



Isolation of potent alpha-glucosidase inhibitor from a novel marine bacterium *Arthrobacter enclensis*

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Abstract

Alpha-glucosidases are involved in the hydrolyzation of glycosidic bond of di- or oligo-saccharides into mono-saccharides, thus help in the breakdown and absorption of sugars. Inhibition of alpha-glucosidases by inhibitors tend to slow break down and release of sugars into the bloodstream and can be used as therapeutic agents in the treatment of diabetes and obesity. In particular, some of the inhibitors are used in the treatment mainly acarbose, voglibose, and miglitol. In this study, we have reported the alpha-glucosidase inhibitory compound from a newly described marine bacterium *Arthrobacter enclensis*. The purified compound from *A. enclensis* was identified by HPLC, and further FTIR and tandem mass spectrometry (MS/MS) methods. The purified compound was annotated and identified by a Web tool CFM-ID (Competitive Fragmentation Modeling for Metabolite Identification). From analysis it was found that the compound showed high similarity with acarbose which is a C₇N aminocyclitol compound. Further, we analyze the draft genome of *A. enclensis* using anti-SMASH. We observed that it matches the homology with biosynthetic gene cluster of acarviostatin and acarbose with 11% and 7% respectively. We demonstrated that, the purified compound from *A. enclensis* shows the inhibitory activity against alpha-glucosidase with an IC₅₀ value of 500 ± 0.142 µg/ml as compared to standard acarbose, which showed an IC₅₀ value of 200 ± 0.012 µg/ml. This result suggests that *A. enclensis* has a tendency to produce a C₇N aminocyclitol like molecule which matched to acarbose and it uses different biosynthetic gene cluster for the synthesis of C₇N aminocyclitol like molecule, which can be further used for the production and treatment purpose.

Keywords Alpha-glucosidase · *Arthrobacter enclensis* · Marine bacterium

1 Introduction

Alpha-glucosidase (EC. 3.2.1.20) is the enzyme of glycoside hydrolase family which hydrolyzes the alpha-glycosidic bonds present in the carbohydrates. It cleaves the α-1, 4 linked terminal non-reducing end of a sugar moiety [10]. It helps in the breakdown of carbohydrates. Inhibition of alpha-glucosidase helps in slow release of monosaccharides into the bloodstream after a meal. In diabetes mellitus type 2 hyperglycemia conditions occurs after the meal due to the action of insulin resistance. To control the

hyperglycemic condition after the meal, alpha-glucosidase inhibitors are used as therapeutic agents [1, 20].

Inhibitors of alpha-glucosidase are naturally produced by many organisms and several inhibitors have been reported till date, some are being already used for the treatment purpose. Many plants and microorganisms have been reported to produce glycosidases inhibitors, but those which produce compounds that mimick the structure of monosaccharide or oligosaccharide are very efficient because of their resemblance to the sugar moiety of natural substrate. C₇N aminocyclitol are metabolites which contains oligosaccharides with amine derivatives.

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These compounds have a pseudosaccharide cyclitol core unit to which different numbers of sugars molecules are attached [12]. An example of this is Acarbose, which is an inhibitor for alpha-glucosidase which comprises an acarviosine moiety and maltose unit. Acarviosine is composed of an unsaturated cyclitol (valienol or valienamine) ring which is attached to the 4-amino-4,6-dideoxyglucose unit with an amide linkage [23]. This amino-oligosaccharide family contains other compounds as well which showed the inhibitory activity against glycosidase enzymes. This class of compounds is isolated from the *Actinoplanes* sp. [21] and some *Streptomyces* sp. [7, 14].

The genus *Arthrobacter* was described by Conn and Dimmick [4] for aerobic, Gram positives, rod shaped (young culture) to coccus shaped (in older cultures) bacteria. It had been demonstrated that, species of *Arthrobacter* were able to degrade hazardous contaminants and chemicals [6, 9]. Some *Arthrobacter* species have been reported to lyse yeast cells [11] and mycelium of *Fusarium roseum* [15, 19]. Despite of above activities, inhibition of alpha-glucosidase enzymes was not reported from any *Arthrobacter* species till date.

