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Cloning, purification and characterization of a functional anthracycline glycosyltransferase

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Abstract

We have cloned the gene that encodes a novel glucosyl transferase (AraGT) involved in rhamnosylation of the polyketide antibiotic Aranciamycin in *Streptomyces echinatus*. AraGT comprises two domains characteristic of bacterial glycosyltranferases. AraGT was synthesized in *E. coli* as a decahistidinyl-tagged polypeptide. Purified AraGT is dimeric, displays a T_{mapp} of 30 °C and can glycosylate the aglycone of an Aranciamycin derivative as shown by liquid chromatography and mass spectrometry. The availability of functional AraGT will allow the generation Aranciamycin-based combinatorial libraries.

Keywords: Aranciamycin; Glycosyl transferase; Antibiotics; Anthracyclin; *Streptomyces*

1. Introduction

Historically, natural products have been the most successful source for the development of antimicrobial and antitumor drugs (Newman et al., 2003). Especially anthracyclines which belong to the aromatic polyketide class of natural products have shown excellent antibiotic activity and anthracyclines like adriamycin (doxorubicin), epirubicin (the 4′-epimer of adriamycin), daunomycin, and mitoxantrone are currently used for cancer chemotherapy (Rabbani et al., 2005). Most of these anthracyclines have been isolated from gram-positive soil bacteria belonging to the order *Actinomycetales*. The backbone of these compounds is iteratively generated by the so called polyketide synthases (Staunton and Weissman, 2001). Subsequently,

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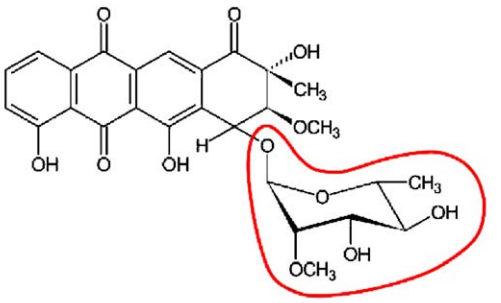
"tailoring enzymes", like glycosyltransferases (GT), introduce various modifications to the backbone (Rix et al., 2002). The aromatic polyketide backbone of anthracyclines is usually decorated with one or more sugar moieties. These sugars not only increase solubility but are also important for the binding to DNA sequences and most often affect the biological activity of the compound (Temperini et al., 2005). The artificial combination or deletion of genes involved in the biosynthesis of the sugar moieties of polyketides is a powerful tool for the manipulation of the sugar moieties or the length of the oligosaccharide chain attached to the anthracycline backbone (Lombo et al., 2004; Luzhetskyy et al., 2005a,b,c). Structural diversity can be achieved due to a pronounced substrate flexibility of the GT attaching the sugar moieties to the anthracycline backbone (Mendez and Salas, 2005). Usually, these GTs do not only accept different activated sugars but are also able to transfer them to various polyketide backbones. Therefore these glycosyltransferases can be utilized for the generation of small compound libraries which can be helpful in drug discovery.

Significant progress has been made recently using genetic and biochemical methods to carry out glycosylation in vivo. For example, the re-engineering of sugar biosynthetic machineries to generate diverse sugar structures and the heterologous expression of foreign glycosyltransferase genes into appropriate strains to facilitate coupling between various sugar donors and aglycone acceptors in a combinatorial manner have been demonstrated (Luzhetskyy et al., 2005a,b,c). However, the general applicability of such an in vivo approach for the production of many antimicrobial agents is limited due to inhibited cell growth or death. Another way to attach unnatural sugars to natural products is by using chemical glycosylation methods. These methods have serious limitations for glycosylating complex systems. Yields are generally low and the stereochemical control is often poor. Thus, although dramatic progress is being made in some areas of oligosaccharide synthesis such as automated oligosaccharide synthesis, the substitution of one sugar for another on a complex natural product remains difficult (Kahne, 1997; Plante et al., 2001; Seeberger and Haase, 2000). One solution to overcome these hurdles is to carry out glycosylation in vitro using an isolated GT. This approach would also enable the use of mutant GTs with the desired substrate specificity. With available aglycone variants of TDP-hexoses, it should be possible to use purified GTs to create libraries of novel glycosylated products that are promising as antibiotic candidates.



(A)

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MRVLITVPPLDAHFNGSVPLAWALRTAGHEVRVAAQPPLTAGITSAGLTAVPVGDDPGLA   60
AVMARKGPRIYAHHEGRDLRRAATAPDGPEFLRTSSALLTDAFYSRIGSEETVDDLVAFC  120
RAWRPDLVVWETFTFAGAIAAHVTGVPHARLLWGPDLFAFMRDALLDHHRRAGVDAGSTP  180
GADPLRDWITGQLARFGEEFTEAVVRGLWRIDQMPEELRLAPGQHTVPLRYVPYNGPVPA  240
VVPPWLRHDPDRPRVCLTKGPSVRTIDSPDGRAVTTDDFFDAVADLDAEVVALLDETDRA  300
ALTTVPANTRVVDFTPLHVLLPTCAAIVHHGGAGTWSTAAVHGVPQLLLASMWDNVFRAV  360
RTEELGAGLFLPPAELTPAALRGALERLLKEPSFADGADELRRAMTSQPAPHTVVTELER  420
LAAQPAA                                                        427
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(B)

Fig. 1. Cloning of *S. echinatus* ssp. *afganiensis* Aranciamycin glucosyltransferase gene. (A) Determined chemical structure of Aranciamycin as described (Bols et al., 1992). The rhamnose group is highlighted in red. (B) Deduced aminoacyl-residue sequence of *S. echinatus* AraGT (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article).

We now describe the purification of the glycosyl transferase AraGT involved in the biosynthesis of the anthracycline Aranciamycin (Fig. 1A). We show that recombinant AraGT is sufficient to glycosylate a variant of the anthracycline backbone of Aranciamycin, indicating that this enzyme can be utilized to generate naturally non-occurring anthracycline derivatives.

2. Materials and methods

2.1. Bacterial strains and recombinant DNA experiments

Growth and manipulation of *Escherichia coli* and *Streptomyces* strains were as described (Ausubel et al., 1994).

2.1.1. Cloning of AraGT in E. coli

The anthracycline Aranciamycin is produced by *Streptomyces echinatus* ssp. *afghanensis* (Tü303; DSM

40730) (Bols et al., 1992; Keller-Schierlein et al., 1970). The Aranciamycin biosynthesis cluster in this organism was recently identified (Bechthold et al., in preparation). Within this cluster lies a gene (*orf21*) (Fig. 1B) that is homologous to glycosyltransferases (A. Bechthold, personal communication; manuscript in preparation).

