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Cloning, Expression, and Characterization of the *Micromonospora viridifaciens* Neuraminidase Gene in *Streptomyces lividans*

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We have cloned the *Micromonospora viridifaciens* neuraminidase (EC 3.2.1.18) gene (*nedA*) in *Streptomyces lividans*. This was accomplished by using the vector pIJ702 and *BglII-BclI* libraries of *M. viridifaciens* chromosomal inserts created in *S. lividans*. The libraries were screened for the expression of neuraminidase by monitoring the cleavage of the fluorogenic neuraminidase substrate 2′-(4-methylumbelliferyl)-α-D-*N*-acetylneuraminic acid. Positive clones (BG6, BG7, BC4, and BC8) contained the identical 2-kb *BclI-BglII* fragment and expressed neuraminidase efficiently and constitutively using its own promoter in the heterologous host. From the nucleotide sequence analysis, an open reading frame of 1,941 bp which encodes a polypeptide with an M_r of 68,840 was detected. The deduced amino acid sequence has five Asp boxes, -Ser-X-Asp-X-Gly-X-Thr-Trp, showing great similarity to other bacterial and viral neuraminidases. We have also identified the catalytic domain by using truncated proteins produced in *S. lividans*.

Neuraminidase (EC 3.2.1.18) is a glycosidase which cleaves an α-ketosidic linkage between sialic acid and the adjacent sugar residue on glycoproteins, glycolipids, and oligosaccharides. It is widely distributed in microorganisms such as viruses, bacteria, and actinomycetes and in various tissues from fowls and mammals. For myxovirus it is an important surface antigen and also plays a role in facilitating the movement of the virus both to and from the site of infection (1). Bacteria and actinomycetes which produce neuraminidase can release sialic acids from glycoconjugates for use as carbon and energy sources. In pathogenic microorganisms, neuraminidase activity has a strong correlation with pathogenesis (27).

Recently, neuraminidase genes were cloned from several pathogenic microorganisms, including *Clostridium sordellii* G12 (25), *Clostridium perfringens* A99 (24), *Salmonella typhimurium* LT-2 (23), *Vibrio cholerae* 395 (29), and *Bacteroides fragilis* TAL2480 (26). From examination of their deduced amino acid sequences, a conserved sequence of 12 amino acids (Asp box) which was repeated at four or five positions was found (23). This conserved sequence shows similarity to the neuraminidase of influenza virus A H7N1 and H13N9. The structures of neuraminidases from nonpathogenic bacteria have not been characterized. In a recent report, neuraminidase was widely found in culture fluids of actinomycetes (3). For *Micromonospora* spp., 10 of the 39 strains examined exhibited neuraminidase activity. *Micromonospora viridifaciens* showed the highest neuraminidase activity among this group. Neuraminidase from *M. viridifaciens* was purified, and its properties were analyzed (2). Neuraminidase activity in *M. viridifaciens* was detected in the culture broth only when a substrate such as colominic acid or the product *N*-acetylneuraminic acid was present in the culture medium (2).

We would like to understand the regulation of neuraminidase gene (*nedA*) expression and the properties of its product in *M. viridifaciens* and *Streptomyces lividans*. As a first step in these analyses, we have cloned *nedA* from *M. viridifaciens* in *S. lividans* by using pIJ702 (15) as a vector. The cloned gene was expressed efficiently and constitutively in the heterologous host *S. lividans*. In this study, we report the cloning and sequencing of *nedA*; the structure-function relationship was analyzed with the recombinant enzyme produced in *S. lividans*. During analysis of the nucleotide sequence, we found another open reading frame (ORF) in the region upstream of *nedA*. The deduced protein structure shows some structural similarity with the Tn3 repressor (9).

MATERIALS AND METHODS

Bacterial strains and plasmids. *M. viridifaciens* ATCC 31146 was used as the source of the gene that codes for the neuraminidase. The following bacterial strains, phage, and plasmids were used; *S. lividans* TK23 and *Escherichia coli* MV1184, M13KO7 phage, and plasmids pIJ702 and pUC118.

Media and culture conditions. *M. viridifaciens* was grown in a medium containing 0.2% colominic acid (or 1% milk casein), 0.2% peptone, 0.1% meat extract, and 0.1% yeast extract (pH 7.2) for the production of neuraminidase (2). *S. lividans* was grown in YEME (11) or SKNo2 medium, which contained (per liter) 20 g of StabiloseK (Matsutani Kagaku Co., Ltd.), 5 g of glucose, 5 g of yeast extract (Nippon Seiyaku Co., Ltd.), 5 g of peptone (Nippon Seiyaku), 3 g of meat extract (Kyokuto Kagaku Co. Ltd.), 0.2 g of KH_2PO_4, and 0.6 g of $MgSO_4 \cdot 7H_2O$) (pH 7.6). *M. viridifaciens* and *S. lividans* were grown in triple-baffled flasks at 30°C in a rotary shaker at 220 rpm. *E. coli* MV1184 was grown in Luria broth (LB) or on LB agar at 37°C. For the production of neuraminidase from *S. lividans* TK23-BG6 and TK23-BC4, 300 ml of a 72-h culture grown in YEME broth was inoculated into a 5-liter jar fermentor containing 2.7 liters of SKNo2 medium. Cultivation was carried out at 30°C with vigorous aeration. Antibiotics were added to media at the following concentrations when used: ampicillin, 100 mg/liter; thiostrepton, 10 mg/liter.

