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DIHYDROCHALCOMYCIN PRODUCTION AND GLYCOSYLTRANSFERASE FROM STREPTOMYCES SP. KCTC 0041BP

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Abstract
The dihydrochalcomycin (GERI) synthetic gene cluster from Streptomyces sp. KCTC 0041BP has been isolated. Two open reading frames (ORFs), designated gerT1 and gerT2 as glycosyltransferase genes, has been identified by sequence analysis. GerT1 encodes for the protein function as dTDP-deoxyallosyltransferase and it is responsible to the attachment of dTDP-allose to the macrolide ring. Similarly, gerT2 encodes for peptide named as dTDP-chacosyltransferase which can transfers the dTDP-4,6-dideoxyglucose to macrolactone core. During process of compound isolation, a new compound has been isolated with molecular weight m/z 755 [M+Na+]. This compound could be the dihydrochalcomycin derivative. The compound has been shown the same antibacterial activity as GERI compound.

INTRODUCTION

Dihydrochalcomycin, a sixteen-membered macrolide antibiotic produced by Streptomyces sp. KCTC-0041BP, have important clinical application in the treatment of both Gram-positive and Gram-negative bacterial infections (Kim et al, 1996). The structure of the compound was speculated as to be hydrogenated analogue of chalcomycin antibiotic at C9-C10 double bonds (Asolkar et al, 2002). Similar to chalcomycin, the 8(S)-OH group and the 2,3-trans double bond presenting in GERI compound may have overall potency for the contribution to the bioactivity of these compounds (Woo et al, 1996). Dihydrochalcomycin is integrated by macrocyclic lactone to which two deoxygar residues attached by the O-glycosydic linkages. The neutraral chalcose substituted at C5 of macrolactone ring and it is thought that 2'-OH group plays important role in the contribution to the binding of macrolide to domain V of bacterial ribosome (Poehlsgaard et al, 2003). Finally, the presence of sugar micynose substituted at C-14 of macrolide core and it was demonstrated that the micinose moiety makes contact with domain II of the ribosome and contributes to enhanced binding of the macrolide (Hansen et al, 2002). We have recently isolated the gene cluster involved in dihydrochalcomycin biosynthesis from Streptomyces sp. KCTC 0041BP (GeneBank accession no AY118081). The study of dihydrochalcomycin biosynthesis gene cluster has reveled two glycosyltransferase gerT1 and gerT2. The gerT1 is responsible to attachment of unusual sugar D-allose to the macrolactone at C-20 and requires a primary hydroxyl group at C20 of the macrolactone with the activity of a P450 enzyme and gerT2 is responsible for the attachment of L-micynose moiety to the macrolactone core at C-5 position (Jaishi et al, 2006).

To better understand the biosynthesis pathway of dihydrochalcomycin and its applications, we have shown here the sequence analysis of two glycosyltransferase by using BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/). We have isolated the GERI compound and it derivatives.

MATERIALS AND METHODS

Bacteria strains and media

Streptomyces sp. KCTC-0041BP (formally reported as Streptomyces sp. GERI-155) (Kim at el, 1996) was used as host strain for sources of DNA and for isolation of products. The ISP2 media is to use as seed culture media, while R2YE and rich protein source media containing glucose 2%, soluble start 1%, meat extract 0.1%, yeast extract 0.4%, soybean meal 2.5%, NaCl 0.2% and K2HPO4 0.005% (Kim et al, 1996) was used for production media. E coli XL1-Blue (MRF) (Stratagene, USA) was used as a host cell for the preparation of the recombinant plasmids and DNA manipulation. E. coli was grown at 37°C in Luria-Bertani (LB) broth, or on an agar plate supplemented with the appropriate amount of antibiotics whenever necessary for the selection or maintenance of the recombinant plasmids (Sambrook et al, 2001).

Fermentation and isolation of production

Well grown seed culture of S. sp KCTC 0041BP was transferred in to production media and inoculate in the 2.5 liter jar fermentor which was operated for 74 days at 28oC (pH 7.4 and 250 rpm) with supplying oxygen (2.5 liters/min). The culture broth was extracted twice with ethylacetate and evaporated to dryness until remaining oil residue and finally result in methanol for further analysis.

The crude extract was subjected to silica gel column chromatography using CHCl3/CH3OH gradient from 2% to 10% methanol. The fraction eluted at 10% methanol was further applied to PTLC to provide GERI compound and its derivatives. The solvent system for PTLC is chloroform and acetone (0.7:0.3)

Bioassay was carried out using paper disk method and agar overlay for testing the antibacterial of the bioactive compound.

