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SanJ, an ATP-dependent picolinate-CoA ligase, catalyzes the conversion of picolinate to picolinate-CoA during nikkomycin biosynthesis in *Streptomyces ansochromogenes*

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Abstract

Nikkomycins, a group of peptidyl nucleoside antibiotics, are competitive inhibitors of chitin synthase. The nikkomycin biosynthetic gene cluster has been cloned previously from *Streptomyces ansochromogenes*. The cluster contains 25 complete ORFs including *sanJ*. The *sanJ* gene was inactivated by the insertion of a kanamycin resistance gene and the resulting disruption mutants failed to produce nikkomycins. Moreover, the nikkomycin production was restored by complementation with a single copy of *sanJ*. The deduced product of *sanJ* bears striking sequence similarity with enzymes belonging to the adenylate-forming superfamily. *sanJ* was overexpressed as a His6-tagged fusion protein in *Escherichia coli* and purified to apparent homogeneity by affinity chromatography. The purified SanJ demonstrated adenylate ligase activity in the presence of picolinate or its analogs (benzoate, nicotinate, 4-methoxybenzoate, 4-hydroxybenzoate), ATP and Mg^{2+}. SanJ was also found to catalyze the conversion of picolinate, benzoate, nicotinate to their corresponding CoA esters and 4-methoxybenzoate, 4-hydroxybenzoate to their respective AMP derivatives in vitro. This was unambiguously shown by using HPLC and electrospray ionization mass spectrometry (ESI-MS) or by comparing the reaction product with an authentic standard of benzoyl-CoA. These results indicated that *sanJ* encodes an ATP-dependent picolinate-CoA ligase which is essential for nikkomycin biosynthesis.

Keywords: Nikkomycin biosynthesis; *Streptomyces ansochromogenes*; *sanJ*; Picolinate-CoA ligase; Picolinate; Adenylate-forming enzymes

1. Introduction

Nikkomycins, a group of peptidyl nucleoside antibiotics, are produced by *Streptomyces ansochromogenes* (Chen et al., 2000) and *Streptomyces tendae* (Brillinger, 1979). Because of their structural similarity to UDP-*N*-acetylglucosamine, a natural substrate of chitin synthase, nikkomycins act as strong competitive inhibitors of the enzyme and inhibit the growth of filamentous fungi, insects, acarids and yeasts (Dahn et al., 1976). Nikkomycins X and Z, the main components produced by *S. ansochromogenes*, consist of a peptidyl moiety covalently linked to a nucleoside moiety (Fig. 1A). Particular attention has been drawn to nikkomycin Z for its efficient activity against the highly chitinous, pathogenic, dimorphic fungi *Coccidioides immitis* and *Blastomyces dermatitidis* (Hector et al., 1990) and human microsporidian species *Encephalitozoon hellem* (Bigliardi et al., 2000). Meanwhile, nikkomycin Z and echinocandins have synergic effect against clinical isolated *Aspergillus fumigatus* (Ganesan et al., 2004). Since nikkomycins display low toxicity to mammals and bees, and are easily degraded in nature, they can be used as ideal insecticides and acaricides in agriculture and as antifungal agents in human therapy.

Studies demonstrated that an aminotransferase was essential for the conversion of L-lysine to piperideine-2-carboxylate (P2C) in peptidyl moiety initiation of nikkomycin biosynthesis (Bruntner and Bormann, 1998). A monomeric sarcosine oxidases (NikD) was found to be