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DNA isolation. Total DNA from *M. viridifaciens* was prepared as described by Hopwood et al. (12). Plasmid DNA from *S. lividans* or *E. coli* was isolated by the method of Kieser (16).

Gene library construction. Chromosomal DNA of *M. viridifaciens* was completely digested with *Bgl*II, *Bcl*I, or *Bam*HI and ligated into the unique *Bgl*II site of plasmid pIJ702. Chromosomal DNA was also completely digested with either *Pst*I or *Sac*I and ligated into the unique *Pst*I site or *Sac*I site of pIJ702. The ligation mixture was used directly for transformation of *S. lividans* TK23 as described by Hopwood et al. (12). After transformation, protoplasts were plated on R5 medium (11), except that colominic acid (0.2%) was used as the sole carbon source, and allowed to regenerate for 15 h at 30°C. Then an overlay of 2.5 ml of molten 0.7% agar containing 300 mg of thiostreptone was added. The transformants were screened for 2'-(4-methylumbelliferyl)-α-D-*N*-acetylneuraminic acid (4MUNANA) cleavage, as described below.

Assays for neuraminidase. Neuraminidase activity was assayed as follows. The reaction mixture contained 4 mg of colominic acid, 20 mmol of citrate-Na_2HPO_4 buffer (pH 5.0), and enzyme preparation in a total volume of 0.5 ml. Incubation was carried out at 37°C for 15 min and stopped by boiling for 3 min. The amount of *N*-acetylneuraminic acid liberated was determined with *N*-acetylneuraminate aldolase and *p*-dimethylbenzaldehyde (22). One unit of enzyme activity was defined as the amount of enzyme that produced 1 μmol of *N*-acetylneuraminic acid per min. A plate assay for neuraminidase expression utilized 4MUNANA, which is a fluorogenic substrate for many exoneuraminidases (18). To screen the *M. viridifaciens* gene library for neuraminidase expression, transformants were overlaid with 2.5 ml of molten 0.7% agar containing 4MUNANA (1 mg/ml), 50 mM sodium acetate (pH 5.5), 150 mM NaCl, and 4 mM $CaCl_2$. *ned*$^+$ clones were detected by their green-blue fluorescent halos when excited with UV light.

Nucleotide sequence analysis. The 2.5-kb *Bgl*II fragment from pNEDBG6 and the 0.6-kb *Bgl*II fragment from pNEDBC4 were subcloned into the *Bam*HI site of pUC118. The 0.6-kb *Pst*I-*Kpn*I flagment from pNEDBC4 was subcloned into the large fragment resulting from the digestion of pUC118 and pUC119 with *Kpn*I and *Pst*I. Deletion derivatives were constructed by the method of Henikoff (10). The deletion clones were screened for size and recovered as single-stranded DNA by using helper phage KO7 (28). The nucleotide sequence was determined by the dideoxy-chain termination method, using the Sequenase (United States Biochemical Corp.) kit. dGTP and dITP were routinely used. Reaction mixtures were separated on an 8% polyacrylamide sequencing gel, using an LKB Macrophor system at 55°C.

Purification of neuraminidase. All operations were done at 4°C, and the buffer used was 10 mM phosphate buffer (10 mM Na_2HPO_4, KH_2PO_4 [pH 7.0]). The culture broth of *S. lividans* TK23-BG6 or TK23-BC4 was centrifuged at 12,000 × *g* to remove the mycelia. The culture supernatant (40 ml) was mixed with $(NH_4)_2SO_4$ to 60% of saturation. After 12 h, the precipitate formed was collected by centrifugation at 12,000 × *g*, dissolved in 10 ml of the buffer, and dialyzed against two changes of 2,000 ml of the buffer overnight. The dialyzed enzyme solution was put on a column (1 by 10 cm) of DEAE-Sepharose CL6B, preequilibrated with the buffer. After the column was washed with the buffer, the adsorbed materials were eluted with a linear gradient (from 0 to 0.2 M) of NaCl (total volume for gradient, 200 ml). The active fractions were combined, and the enzyme was precipitated by the addition of $(NH_4)_2SO_4$ to 60% of saturation. The precipitate was collected by centrifugation at 12,000 × *g* for 20 min, dissolved in 5 ml of the buffer, and dialyzed against