The aim of the study was to isolate alpha-glucosidase inhibitor from a novel marine bacterium *Arthrobacter enclensis*, which was previously, reported [5]. We had investigated its crude complex inhibitory activity against alpha-glucosidase. We have isolated and identified the compound that showed similarity with an acarbose, with a potent alpha-glucosidase inhibition activity.

2 Materials and methods

2.1 Microorganism and enzyme

Arthrobacter enclensis sp. nov., which was isolated from marine sediment sample from Chorao Island, Goa, India, was used in this study. *Arthrobacter enclensis* was stored at -80°C in 20% (v/v) sterile glycerol until further use. Prior to the study, *A. enclensis* was activated on the nutrient agar plate at 30°C for about 24 h. The alpha-glucosidase enzyme from *Saccharomyces cerevisiae* (E.C.3.2.1.20), p-nitrophenyl- α -D-glucopyranoside (pNPG) and acarbose was purchased from Sigma (Merck). Other chemicals used were of analytical grade.

2.2 Fermentation of *Arthrobacter enclensis*

The seed culture was prepared by shaking at 30°C for 24 h in a medium consisting of (g/L): glucose 15.0; peptone 7.5; $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$ 1.0 and NaCl 5.0. The seed culture was then transferred to the fermentation medium consisting of (g/L): glucose-30.0; maltose-15.0; peptone-5.0;

monosodium glutamate-3.0; $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$ -1.0; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ -1.0; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ -0.02 and NaNO_3 -3.0. The fermentation was carried out in shaking condition at 30°C for 168 h (7 days) at 140 rpm. The growth parameter of *A. enclensis* and the effect of their crude fermentation broth on alpha-glucosidase were monitored for 168 h (7 days) with an time interval of 24 h. Growth was measured as an optical density at spectrophotometer with wavelength of 600 nm and the inhibition were measured as a % inhibition.

2.3 Purification of alpha-glucosidase inhibitor

The culture broth was centrifuged at 8000 rpm for 10 min. The filtrate was then incubated with *S. cerevisiae* beads for 24 h at 37°C . The *S. cerevisiae* beads were prepared according to the method described by [22]. The *S. cerevisiae* beads were removed by centrifugation at 6000 rpm for 10 min. The resulting supernatant was mixed with an equal volume of absolute cold ethanol and kept for 30 min to remove any soluble dextran. The mixture was centrifuged again and filtered through Whatman filter paper to remove the precipitated material.

The water fraction was then subjected to the gel permeation chromatography. The fraction was loaded on Bio-gel P-2 (extra fine) column (1.5×90 cm) and eluted with deionized water at a flow rate of 0.1 ml/min, to collect 10 ml fractions. The fractions which showed the inhibitory activity against alpha-glucosidase were pooled together and concentrated for further analysis.

2.4 Assay for alpha-glucosidase inhibition

The alpha-glucosidase inhibition assay was carried out at a 96 well microtiter plate based spectrometry (Thermo Multiskan EX). A volume of 50 μl sample and 100 μl of enzyme solution (1U/ml) prepared in 0.1 M sodium phosphate buffer of pH 6.9, were incubated in microtiter plates for 10 min at 25°C . After incubation, 50 μl (5.0 mM) of para-nitrophenyl alpha-D-glucopyranoside (pNPG) solution prepared in 0.1 M sodium phosphate buffer of pH 6.9, was added in each well and further incubated for 5 min at 25°C . The absorbance was recorded at 405 nm by using microtiter plate reader. The absorbance of samples was compared with the absorbance of control, which contains 50 μl of 0.1 M phosphate buffer of pH 6.9 in place of sample. The enzyme inhibition was expressed as % inhibition using the following equation.

$$\% \text{ Inhibition} = \frac{[A_{405} \text{Control} - A_{405} \text{Sample}]}{A_{405} \text{Control}} \times 100$$

2.5 High performance liquid chromatography (HPLC) analyses

The fractions collected from Biogel P-2 chromatography were further analyzed by HPLC along with standard acarbose. The samples were injected into HPLC system (Thermo, UHPLC Ultimate 3000); Carbohydrate column (Waters) 3.9×300 mm, $10 \mu\text{l}$ of injection volume was employed for HPLC analysis with a flow rate of 1 ml/min and a wavelength at 210 nm. The mobile phase used was acetonitrile: phosphate buffer (0.6 g of KH_2PO_4 and 0.48 g of Na_2HPO_4) with a ratio of 70:30.