To overexpress AraGT in *E. coli* a decahistidinyl tag was fused N-terminally to the AraGT ORF. To this end, the AraGT ORF was amplified by PCR using the primers X266 (5′-GGGAATTC**CATATG**CGTGTTCTGATCACCGTGCCGCCAC-3′) and X267 (5′-GCG**GGATCC***TTA*GGCCGCCGGCTGTGCGGCGAGCCG-3′) and cosmid 41-2C06 containing *S. echinutus* DNA as template. A NdeI restriction site (in bold) was included in the forward primer, while a BamHI site and a second in-frame stop codon (in bold and underlined, respectively) were introduced in the reverse primer. A PCR reaction was done by Taq polymerase in a buffer containing 10% DMSO in order to facilitate the melting of the *AraGT* GC-rich DNA double strands. The 1.3 kb PCR fragment was digested by NdeI and BamHI and cloned to the corresponding sites of pET16b (Novagen) resulting in pIMBB497. Constructs were verified by nucleotide sequencing.

2.2. Bacterial growth and heterologous expression of recombinant AraGT

pIMBB497 DNA was used to transform two different *E. coli* expression strains, BL21(DE3)/pLysS and JM109(DE3)/pLysS. Cells were grown in LB medium in the presence of 100 μg/ml ampicillin and 25 μg/ml chloroamphenicol at 37 °C and AraGT biosynthesis was induced by addition of 0.5 mM IPTG at OD_{600} 0.5 for 3 h at 30 °C. Cells were harvested by centrifugation and protein expression was determined by SDS-PAGE.

2.3. Protein purification and chromatography

All chromatography resins and molecular weight markers were from Amersham. Purified proteins were stored at −20 °C. For AraGT purification JM109(DE3)/pLysS cells harboring the plasmid pIMBB497 were grown in LB (4 L) as described (see Section 2.2) and cells (15 g) were resuspended in 150 ml buffer A (50 mM Tris pH8.0, 1 M NaCl, 20% glycerol, 10 μg/ml DNaseI, 1 mM PMSF, 5 mM β-mercaptoethanol, 20 mM imidazole) and broken by ultrasonication (30 min at 500 W in a Vibra-cell ultrasonicator under constant cooling). The soluble cytosolic fraction was separated from the insoluble material by ultracentrifugation (50,000 × *g*; 1 h). The soluble fraction containing His_{10}-AraGT was loaded on 0.5 ml Ni^{2+}-NTA resin (QIAGEN) pre-equilibrated with buffer A. The resin was washed twice with 40 column volumes of buffer A, whereas in the second pass the concentration of NaCl was decreased to 50 mM. His_{10}-AraGT was eluted with five column volumes of buffer A containing 200 mM imidazole. Polypeptides from the different purification steps were visualized by 12% SDS-PAGE and immunostaining with anti-His antibodies (Serotec).

2.4. Intrinsic fluorescence-thermal stability

Tryptophan fluorescence emission of AraGT (50 μg/ml; 50 mM Tris–HCl pH 8, 50 mM KCl, 1 mM dithiothreitol) was monitored as a function of temperature (4–82 °C; 0.8 °C/min) at 345 nm (exc. 297 nm; data interval 0.5 min; slits were set at 2.5/20) in a Varian Eclipse instrument. Data were analyzed by non-linear regression as described (Vrontou et al., 2004). AraGT has nine Trp residues.

2.5. Generation of Streptomyces cytosolic extract

To provide an optimal environment for the enzymatic activity, biocatalytic reactions were performed in an extract of *Streptomyces albus*. Cells from a culture of *S. albus* in a 250 ml Erlenmeyer flask with one spiral (100 ml E1 medium, 96 h, 28 °C, 160 rpm) inoculated with 5 ml of a preculture (TSB medium, 72 h, 28 °C, 160 rpm) were disrupted by a two-fold passage through a French press and sonification (3.30 min, five cycles by 10%). The supernatant was clarified by centrifugation at 4 °C and used for biocatalysis experiments.

2.6. Biocatalysis assay of AraGT function by mass spectrometry

To test the activity of the purified heterologously expressed glycosyl transferase, the enzyme was incubated with CBS000020 (see Supplementary Fig. 1), a non-glycosylated Aranciamycin derivative (manuscript in preparation) and TDP-L-rhamnose (see

Supplementary Fig. 2). TDP-L-rhamnose di-sodium salt (1.1 mg) was dissolved in cytosolic extract from *S. albus* (200 μl) before addition of CBS000020 (100 μg) and purified recombinant AraGT at the indicated concentrations (0.5 mg/ml; >90% pure). The reaction mix was shaken (14 h; 30°) and afterwards extracted with ethylacetate (400 μl). The organic phase was finally evaporated to dryness and the residue analyzed by LC-DAD-MS (see Supplementary Fig. 4).

3. Miscellaneous

Chemicals were from Sigma. DNA enzymes were from Minotech and oligonucleotides from the IMBB microchemistry facility. A model of a predicted 3D structure of AraGT was carried out using coordinates from the structure of GtfD from A. *orientalis* (pdb code: 1RRV) (Mulichak et al., 2004) and the program Swiss-Model (Schwede et al., 2003).

4. Results

4.1. A gene encoding a putative Aranciamycin glycosyltransferase

A putative Aranciamycin biosynthetic cluster endoding genes with homologues in several other antibiotic biosynthetic pathways was recently identified in *S. echinatus* ssp. *afghanensis* (Tü303; DSM 40730) (A. Bechtold, personal communication; manuscript in preparation). One of the genes in this regulon encodes a polypeptide (Fig. 1B) that has sequence homology to bacterial GTs involved in several glycosyl transfer reactions including those involved in antibiotic biosynthesis. This protein (hereafter AraGT) could be important for transfer of the rhamnose moiety to the Aranciamycin aglycone (Fig. 1A). A structural homology model of AraGT could be derived using the structure of the homologous (35% identity and strong similarity at the primary aminoacid sequence) enzyme GtfD from *Amycolatopsis orientalis* (Mulichak et al., 2004) (data not shown). GtfD transfers vancosamine from a TDP-L-vancosamine donor to the monoglycosylated acceptor desvancosaminyl vancomycin to form a disaccharide moiety. AraGT is predicted to contain two characteristic domains: an aminoterminal GT family 28 domain (interpro code: IPR004276) fused to a domain with homology to that of other bacterial GTs (interpro code: IPR010610). Several short insertions in linker stretches differentiate the two structures.