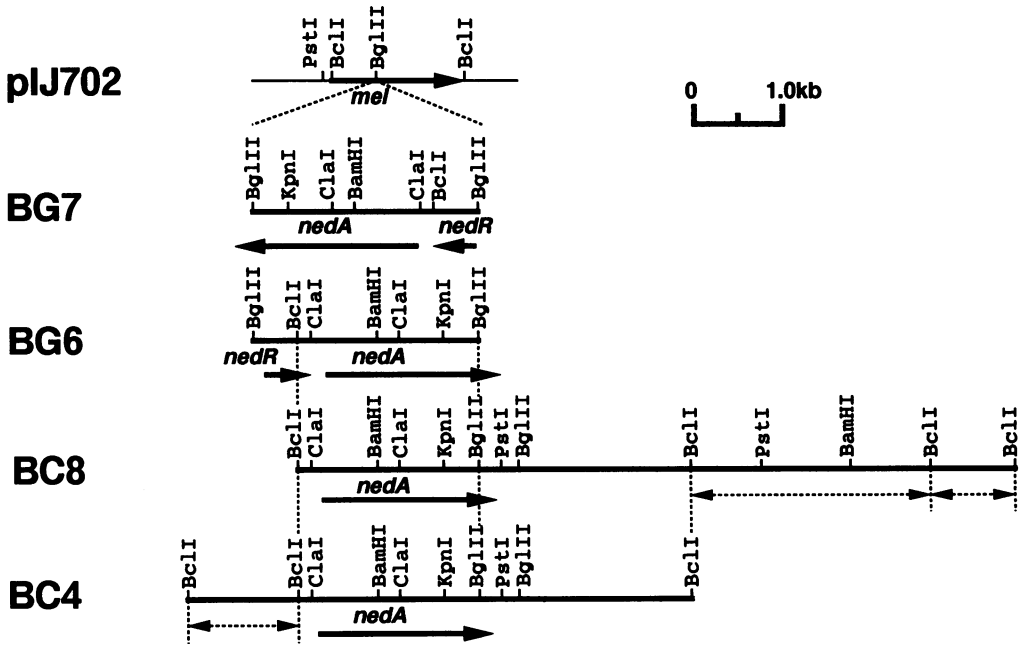

FIG. 1. Restriction maps of chromosomal DNA fragments from *M. viridifaciens*, cloned in the *Bgl*II site of pIJ702, which carry the genetic determinant for neuraminidase overproduction. *Bcl*I fragments of clones BC8 and BC4 (broken arrows) were created by ligation to vector DNA.