2.6 Characterization of the alpha-glucosidase inhibitor

The isolated compound was identified using FTIR spectrometry, and by tandem mass spectrometry and compared with standard acarbose. The FTIR was investigated on BRUKER- Tensor37 system with a wavelength range from 4000 to 500 cm^{-1} . The system was controlled by OPUS v.6.5 software. Mass detection (MS) and tandem mass spectro-metry (MS/MS) spectra were performed on a Thermo Q-Exactive Orbitrap mass spec-trometer, equipped with an ESI source and an ion trap mass analyzer. The mobile phase methanol: water (90:10) with 0.1% formic acid was used at a flow rate of 0.350 ml/min. The injection volume of $1.5 \mu\text{l}$ was used for positive mode ionization. The mass range of m/z is from 100 to 1500. For MS/MS analysis the collision energy was set at 35 V and system software was controlled by X-calibur v.3.0.

2.7 Anti-SMASH analysis of whole genome of *A. enclensis*

The draft genome of *A. enclensis* was generated using Illumina sequencing at DOE Joint Genome Institute (JGI), Walnut Creek, California, USA. By using Illumina HiSeq2000 platform, shotgun library and sequencing were constructed which generates 8,861,546 reads of totalling 1338.1 Mb data. The final assembly contained 19 contigs in 18 scaf-folds. The length of circular map genome consists of 4,226,231 bp with G + C content of 67.08% (Fig. 1). The general features of draft genome statistics are summarized in (Table 1). This draft genome sequence of *A. enclensis* was deposited in DDBJ/ENA/GenBank under the accession number LNQM00000000 [16]. This draft genome of *A.enclensis* was analyzed using anti-SMASH (Antibiotics and Secondary Metabolite Analysis Shell) v.4.0.

3 Results and discussion

3.1 Effect of crude fermentation broth on alpha-glucosidase and growth parameters of *A. enclensis*

Production of alpha-glucosidase inhibitors has gained large commercial interest due to its beneficial properties in the human health. The alpha-glucosidase inhibitory complex was isolated from the culture broth of *A. enclensis*. The starting inhibitory activity was observed from 24 h of fermentation which was then exceeded

Fig. 1 Graphical circular map of *A. enclensis* genome. This genome consists of 4,226,231 bp and 3888 predicted protein coding sequence. From the outside to the center: protein coding sequences (blue colour), RNA genes (tRNA red, rRNA pink and others grey) on the forward strand, G+C content (black colour) and GC skew (purple negative value and olive positive value)