4.2. Functional expression of AraGT in E. coli

To achieve high-level expression of AraGT we cloned *AraGT* in a pET system vector such that AraGT is expressed as a decahistidinyl fusion protein in *E. coli*. A novel polypeptide of ~45 kDa was seen in cultures grown in the presence (Fig. 2, Panel A, lanes 3 and 5) but not in the absence (lanes 2 and 4) of the inducer IPTG. This polypeptide had an apparent M_r that was similar to that deduced from the nucleotide sequence of the ORF and could be immunostained by anti-hexahistidinyl antisera (lanes 8 and 10). These data indicated that the protein of 45 kDa is the recombinant AraGT. Only small amounts (~5% of total) of recombinant AraGT was found in the soluble cytosolic fraction (Fig. 2, Panel B, lanes 3 and 5) of cells disrupted by ultrasonication. Most of the recombinant protein remained insoluble (lanes 4 and 6), presumably in inclusion bodies, and appeared to undergo proteolytic degradation (lane 6; bracket). Attempts to overcome the solubility problem by genetic fusions to the maltose binding protein of *E. coli* or with the co-expression of various chaperones failed (data not shown). Moreover, the protein could not be recovered in an active form after urea denaturation and refolding (data not shown). Reduction of the concentration of IPTG down to 50 μM and of the growth temperature (20 °C) resulted in some amelioration with up to 10% of AraGT ending up in the soluble fraction. However, under these conditions overall yield was also reduced (0.1–0.25 mg/l were obtained in a typical purification experiment).

4.3. Purification of recombinant AraGT

We next proceeded to purifying recombinant AraGT using a rapid single-step scheme from the soluble fraction using metal-affinity chromatography (Fig. 3; see Section 2). AraGT was thus purified to more than 90% purity as judged by Coomassie-staining of SDS-PAGE gels (lane 5). The purified polypeptide can be immunostained with an anti-hexahistidinyl antiserum (data not shown) and therefore retains the N-terminal residues

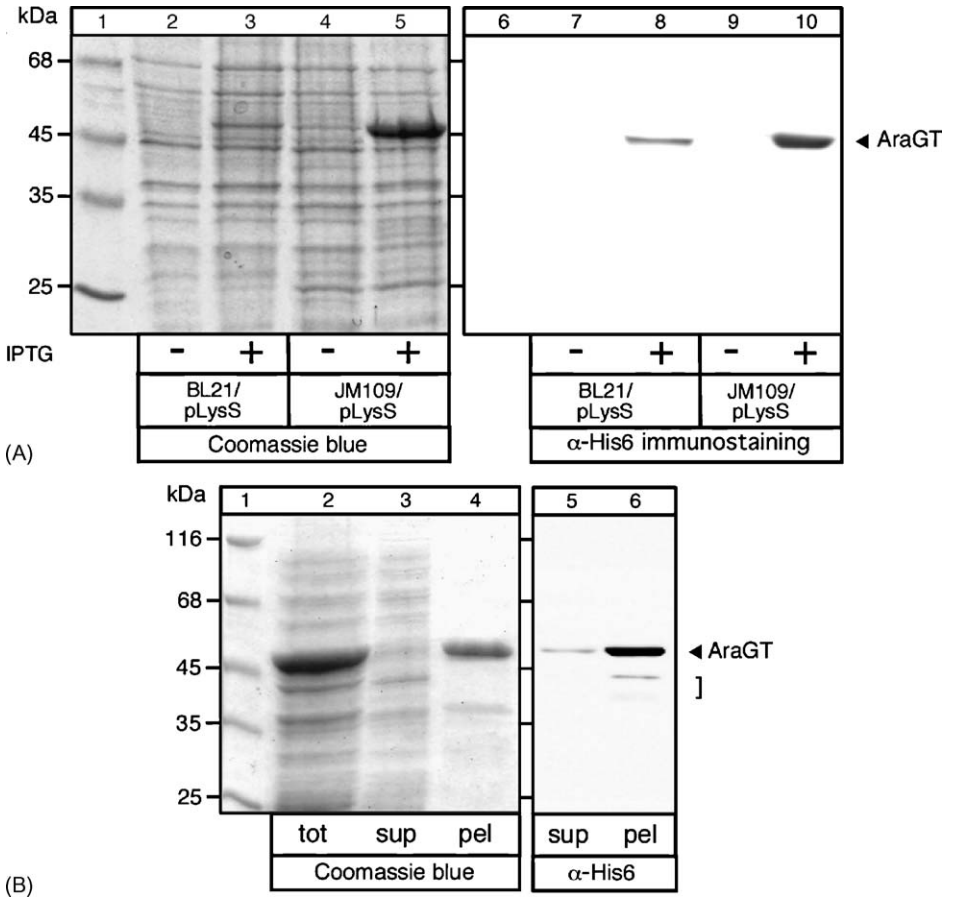


Fig. 2. Heterologous expression and solubility of AraGT in *E. coli*. (A) Induced and non-induced cell samples from AraGT expression in *E. coli* strains BL21(DE3)pLysS and JM109(DE3)pLysS were analyzed by SDS-PAGE and either stained with Coomassie Brillant Blue or immunostained with anti-hexahistidinyl antibody as indicated. (B) Solubility of AraGT. The soluble and insoluble fractions from expressed AraGT in JM109(DE3)pLysS were analyzed as in (A). Lane 1, molecular weight markers: β-galactosidase (116 kDa), bovine serum albumin (68 kDa), ovalbumin (45 kDa), lactate dehydrogenase (35 kDa), restriction endonuclease *Bsp98I* (25 kDa).

where the decahistidinyl-tag lies and does not undergo proteolytic processing.

4.4. Physical characterization of recombinant AraGT

We next examined the oligomeric state and thermal stability of recombinant AraGT. Native PAGE electrophoresis revealed that AraGT migrates as a single monodisperse species without any visible signs of higher order aggregates (Fig. 4A; lanes 3 and 4). AraGT migrates with an apparent native M_r of ~80 kDa and therefore would be dimeric. Recombinant AraGT displays a main apparent T_m of 30 °C and two minor T_{mapp} of 15, 8 and 25 °C as revealed by monitoring changes in intrinsic tryptophan fluorescence during thermal denaturation (Fig. 4B).

4.5. Enzymatic characterization of recombinant AraGT

We next examined the catalytic activity of recombinant AraGT. The ability of recombinant AraGT to transfer activated sugars to a polyketide backbone (Fig. 5A) was tested by biocatalysis and analyzed by LC-DAD-MS (see Supplementary Fig. 4). To

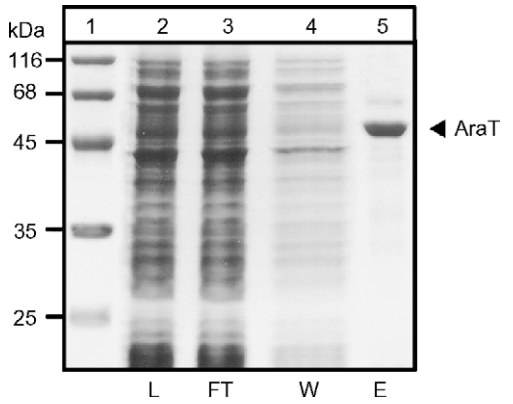


Fig. 3. Purification of recombinant *S. echinatus* AraGT. Protein samples (10-20 μg/lane) from the various purification steps on Ni-NTA (see Section 2) were analyzed and stained with Coomassie Brillant Blue. Lane 1: MW markers as in Fig. 2. L, loading material; FT, flow through; W, wash; E, elution.