RESULTS AND DISCUSSION

Sequence analysis of gerT1 and gerT2 genes

Open reading frames gerT1 (1257 bp) and gerT2 (1278 bp) are located within the dihydrochalcomycin biosynthesis gene cluster (**figure 1A**), the whole 75.5 kb region encoding the genes for dihydrochalcomycin biosynthesis has been deposited in the GenBank with the accession number AY118081. GerT1 is flanked downstream by gerR and upstream by gerM3 and GerT2 is flanked downstream by gerK2 and upstream by gerY. Using the Clustal X program, the determined sequence analysis of the dihydrochalcomycin gene cluster revealed a deduced amino acid sequence (419 amino acids) encoded for allosyltransferase GerT1, which displays a very high degree of similarity to a number of the known glycosyltransferase genes in the GeneBank database (**figure 1A**), including ChmN from Streptomyces bikiniensis (95% identity) (GeneBank accession no AY509120) (Ward et al., 2004), TylN from Streptomyces fradiae (66% identity) (GeneBank accession no AF055922) (Fouces et al., 1999 ; Vanessa et al., 1998), mycD from Micromonospora griseorubida (63% identity) (GeneBank accession no AB089954) (Anzai et al., 2003), and ORF 11 Streptomyces neyagawaensis (47% identity) (GeneBank accession no DQ149987). Similarly, the deduced amino acid sequence (426 amino acid) encodes for the protein named as chalcosyltransferase GerT2. This protein also displays similarities to glycosyltransferase from different sources, including chmCIII from Streptomyces bikiniensis (93 % identity) (GeneBank accession no AY509120) (Ward et al., 2004), TylMII from Streptomyces fradiae (61% identity) (GeneBank accession no X81885) (Charles et al., 2004), DesVII from Streptomyces venezuelae (56% identity) (GeneBank accession no AF079762) (Xue et al., 2001), NbmD from Streptomyces narbonensis (55% identity) (GeneBank accession no AF521878). In addition, analysis of amino acid sequences of GerT1, GerT2 and several GTRs involved in the biosynthesis of poliketides showed a very well conserved domain including histidine residue rich region which depends on the GTF (**figure 1A** and **figure 1B**). It is suggested that the histidine amino acid present at this conserved region could play important role in the catalytic activity of the enzyme and histidine residues have been showed to be important active site of substrate binding and transition state stabilization in some oligosaccharide-independent GTFs (Quiros et al, 2000 a, b).

Figure 1A. Multi-alignment analysis of the deduced amino acid sequence of GerT1 with the known glycosyltransferase from different strains.