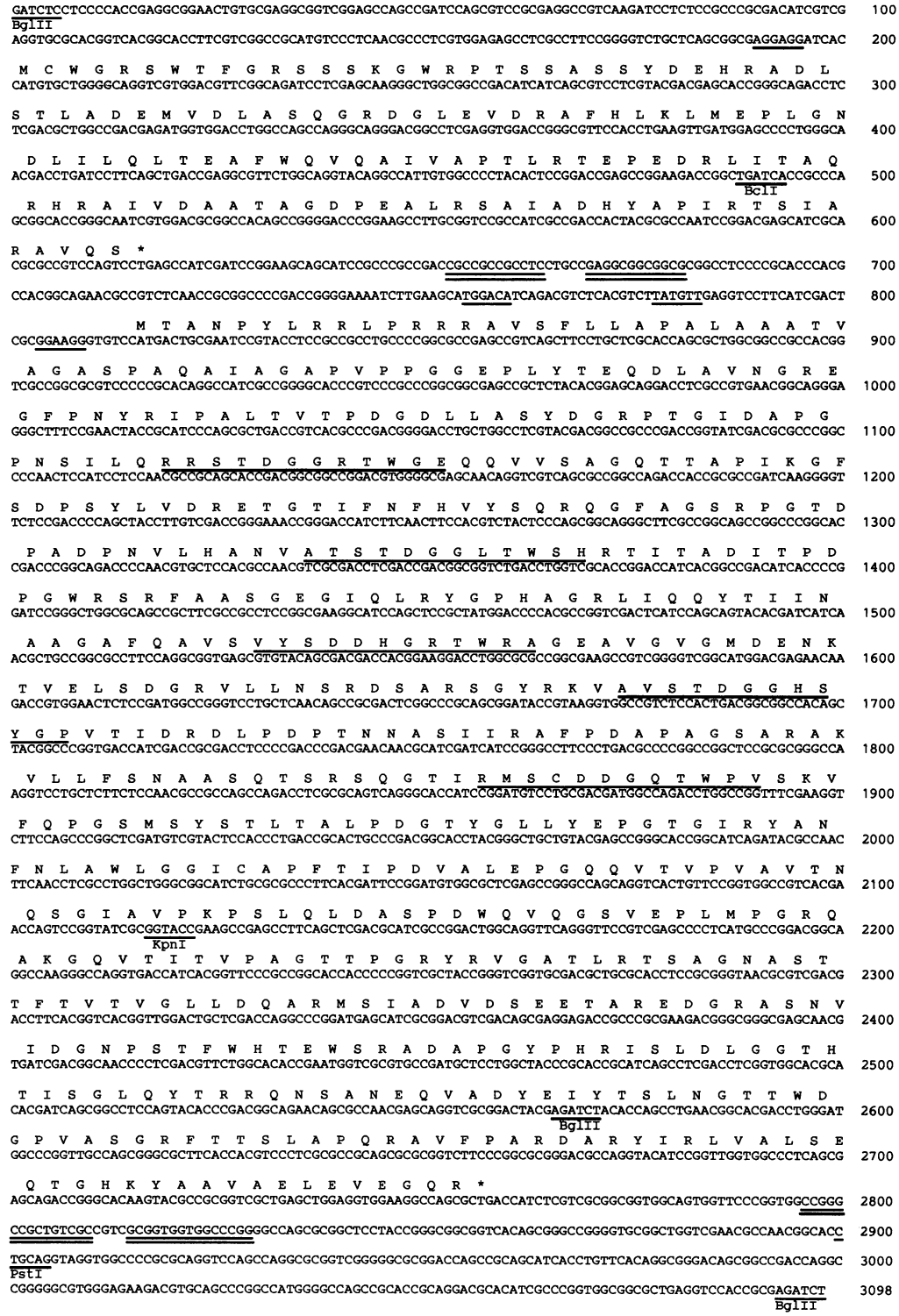

FIG. 2. DNA sequence analysis of the *ned* genes. Nucleotide and deduced amino acid sequences of *nedA* and *nedR*. The putative RBS and promoter sequence are underlined. Two long inverted repeat sequences are indicated by double underlines. The five Asp boxes are indicated by lines under the amino acid sequence.

two change of 2,000 ml of the buffer overnight. The dialyzed solution was used as the enzyme sample.

Protein sequencing. Purified proteins were sequenced with an automatic gas phase sequencer (Applied Biosystems, Model 470A). Amino acid compositions were analyzed with the Picotag System (Waters) after vapor phase hydrolysis.

SDS-PAGE. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was done in 15% polyacrylamide slab gels, using the Tris-glycine buffer system described by King and Laemmli (17). The molecular weight of the enzyme was calculated by using the relative mobilities of standard proteins (Pharmacia LKB Biotechnology, Uppsala, Sweden).

Nucleotide sequence accession number. The sequence described here has been submitted to the GenBank/EMBL/DDBJ data bases and assigned nucleotide sequence accession number D01045.

RESULTS

Cloning of *nedA*. A gene library of chromosomal DNA derived from *M. viridifaciens* was constructed by using the plasmid vector pIJ702. *S. lividans* was negative for 4MUNANA cleavage as determined by the plate assay and was chosen as a host strain.

Five libraries (*Bgl*II, *Bcl*I, *Bam*HI, *Pst*I, and *Sac*I) were constructed as described in Materials and Methods. One hundred twenty-one clones from the *Bgl*II library and 27 clones from the *Bcl*I library were positive for 4MUNANA cleavage by the plate assay. No positive clones were obtained from the *Bam*HI, *Pst*I, or *Sac*I library.

To demonstrate that cleavage of 4MUNANA was actually due to hydrolysis catalyzed by a neuraminidase, 12 clones from the *Bgl*II library (clones BG1 to -12) and 8 clones from the *Bcl*I library (clones BC1 to -8) were cultured in YEME liquid medium (without inducer). The supernatants of the culture broths were examined by the assay described in Materials and Methods. All 20 clones showed 20 to 30 U of neuraminidase activity per ml. This result suggested that the cloned genes expressed neuraminidase constitutively in *S. lividans*.

Restriction maps of four positive clones (clone BG6, BG7, BC4, and BC8) were determined. As shown in Fig. 1, clones BG6 and BG7 have identical 2.5-kb inserts in the *Bgl*II site of pIJ702 but in the opposite orientation. Clones BC4 and BC8 have 5.6- and 7.9-kb inserts, respectively, and share an identical 4.5-kb *Bcl*I-*Bcl*I fragment. All four clones share an identical 2-kb *Bcl*I-*Bgl*II fragment and produced neuraminidase in almost the same amount in *S. lividans* (data not shown). Thus, it seems that the expression of neuraminidase is not driven from the *mel* promoter on the vector pIJ702 but from its own promoter.

DNA sequencing. The 2.5-kb *Bgl*II fragment of clone BG6 was subcloned into the *Bam*HI site of pUC118 in both orientations (pUC118NEDA and -B). Deletion clones of pUC118NEDA and pUC118NEDB were constructed as described in Materials and Methods. An ORF starting at the ATG codon at nucleotide (nt) 816 and continuing to the end of the fragment was identified. The 0.6-kb *Kpn*I-*Pst*I fragment and the 0.6-kb *Bgl*II-*Bgl*II fragments were then cloned into pUC118 and pUC119 and sequenced. In this way an ORF of 1,941 bp with a TGA stop codon at position 2757 was identified. The deduced amino acid sequence for this ORF agreed with the amino acid composition of purified protein from clone BC4, as described below. Therefore, clones BG6 and BG7 have a deletion at the 3′ end of the *nedA* ORF,