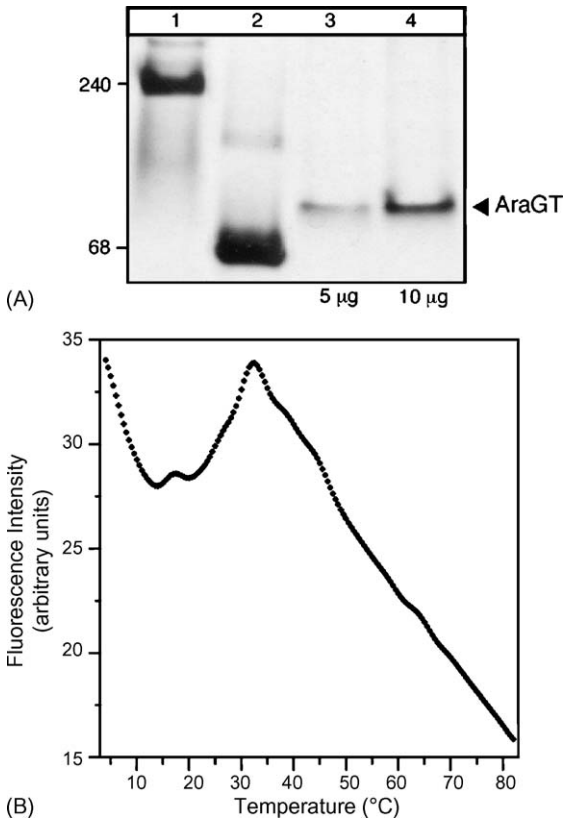
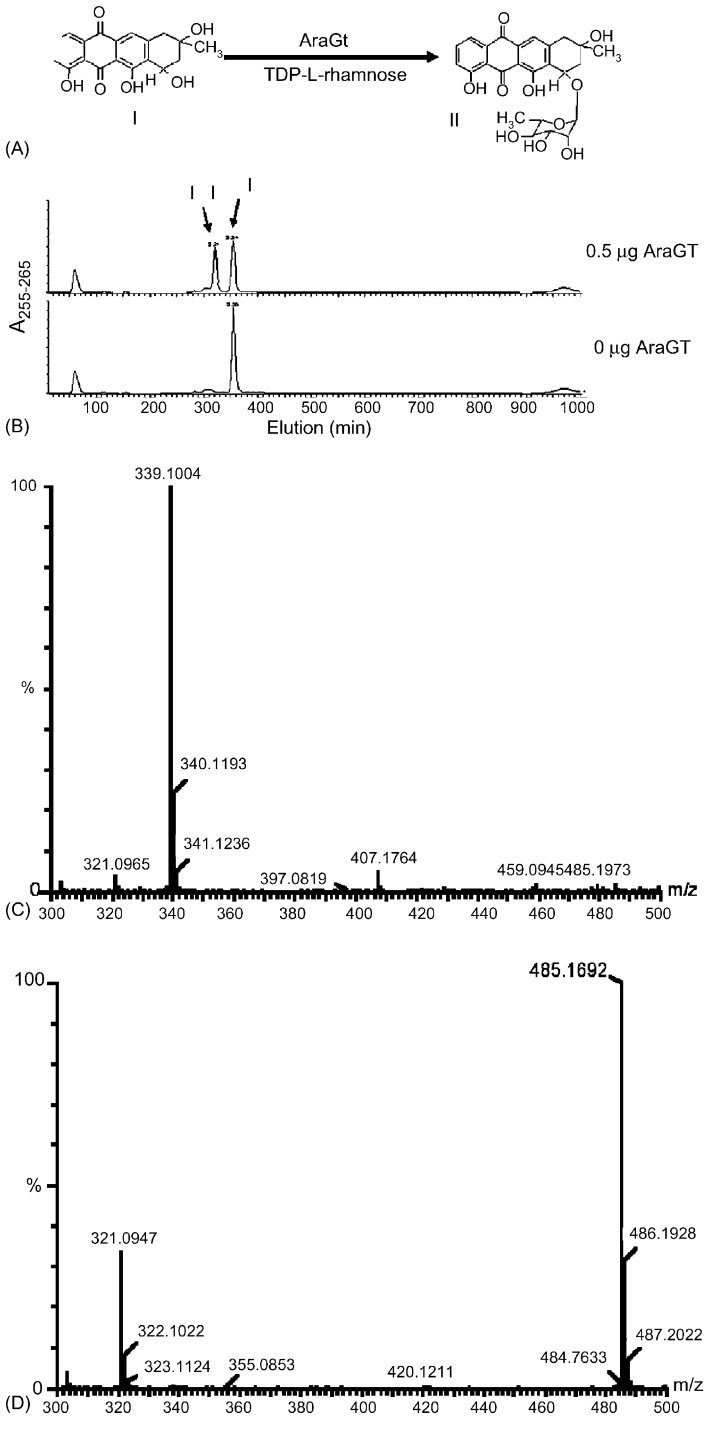


Fig. 4. Physical characterization of recombinant AraGT. (A) Native PAGE. Purified AraGT at the indicated amounts was analyzed by 8% native PAGE (lanes 3 and 4) and stained with Coomassie Brillant Blue. Lane 1 catalase (240 kDa), lane 2 bovine serum albumin (68 kDa). (B) Thermal stability of AraGT monitored by fluorescence. Changes in the intrinsic fluorescence of purified AraGT (50 μg) were followed as a function of temperature as described (Vrontou et al., 2004).

make optimal use of the small amounts of substrate and enzyme available we performed the biocatalysis experiment in a "cell-like" environment, using a cytosolic extract of *S. albus*. A deglycosylated Aranciamycin derivative missing a methoxy-group in position 3 and a keto group in position 1 (CBS000020; see Supplementary Fig. 1) and a six-fold molar excess of TDP-L-rhamnose (see Supplementary Fig. 2) were incubated with purified AraGT (Fig. 5B). In the presence of AraGT conversion of CBS000020 (R_f 3.5 min) to a species that elutes faster (3.2 min) was detectable (upper two UV chromatograms and Supplementary Fig. 5B and C) whereas in its absence the elution profile of CBS000020 remained unaltered (bottom UV chromatogram and Supplementary Fig. 5A). The successful biocatalytic conversion of CBS000020 (Fig. 5C; $[M-H^+]^-$339) to a glycosylated form (Fig. 5D; see Supplementary Fig. 3; $[M-H^+]^-$485) was also confirmed by mass spectrometry analysis of the chromatographic peaks (see Supplementary Fig. 5B and C). The glycosylated product could not be isolated in sufficient quantities to determine experimentally the precise position on the backbone where glycosylation had occurred although we hypothesize this to be position 4 (see Supplementary Fig. 3; see Section 5).

