomic DNA of