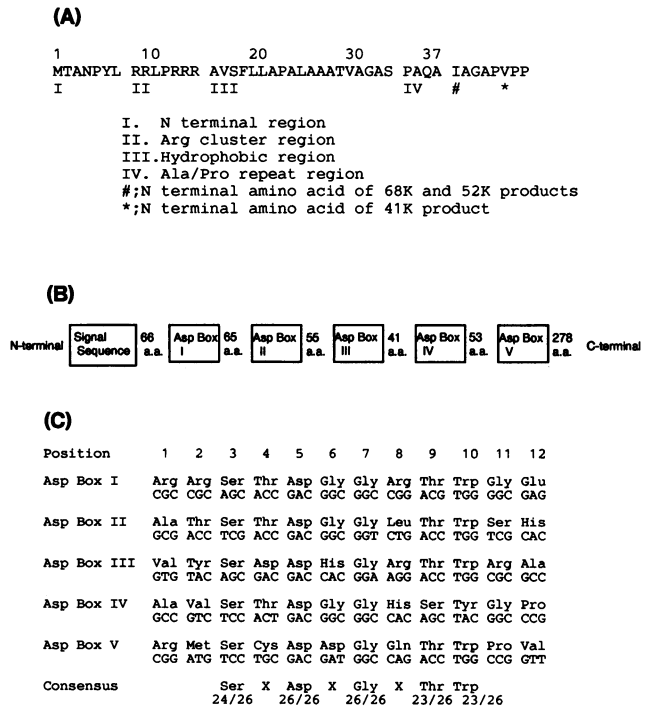

FIG. 3. Analysis of the NEDA protein. (A) Signal sequence of the NEDA protein of *M. viridifaciens*. The signal sequence was deduced from the nucleotide sequence and the N-terminal amino acid sequencing of the mature enzyme. (B) Organization of the NEDA protein. (C) Nucleotide and deduced amino acid sequences for the five Asp boxes found in the NEDA protein.

corresponding to loss of the C-terminal 62 amino acids. In clone BG6 the *nedA* gene is ligated to the *mel* gene in frame. The deduced product of clone BG6 has 90 amino acids at its C-terminal region that come from the *mel* gene product. The deduced product of clone BG7 has at its 3′ end 89 extra amino acids. The 3,098-bp DNA sequence determined in this study is shown in Fig. 2.

Within the deduced amino acid sequence of *nedA*, we identified five regions which show similarity to the Asp boxes found in other bacterial neuraminidases (23). Figure 3B depicts the positions of the five Asp boxes within the deduced amino acid sequence. Figure 3C gives the nucleotide and deduced amino acid sequences for the *M. viridifaciens* Asp boxes along with the consensus sequence. A distinctive feature found in the *M. viridifaciens* neuraminidase is that it possesses a large C-terminal region downstream of the last Asp box. The amino acid sequence in the N-terminal region shows similarity to that of the *C. perfringens* neuraminidase (24).

The nucleotide sequences which may act as a ribosome-binding sequence (RBS) were located 7 to 12 nt upstream of the ATG triplet of *nedA*. A promoter-like sequence was located upstream of the RBS (Fig. 2). The putative promoter sequence of *nedA* (5′-TGGACA-17 bp-TATGTT-3′) is similar to the *E. coli* consensus sequence (5′-TTGACA-17 bp-TATAAT-3′) (5, 13, 14). Long inverted repeat sequences, which may act as transcription terminators, were observed downstream from the transcriptional termination triplet of the *nedA* gene (Fig. 2).

Cultivation of TK23-BG6 and TK23-BC4 in 51-jar fermentors. As previously reported, *M. viridifaciens* produced