We concluded that recombinant AraGT produced in *E. coli* is biologically fully functional in catalyzing glycosylation of the aglycone anthracycline backbone.

Fig. 5. Assay of glucosyltransferase activity of recombinant AraGT. (A) Theroretical chemical equation of the conversion of CBS00020 "I" to L-rhamnosyl-CBS000225 "II" by AraGT. (B) L-Rhamnose transferred to an aromatic polyketide backbone through AraGT-mediated catalysis rhamnosylation of CBS00020 (100 μg) by the indicated amounts of AraGT. The reactions were separated by RP-HPLC and CBS00020 derivatives were detected at 255–265 nm. The ramnosylation position has not been determined experimentally to be identical to that of the determined chemical structure model (see Panel A). (C and D) Mass spectrometry analysis of peaks "I" and "II". "II": the product CBC000225 ($[M-H^-]^-$ m/z 485) and "I": is the educt CBS000020 ($[M-H^-]^-$ m/z 339).


AraGt
TDP-L-rhamnose
I
II
(A)
0.5 μg AraGT
0 μg AraGT
A255-265
Elution (min)
(B)
339.1004
340.1193
341.1236
321.0965
397.0819
407.1764
459.0945485.1973
m/z
(C)
485.1692
321.0947
322.1022
323.1124
355.0853
420.1211
484.7633
486.1928
487.2022
m/z
(D)

5. Discussion

Glycosylation is important in maintaining the biological activity of numerous natural products. Nevertheless, only a few GTs have been studied biochemically, including glycopeptide Gtfs (GtfA-E) (Losey et al., 2002; Lu et al., 2004; Mulichak et al., 2003), the novobiocin GT NovM (Freel Meyers et al., 2003), the macrolide GTs (OleD and DesVII) (Borisova et al., 2004; Quiros et al., 2000) and anthracycline AknK and AknS (Lu et al., 2005). The difficulty in obtaining highly purified active Gtfs, aglycones, and NDP-deoxysugars has hindered biochemical studies. Firstly, limited accessibility of activated deoxysugars often restricts biochemical studies. Secondly the aglycone substrates often have to be purified from blocked mutant strains, obtained by partial degradation of mature glycosides, or isolated after expression of cosmids with appropiate GT mutations. Finally, only few antibiotic GTs have been successfully expressed in heterologous systems. For example, DnrS, from the daunomycin producer, is not soluble when expressed in *E. coli* (Luzhetskyy et al., 2005a,b,c). So far only the anthracycline aglycone GT AknS from the aclacinomycin producer has been successfully overexpressed in *E. coli* (Lu et al., 2005).

Here we report the molecular cloning of a GT involved in Aranciamycin biosynthesis in *S. echinatus* ssp. *afghanensis* (Tü303; DSM 40730). The enzyme was purified to near homogeneity at milligram levels (Fig. 3) in a proteolytically stable form. AraGT was shown to be catalytically active on the C7-OH position of the anthracycline backbone (Fig. 5). The enzyme was able to accept this substrate with surprising efficiency despite fundamental changes in the backbone ring structure where glycosylation takes place. Moreover AraGT did attach L-rhamnose to the anthracycline backbone in contrast to 2-*O*-methyl-L-rhamnose utilized in the natural substrate Aranciamycin (Keller-Schierlein and Muller, 1970). This indicates that in contrast to other GTs like LanGT2, involved in the biosynthesis of the angucyclic antibiotic landomycin, AraGT is flexible with regard to the polyketide substrate as well as the sugar moiety. Similar flexibilities have been described for the GTs UrdGT2 (Dürr et al., 2004) and ElmGT (Blanco et al., 2001) involved in the biosynthesis of urdamycin and eloramycin. Variability in linker regions (Fig. 1B) and an appreciably low T_{mapp} (Fig. 4B) may contribute to these molecular adaptations.

Our data and the low yield of in vitro generated glycosylated product do not allow us to conclusively determine which of the backbone hydroxyls has been glycosylated. Nevertheless, in vivo synthesized Aranciamycin derivatives using AraGT heterogously expressed in other *Streptomycetes* led to generation of polyketide derivatives that were always modified at position 4 (manuscript in preparation). We therefore anticipate that the glycosylated compound generated by the recombinant AraGT is also glycosylated at position 4 (Fig. 1 A). However, additional work will be required to demonstrate this experimentally.

AraGT is homologous to the GTs DnrS, SnoG and AknS (Luzhetskyy et al., 2005a,b,c). These enzymes catalyze the transfer of a sugar to the hydroxyl group in position 7 of anthracyclic aglycons. In contrast to AknS, AraGT is highly active in glycosylation of the Aranciamycin-like aglycone on its own (Fig. 5).

Recent progress in the biochemical characterization of antibiotic GTs, renders chemo-enzymatic approaches attractive for the rapid generation of antibiotic derivatives. Here we show that AraGT can be used to generate novel anthracyclines containing novel oligosaccharide side chains. In combination with enzymes like AknK, AraGT could be used for the enzymatic generation of libraries of differentially glycosylated aromatic polyketides. Such approaches will allow the generation of new anthracycline derivatives with improved pharmacological features.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jbiotec.2006.03.035.