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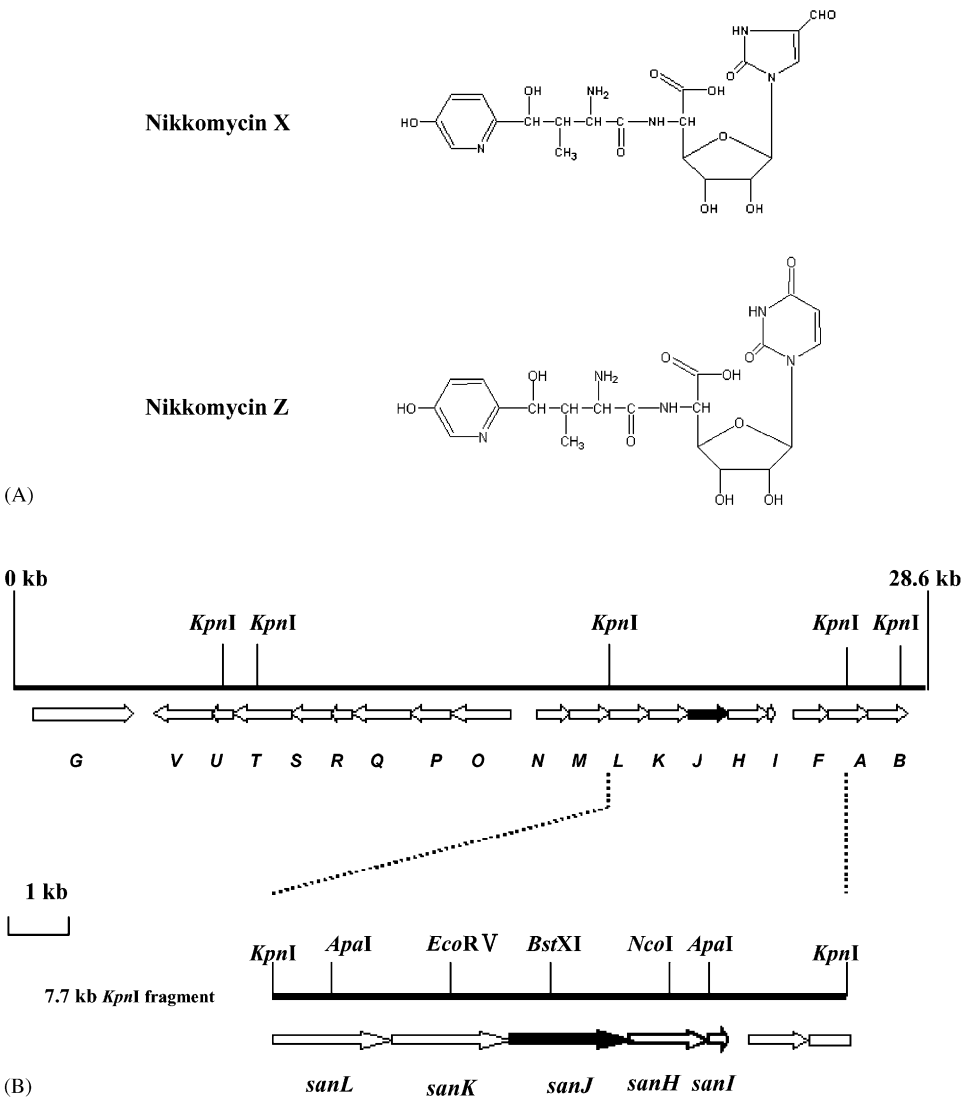

Fig. 1. Chemical structure of nikkomycins and genetic organization of a partial nikkomycin biosynthetic gene cluster in *Streptomyces ansochromogenes* 7100. (A) Chemical structure of nikkomycins X and Z. (B) Organization of a partial nikkomycin biosynthetic gene cluster containing *sanJ*. The solid arrow shows *sanJ* and its orientation. The 7.7 kb *Kpn* I fragment is used as template for PCR amplification of *sanJ* as detailed in Section 2.

responsible for the conversion of P2C to picolinate (Bruckner et al., 2004; Venci et al., 2002). *nikE* gene disruption and its feeding with hydroxypyridylhomothreonine (HPHT) to restore nikkomycin production indicated that *nikE* participated in HPHT formation (Bruntner et al., 1999). Many other enzymatic reactions were proposed to elucidate the biosynthesis of peptidyl moiety from molecular characterization of the nikkomycin biosynthetic genes in *S. tendae* (Bruntner et al., 1999; Lauer et al., 2001). The biosynthetic precursors of the nucleoside moieties of nikkomycins X and Z are ribose and histidine, or ribose and uracil with phosphoenolpyruvate (Schuz et al., 1992; Chen et al., 2002). In *S. ansochromogenes*, we have functionally identified six structural genes involved in the nikkomycin biosynthesis (Chen et al., 2000; Zeng et al., 2002; Wang et al., 2003; Li and Tan, 2003; Li et al., 2004, 2005). Recently, *sanG* located upstream of *sanV* has been characterized as a pathway-specific transcriptional activator for nikkomycin biosynthesis (Liu et al., 2005). However, the pathway of nikkomycin biosynthesis is still not well understood.

In order to obtain greater insight into nikkomycin biosynthesis and to explore the exact destination of picolinate, we have investigated *sanJ*, a gene located in the nikkomycin biosynthetic gene cluster of *S. ansochromogenes*. The product of *sanJ*, SanJ shows homology to adenylate-forming enzymes which could activate different carboxylic acids for their subsequent biochemical biosynthesis (Ishiyama et al., 2004; Namwat et al., 2002; de Crecy-Lagard et al., 1997; Mallonee et al., 1992). An acyl-CoA ligase (SnbA) activates 3-hydroxy-picolinate to adenylate aromatic acids during pristinamycin biosynthesis in *S. pristinaespiralis* (de Crecy-Lagard et al., 1997). It is anticipated that SanJ may function in the activation of