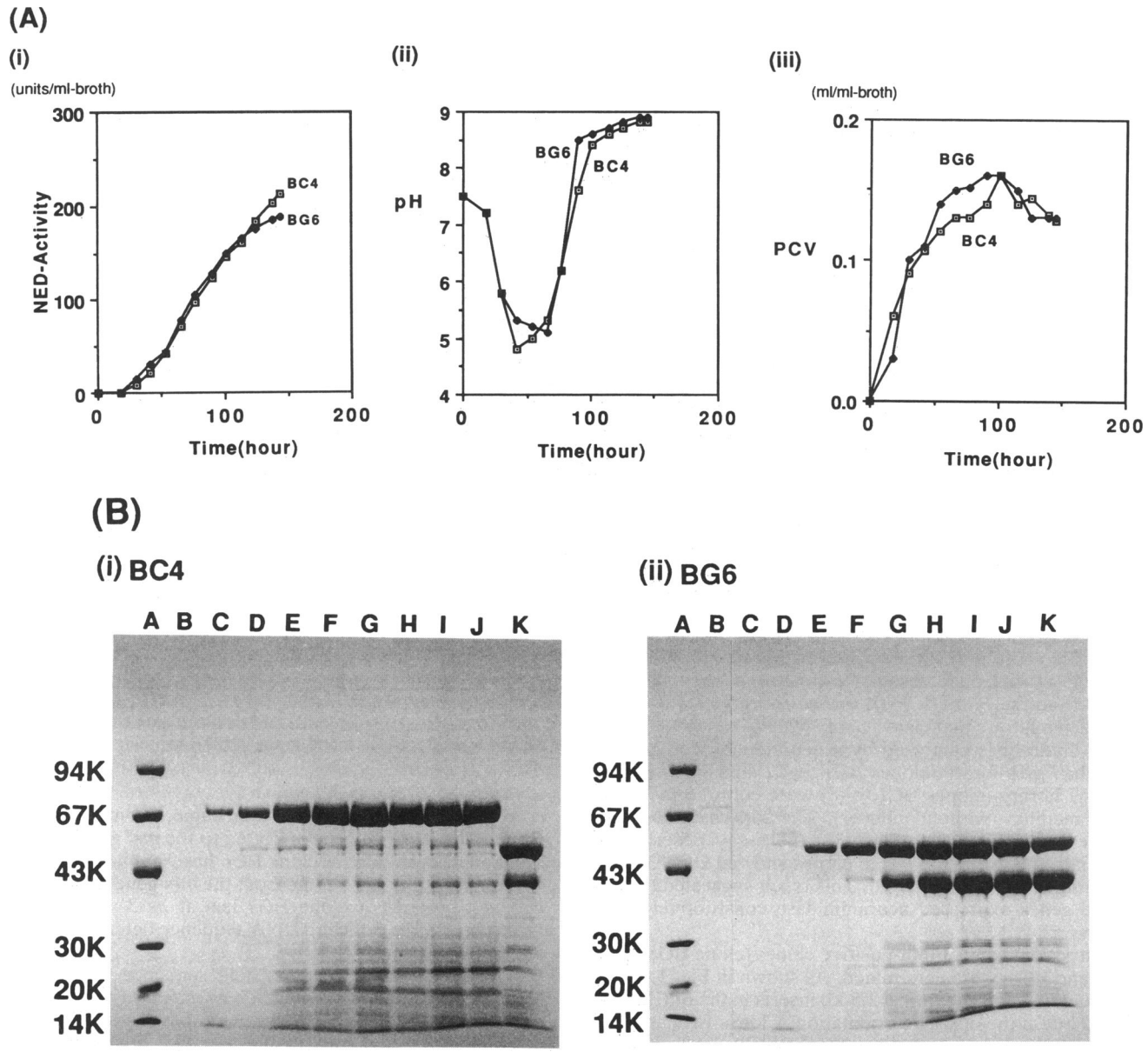

FIG. 4. Kinetics of neuraminidase expression in *S. lividans*. (A) Supernatants of *S. lividans* TK23-BG6 and -BC4 cultures were collected by centrifugation. Neuraminidase activity (i) and change in pH (ii) were measured. Cell growth was monitored by packed-cell volume (PCV) (iii). (B) Estimation of molecular weight of neuraminidase produced by *S. lividans* TK23-BC4 and -BG6. Excreted products from *S. lividans* TK23-BC4 (i) and -BG6 (ii) were fractionated by electrophoresis in a 10% polyacrylamide denaturing gel and visualized by protein staining. (i) Samples of TK23-BC4 were collected after 18 (lane B), 30 (lane C), 54 (lane D), 77 (lane E), 90 (lane F), 101 (lane G), 125 (lane H), 138 (lane I), and 144 (lane J) h of cultivation. Lane K, sample of TK23-BG6 cultivated for 90 h; lane A, size markers. (ii) Lane B, excreted products of *M. viridifaciens* cultivated in milk casein broth. Samples of TK23-BG6 were collected after 18 (lane C), 30 (lane D), 54 (lane E), 77 (lane F), 90 (lane G), 101 (lane H), 125 (lane I), 138 (lane J), and 144 (lane K) h of cultivation. Lane A, size markers. K, kilodaltons.

neuraminidase only when either the substrate, colominic acid, or the product, *N*-acetylneuraminic acid, was added to the culture medium (2). When the concentration of colominic acid in the medium was increased, the level of neuraminidase excreted was increased. The quantity of neuraminidase produced was 1.5 U/ml of broth when 1.0% colominic acid was added. To examine the production of recombinant neuraminidase in *S. lividans*, both TK23-BG6 and TK23-BC4 were cultured in SKNo2 medium, using 51-jar fermentors. The kinetics of neuraminidase activity production in the culture broth are shown in Fig. 4. Both TK23-BG6 and TK23-BC4 produced neuraminidase at up to 200 U/ml of broth without any inducer. This means that the promoter and the secretion signal sequence work well in the heterologous host, *S. lividans*.

The products in the culture supernatant were examined by SDS-PAGE (Fig. 4). TK23-BC4 produced the 68-kDa product; a small amount of a 41-kDa protein appeared in the later half (77 h) of the cultivation. By contrast, 57- and 52-kDa products appeared at early stages (30 h) of cultivation of