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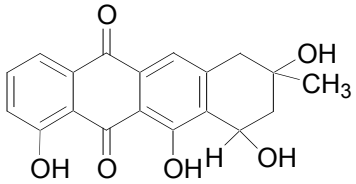
Sianidis et al., Supplementary analytical data

Supplementary Fig. 1

CBS000020



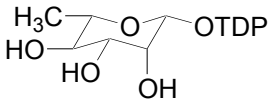
Molecular Formula: $C_{19}H_{16}O_6$

Monoisotopic Mass: 340.0974

Average Mass: 340.3324

Supplementary Fig. 2

TDP-L-rhamnose di-sodium salt (CBC000055)



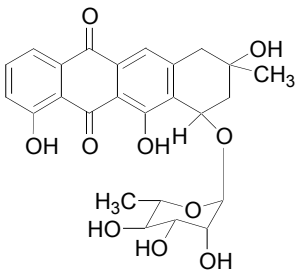
Molecular Formula: $C_{16}H_{24}N_2Na_2O_{15}P_2$

Monoisotopic Mass: 592.0447

Average Mass: 592.2981

Supplementary Fig. 3

Product: CBC0000225



Molecular Formula: $C_{25}H_{26}O_{10}$

Monoisotopic Mass: 486.1526

Average Mass: 486.4754

Supplementary Fig. 4

LC-DAD-MS:

HPLC	MS
Model: Waters Alliance 2790 Column: Grom Sil 120 ODS-4 HE, 3 µm, Dim. 40 x 4 mm Guard column: Phenomenex C18 ODS, 4 mm L x 3.0 mm ID	Model: Micromass Q-TOF 2 Source: ESI all data acquired in negative mode

Gradient system

Solvents:

A water with 0.1% formic acid

B acetonitrile

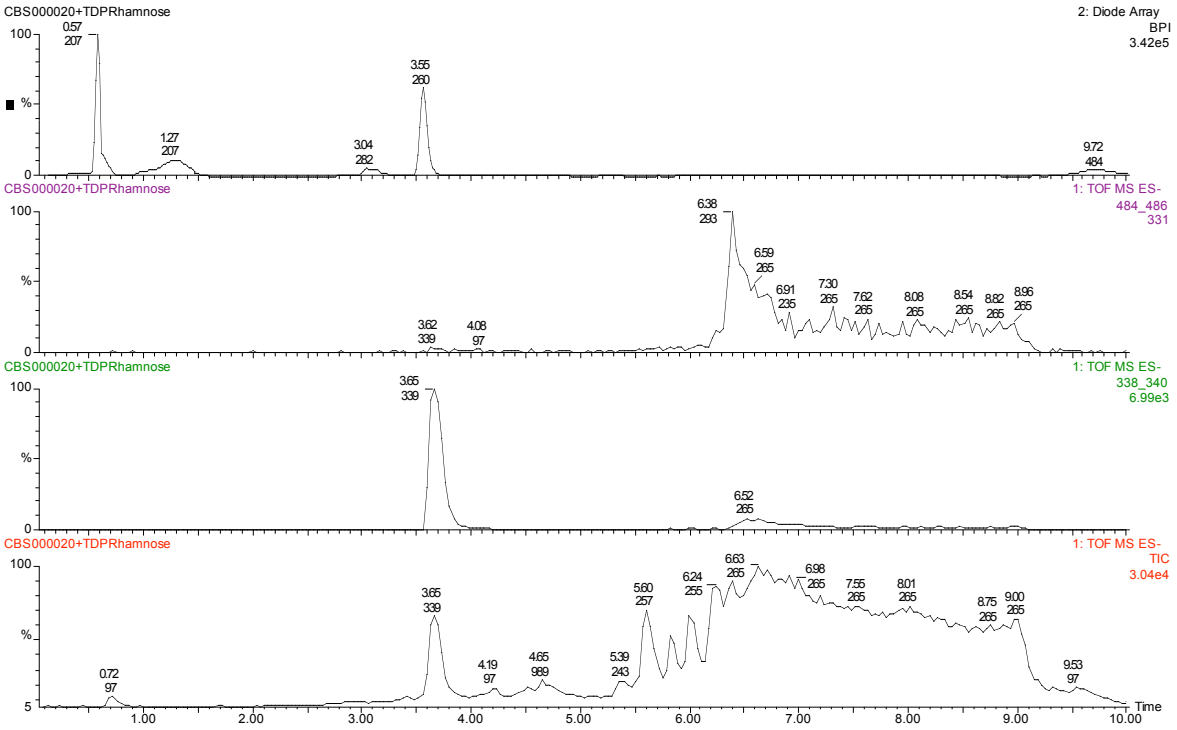
Time	A%	B%	Flow	Curve
0.00	98.0	2.0	1.000	1
5.00	16.7	83.3	1.000	6
6.00	4.0	96.0	1.700	6
8.00	4.0	96.0	1.700	6
8.50	98.0	2.0	1.000	6
10.00	98.0	2.0	1.000	6