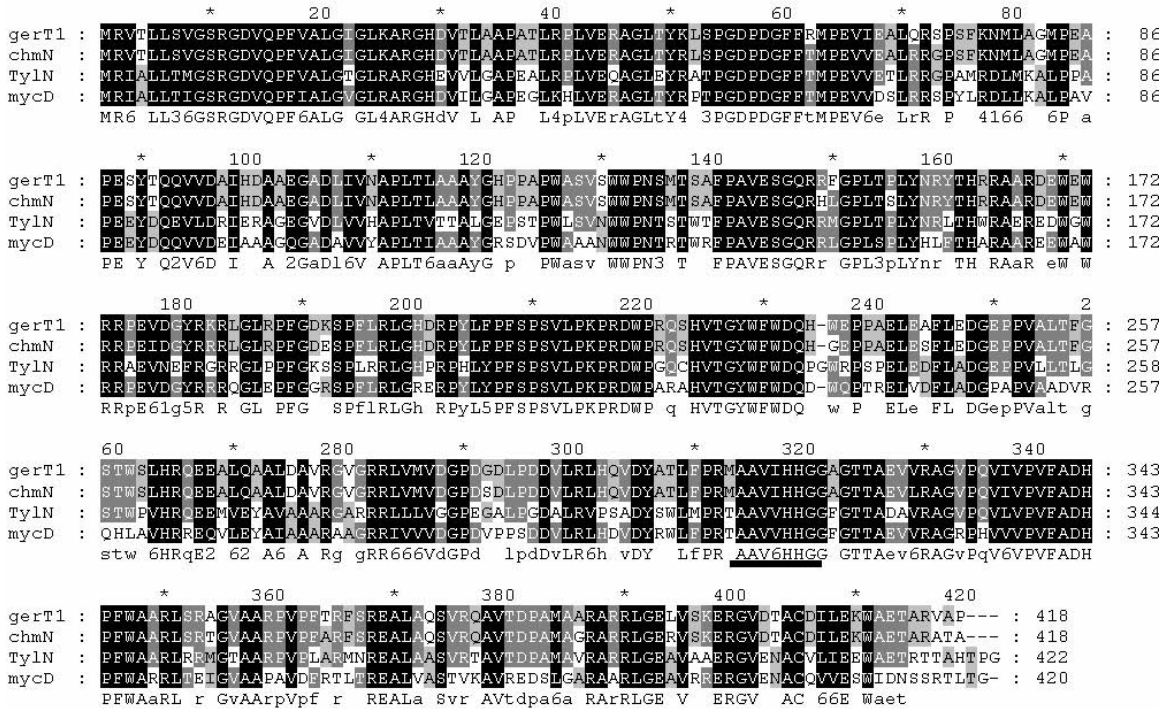
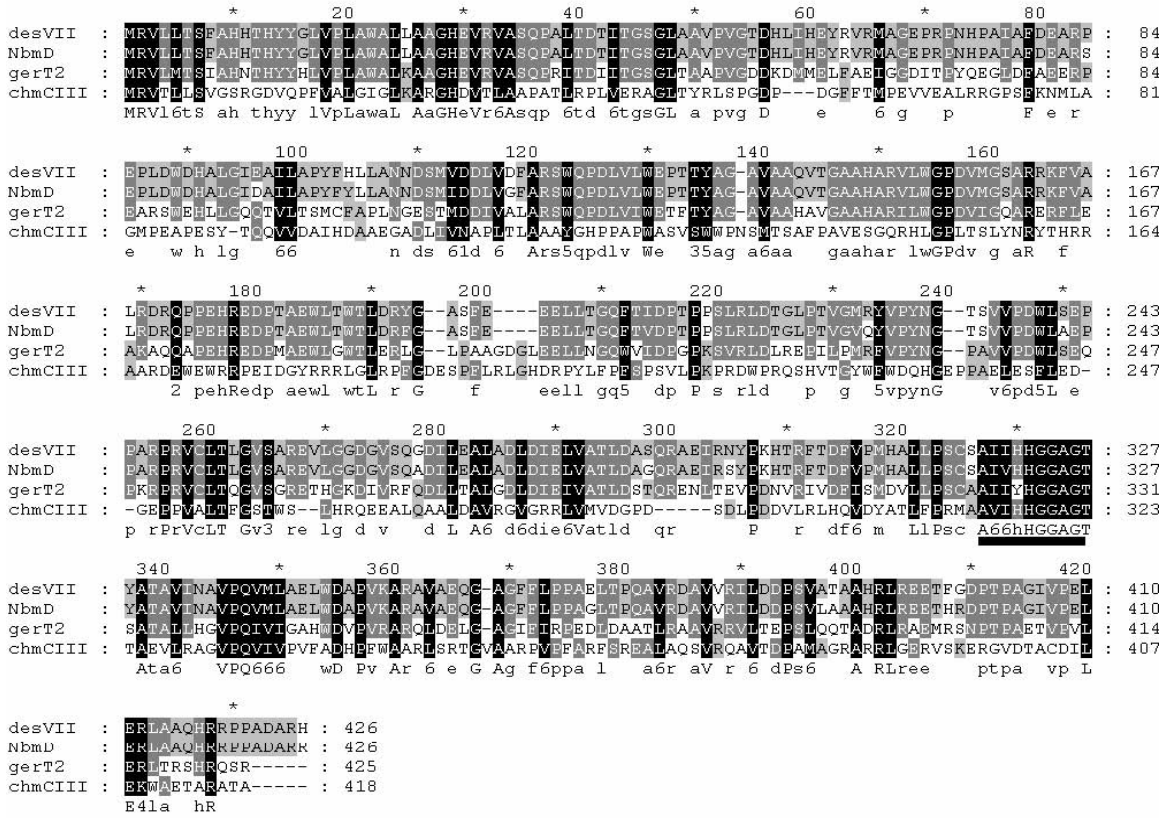


Figure 1B. Analysis of the deduced amino acid sequence of GerT2 in comparisons with the known glycosyltransferase from different strains from GeneBank. (the underlined amino acids are shown as the active sites of the enzyme)



Analysis of production from streptomyces sp. KCTC 0041BP

Three fractions containing active compounds were successively obtained from elution with 2% to 10% methanol as reported (Kim et al, 1996). The third fraction was further purified by PTLC to afford dihydrochalcomycin along with its derivative. ESI-mass analysis showed the peak at m/z=725 [M+Na+] corresponding to the molecular weight of 702 Da as the molecular weight of GERI (**figure 2**) and the compound shows Rf value at 0.3. In addition, the fraction with Rf value at 0.2 containing unknown compound was isolated with m/z = 755 [M+Na+] corresponding to the molecular weight 732 Da as new compound that is not reported previous paper (**figure 3**). The compound was carried bioassay test and it also has same antibacterial activity as dihydrochalcomycin (data has not shown).