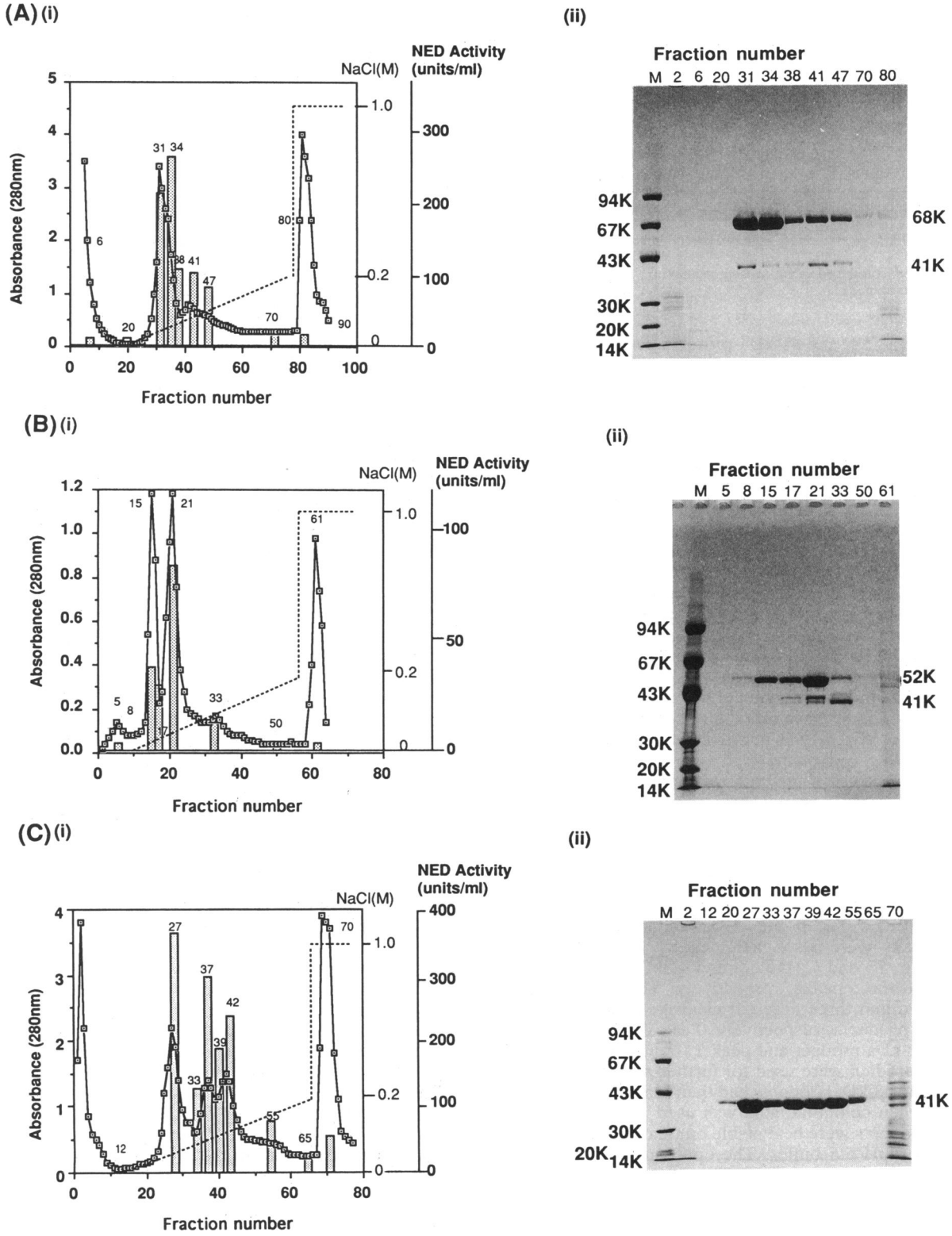

FIG. 5. Purification of 68-kDa (A), 52-kDa (B), and 41-kDa (C) neuraminidase products from *S. lividans*. The 68-kDa product was purified from TK23-BC4; the 52- and 41-kDa products were purified from TK23-BG6. (i) UV light (280 nm) absorption of fractions eluted from DEAE-Sepharose CL6B is indicated by squares, and neuraminidase activities of selected fractions are indicated by shaded boxes. Conditions for chromatography are described in Materials and Methods. (ii) Fractions corresponding to the absorption peaks were analyzed by SDS-PAGE. K, kilodaltons.

TABLE 1. K_m and V_{max} values of purified neuraminidase for the substrate colominic acid

Neuraminidase product[a]	K_m (mM)		V_{max}[b]	
	pH 5.0	pH 7.0	pH 5.0	pH 7.0
M. viridifaciens, 68 kDa[c]	0.1	ND[d]	1.8×10^3	ND
S. lividans TK23-BC4, 68 kDa	0.07	0.05	1.9×10^3	2.4×10^2
S. lividans TK23-BG6, 41 kDa	0.08	0.04	1.2×10^3	1.4×10^2
S. lividans TK23-BG6, 52 kDa	0.10	0.05	ND	ND

[a] The designations 68, 52, and 41 kDa refer to the apparent sizes of the purified polypeptides.
[b] Moles of *N*-acetylneuraminic acid produced per minute per mole of enzyme.
[c] Neuraminidase produced in milk casein medium.
[d] ND, not determined.

TK23-BG6; the 52- and 41-kDa proteins become the major products after 90 h of cultivation. Incubation of BG6 culture supernatant at room temperature also increased the amount of the 41-kDa product. On the basis of the nucleotide sequence analysis, the product of TK23-BG6 should be deleted for its C-terminal 62 amino acids and fused with the C-terminal 89 amino acids of a *mel* gene product. However, only products of 57, 52, and 41 kDa were observed throughout the fermentation. The most likely explanation is that the C-terminal region is unstable and sensitive to attack by proteases from *S. lividans*. The 57-kDa product could be the product cleaved at the *nedA-mel* junction. It is important that both TK23-BG6 and TK23-BC4 produced the 41-kDa products. A similar observation was reported for the neuraminidase produced in *M. viridifaciens*, which produced a 41-kDa product when colominic acid was used as the inducer. However, when milk casein was used as the inducer, a 68- to 72-kDa product was mainly excreted (2). To understand the meaning of these processing reactions, each product (41, 52, and 68 kDa) was purified, and its properties were analyzed as described below.