Supplementary Fig. 5

A) Control

Incubation of CBS000020 with TDP-L-Rhamnose

	CBS000020	TDP-L-Rhamnose diNa	Enzyme
1	0.1 mg / 0.29 μmol	1.1 mg / 1.8 μmol	-



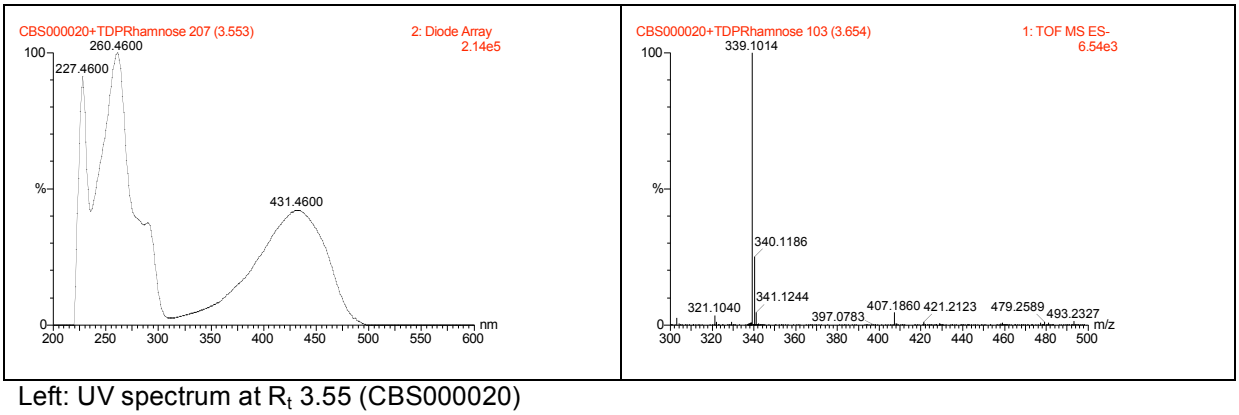
Bottom to top

- total MS ion current

- focused ion chromatogram between m/z 338 and 440

- focused ion chromatogram between m/z 484 and 486

- DAD UV chromatogram between wavelengths 200 and 795 nm



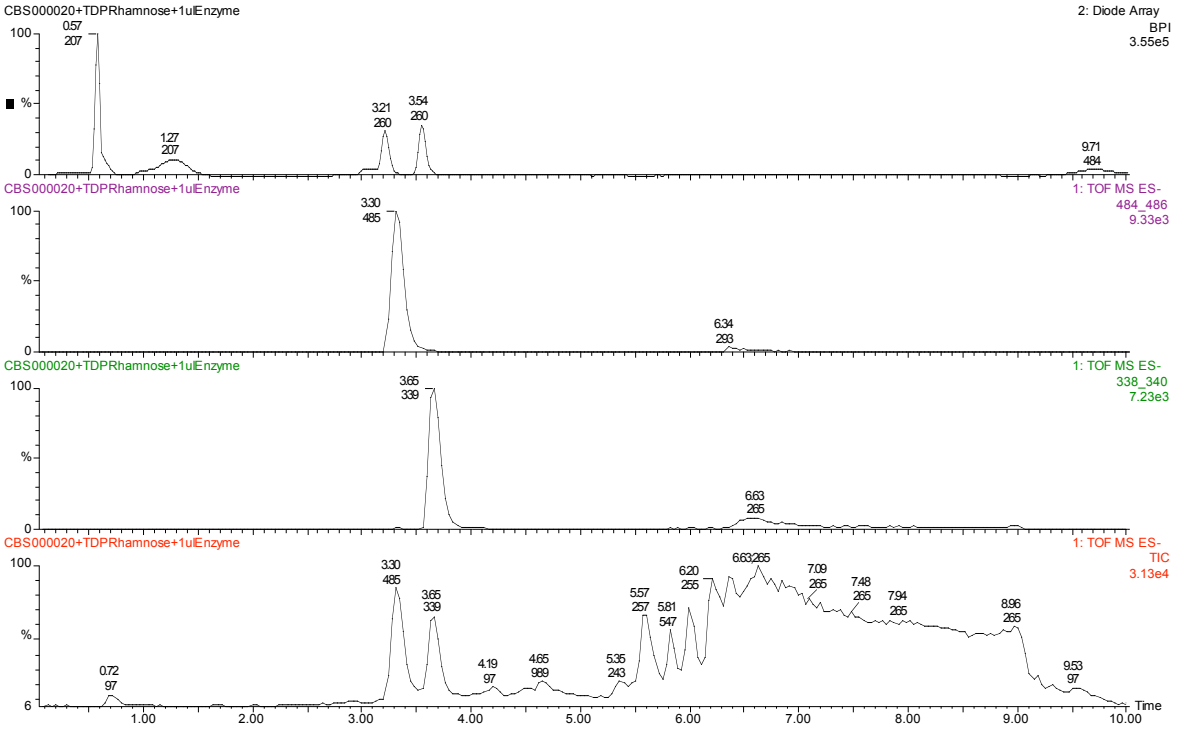
Left: UV spectrum at R_t 3.55 (CBS000020)

Right: corresponding MS spectrum at R_t 3.65 (CBS00020; m/z $[M-H^+]^-$ 339)

B) Biocatalysis

Incubation of CBS000020 with TDP-L-Rhamnose and 0.5µg AraGT

	CBS000020	TDP-L-Rhamnose diNa	Enzyme
2	0.1 mg / 0.29 µmol	1.1 mg / 1.8 µmol	1 µl



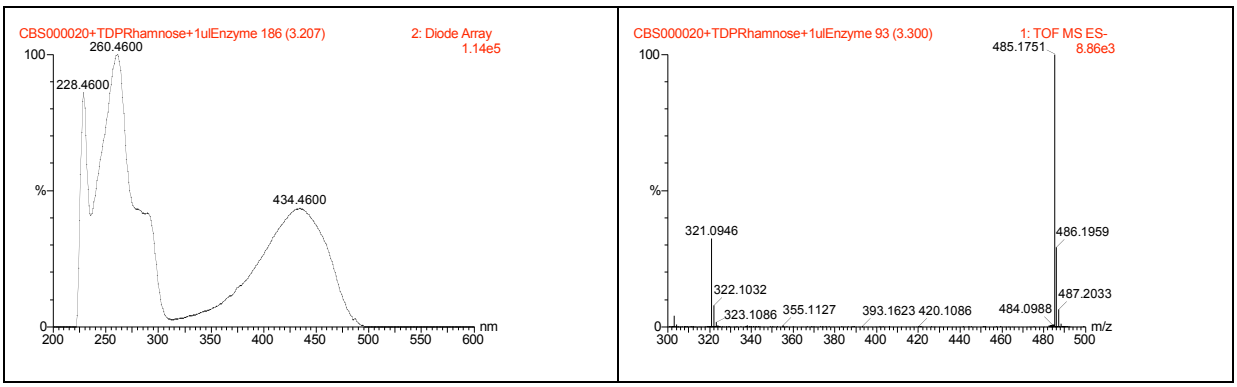
Bottom to top

- total MS ion current

- focused ion chromatogram between m/z 338 and 440

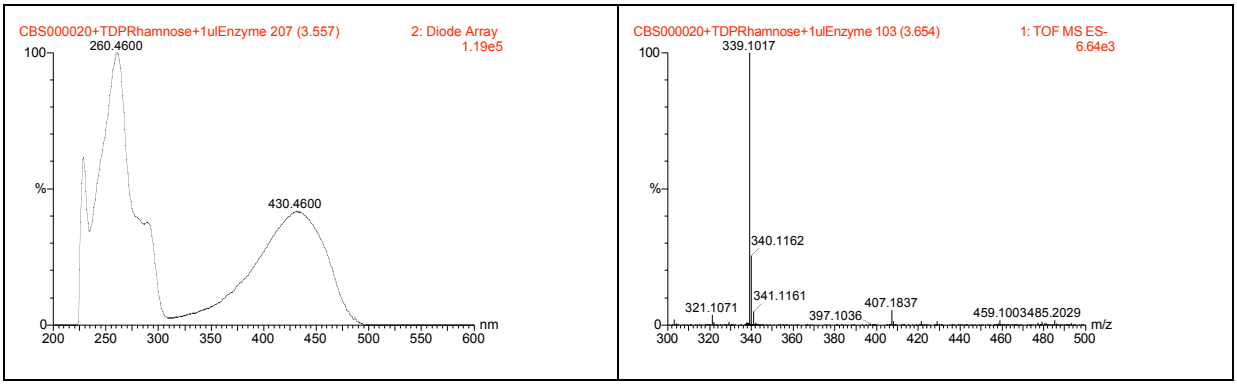
- focused ion chromatogram between m/z 484 and 486

- DAD UV chromatogram between wavelengths 200 and 795 nm



Left: UV spectrum at R_t 3.20 (CBC000225)

Right: corresponding MS spectrum at R_t 3.30 (CBC000225; m/z $[M\text{-}H^+]^-$ 485)