Figure 2. ESI-mass analysis of GERI compound isolated from culture broth m/z =725 in (M+Na+)

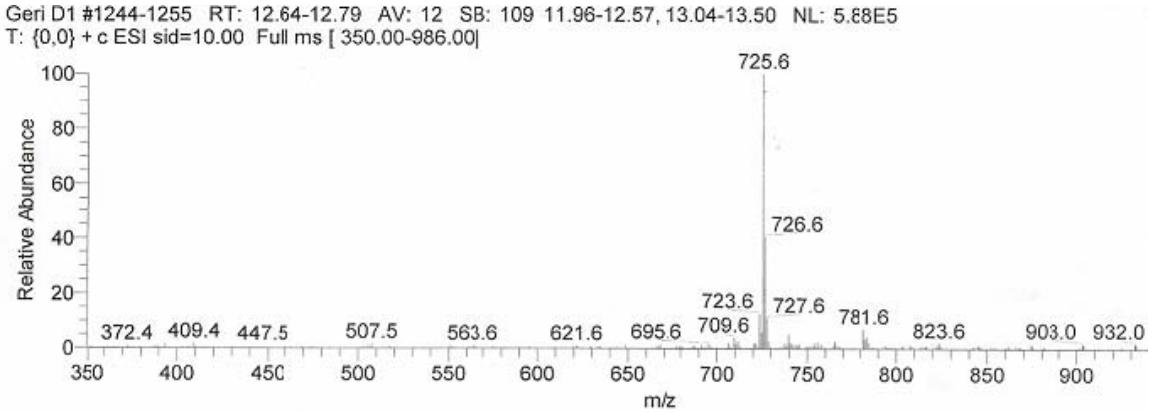
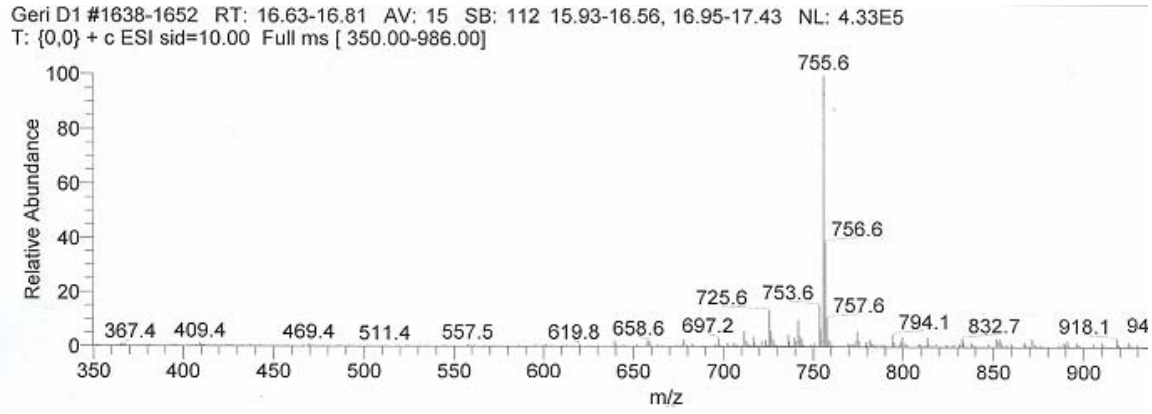


Figure 3. ESI-mass analysis of GERI derivative compound isolated from culture broth m/z =755 in (M+Na+)



From this results we found that the novel compound isolated has molecular weight equal with the dihydrochalcomycin derivative (Rf = 0.2 and MW = 732) in which the macrolactone was attached by two mycinose sugar moieties. This can be suggested that gerT2 may act as a flexible glycosyltransferase to different glycone. Hence, it can accept either dTDP-allose or dTDP-chalcose as glycone donors during macrolactone glycosylation steps. For confirmation of this hypothesis, it is necessary to study more and carry out further analysis about gerT2.

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