Purification and characterization of the 41-, 52-, and 68-kDa products. The 68-kDa product was purified from the 144-h culture broth of TK23-BC4. The 52- and 41-kDa products were purified from the TK23-BG6 broths cultivated for 72 and 144 h, respectively. Figure 5 shows the UV light (280 nm) absorption of eluted fractions from DEAE-Sepharose CL6B, with neuraminidase activities corresponding to the absorption peaks. The products corresponding to the absorption peaks were also examined by SDS-PAGE. As shown in Fig. 5B and C, the 52- and 41-kDa products split into two and three peaks, respectively. This suggests that there are two and three different cleavage sites for the 52- and 41-kDa products, respectively. Peak 2 (fractions 19 to 23) of the 52-kDa product and peak 1 (fractions 25 to 30) of the 41-kDa product were used for further analyses.

The 41- and 68-kDa products had their maximum activities at pH 5.0 when colominic acid was used as the substrate. The three products were heat stable on treatment at 50°C for 15 min in the pH 5.5 buffer. The optimum temprature for neuraminidase activity was 50°C. The apparent K_m and V_{max} values for colominic acid were determined (Table 1). All three products show similar values, values which were also similar to those determined for the neuraminidase produced in *M. viridifaciens* (2). These observations show that the three products have similar neuraminidase activities and stabilities. It could be concluded that the N-terminal 41-kDa portion of the protein was sufficient for neuraminidase activity.

The N-terminal amino acid sequences of the three products were determined as follows: 68-kDa product, Ile Ala Gly Ala Pro Val Pro Pro Gly Gly; 52-kDa product, Ile Ala Gly Ala Pro Val Pro Pro Gly Gly; 41-kDa product, Val Pro Pro Gly Gly Glu Pro Leu Tyr. These results suggest that the N-terminal 37 amino acids of the *nedA* ORF act as a signal sequence (Fig. 3A). The amino acid composition of the purified 68-kDa product was also determined. It agreed with the amino acid composition deduced from the nucleotide sequence.

DISCUSSION

In this study, we have shown that the promoter and secretion signal sequences of *M. viridifaciens* work efficiently in *S. lividans*. The deduced signal sequence of the *nedA* product agrees with the consensus sequence for *Streptomyces* secreted proteins (19).

The expression of neuraminidase in *M. viridifaciens* is repressed by glucose and stimulated by the substrate, colominic acid, or the product, *N*-acetylneuraminic acid. Similar control can be found in the expression of neuraminidase in *C. perfringens*, *V. cholerae*, and *S. typhimurium* LT-2 (29, 30). In *E. coli*, induction of *nanT* and *nanA* are regulated by sialic acid (30). Furthermore, neuraminidase and aldolase in *C. perfringens* appear to be coordinately regulated, suggesting an operon organization (30). Thus, a variety of microorganisms respond to sialic acid by a related catabolic system. It will be interesting to define the genetic controls in these systems, but the details are still unclear. In this study, another potential ORF starting at an ATG at 202 nt and terminating at 616 nt was detected by computer analysis (6) (Fig. 2). This ORF showed a high GC content and a marked bias in codon usage, as described for *Streptomyces* genes (4). The deduced amino acid sequence showed weak similarity to the Tn3 repressor (9) within the entire ORF. In the C-terminal region, sequences similar to DNA-binding domains of known DNA-binding proteins (7, 21) existed. There is a possibility that this gene product works as a regulatory protein. Therefore, we termed this ORF *nedR*. An RBS could be located upstream of the ATG triplet of *nedR*. The cloned neuraminidase gene of *M. viridifaciens* was expressed constitutively in *S. lividans*. The amounts of neuraminidase produced by TK23-BG6 and -BC4 were similar even though TK23-BG6 contains the *nedR* gene. During analyses of intracellular proteins by SDS-PAGE, no 15-kDa protein corresponding to NEDR was observed (unpublished data). It follows that *nedR* is not efficiently expressed in *S. lividans*. Recently, we have established a novel highly efficient gene cloning system for *Micromonospora* strains (8). We are now trying to clarify the function of *nedR* by creating an insertion in the chromosomal *nedR* gene.

The structure of the *M. viridifaciens* neuraminidase showed significant homology to those of other bacterial and viral neuraminidases. In the *M. viridifaciens* neuraminidase, there are five Asp boxes in the N-terminal four-sevenths of the protein. As previously reported, the neuraminidase produced by *M. viridifaciens* is digested to a 41-kDa product. In this study, we have shown that the N-terminal 41-kDa portion must include the catalytic domain of the neuraminidase. The deduced processing site agrees with the C-terminal end of the *C. perfringens* neuraminidase (24) when the sequences are aligned by their homology. The homology found between the *M. viridifaciens* and *C. perfringens* neuraminidases was also consistent with the observations

that the properties of the two enzymes are similar in many respects (2, 20).

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