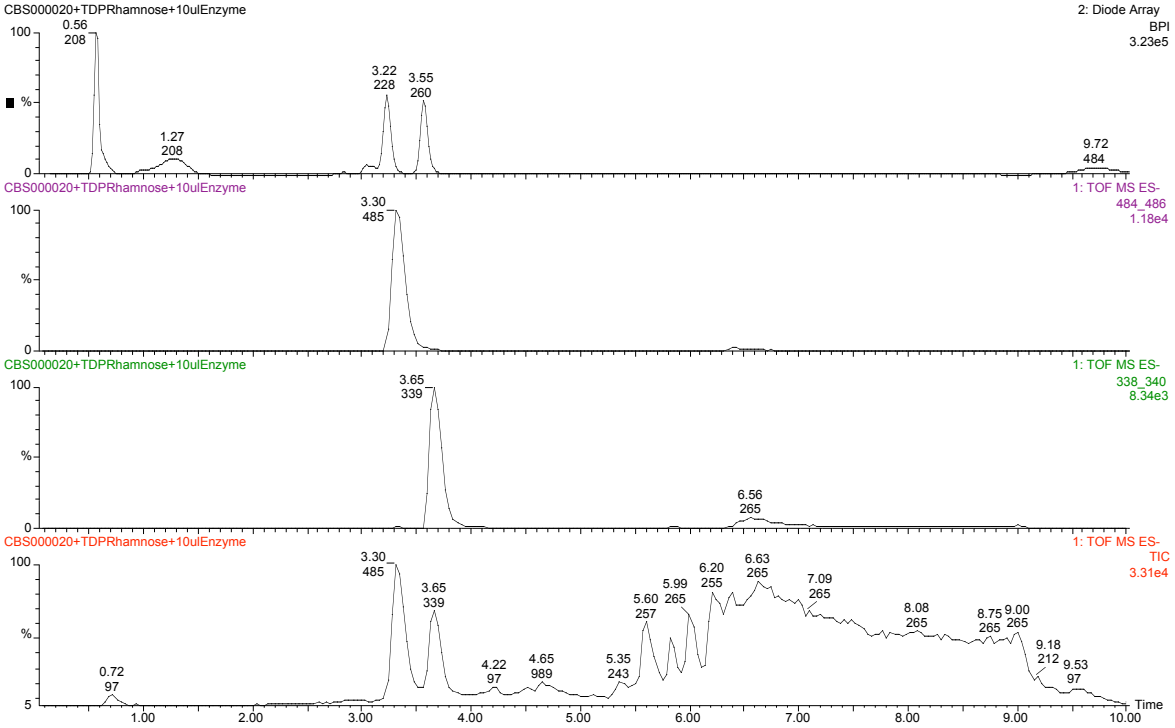
Left: UV spectrum at R_t 3.56 (CBS000020)

Right: corresponding MS spectrum at R_t 3.65 (CBS00020; m/z $[M\text{-}H^+]^-$ 339)

C) Biocatalysis

Incubation of CBS000020 with TDP-L-Rhamnose and 5 µg AraGT

	CBS000020	**TDP-L-Rhamnose diNa**	**Enzyme**
3	0.1 mg / 0.29 µmol	1.1 mg / 1.8 µmol	10 µl



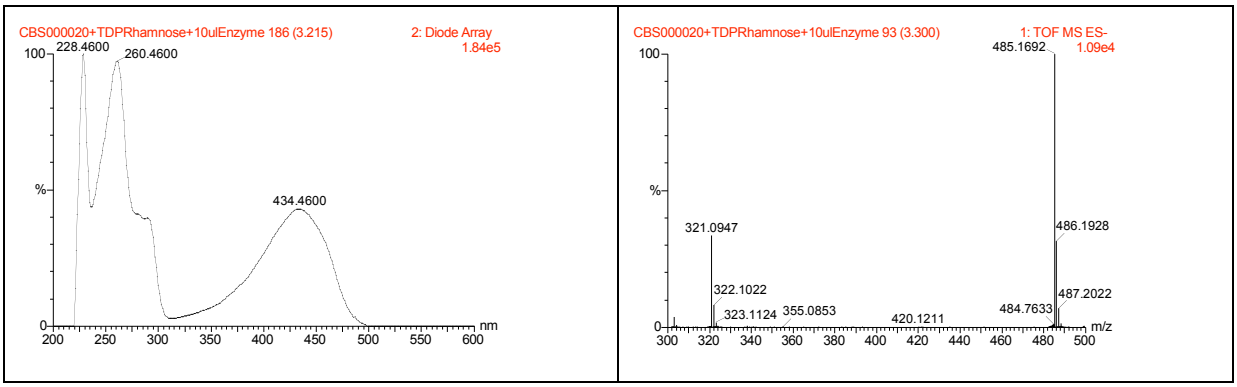
Bottom to top

- total MS ion current

- focused ion chromatogram between m/z 338 and 440

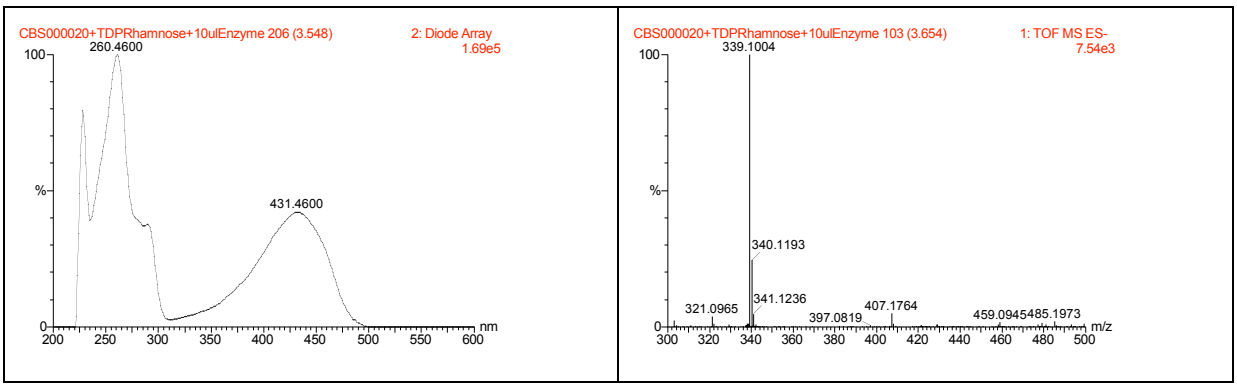
- focused ion chromatogram between m/z 484 and 486

- DAD UV chromatogram between wavelengths 200 and 795 nm



Left: UV spectrum at R_t 3.20 (CBC000225)

Right: corresponding MS spectrum at R_t 3.30 (CBC000225; m/z $[M\text{-}H^+]^-$ 485)



Left: UV spectrum at R_t 3.56 (CBS000020)

Right: corresponding MS spectrum at R_t 3.65 (CBS00020; m/z $[M\text{-}H^+]^-$ 339)