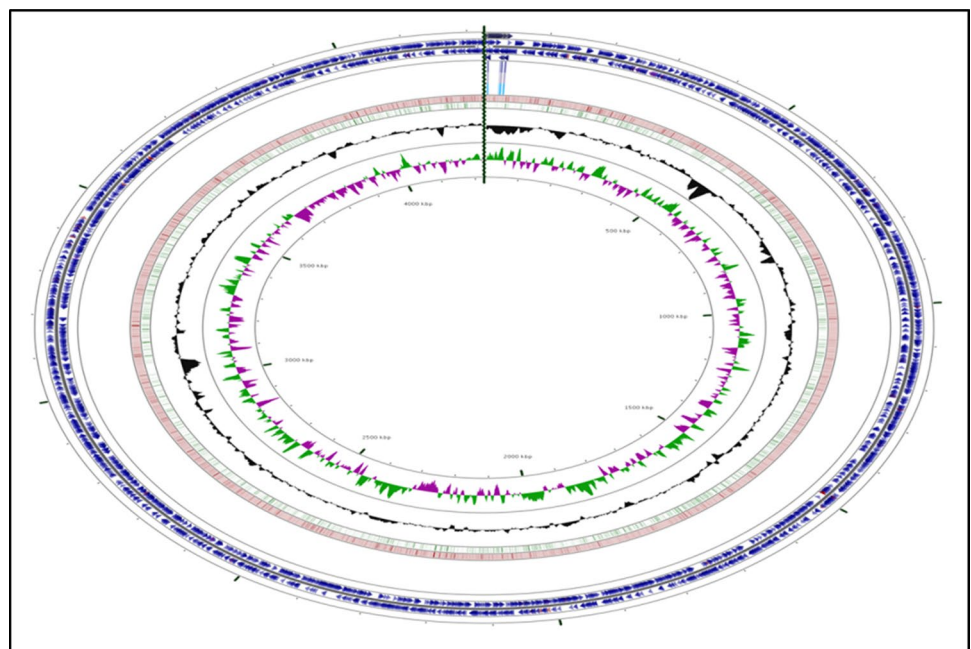


Table 1 General features of draft genome statistics of *A. enclensis*

Attribute	Value	% of total ^a
Genome size (bp)	4,226,231	100.00
DNA G + C content (bp)	2,835,090	67.08
DNA coding region (bp)	3,839,475	90.85
Number of scaffolds	18	
Number of contigs	19	
Total genes	3956	100
RNA genes	68	1.72
rRNA genes	10	0.25
tRNA genes	50	1.26
Pseudogene	55	1.39
Protein-coding genes	3888	98.28
Genes with function prediction	3057	77.28
Genes assigned to COGs	2660	67.24
Genes assigned to Pfam domains	3245	82.03
Genes assigned to TIGRfam domains	1027	25.96
Genes with peptide signals	174	4.40
Genes coding transmembrane proteins	1015	25.66
CPISPR repeats	0	

^aThe total is based on either the size of genome in base pairs or the total number of genes in the predicted genome

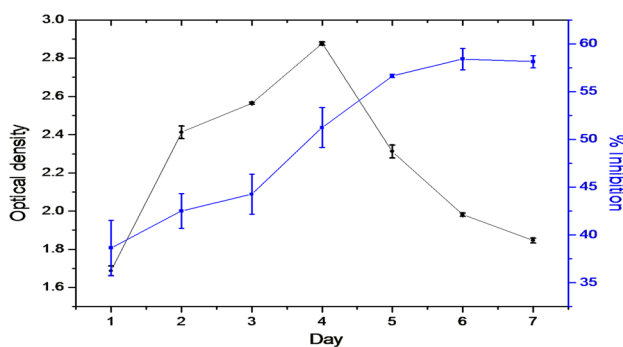


Fig. 2 Growth pattern of *A. enclensis* with % Inhibition of alpha-glucosidase from 1st to 7th day of fermentation. Growth was measured as an optical density at a wavelength of 600 nm and the inhibitory activity was measured as a % inhibition. The data were measured as triplicates

from 96 h to the 168 h. The growth pattern of *A. enclensis* was measured as an optical density with a wavelength of 600 nm. The maximum growth of an organism was observed at 96 h of fermentation on which 50% of inhibition was achieved. The decline of growth was observed from 120 h of fermentation and effect of inhibition was seemed to be constant to the rest of days. The correlation

between growth of bacteria to the inhibition against an enzyme is shown in (Fig. 2). It was then purified by Biogel P-2 gel permeation chromatography. The fractions from 8 to 12 showed the inhibition and was then pooled and further analyzed with HPLC, FTIR and MS/MS.

3.2 HPLC analysis of purified compound

The HPLC chromatogram of standard acarbose and purified compound of *A. enclensis* and unfermented broth as a control is shown in (Fig. 3). The standard acarbose showed a peak at a retention time of 6.61 min. The unfermented broth shows peaks mostly at ranging 2.0–4.4 min, while purified compound showed peaks at different retention times apart from 2.0 to 4.4 min, suggesting that some new peaks appeared which were not observed in unfermented broth. The purified compound showed a peak at a retention time 6.28 min. Apart from this, some additional peaks were also observed which might be of other components produced by *A. enclensis*.

3.3 IR of purified compound

The IR spectra of standard acarbose and purified compound of *A. enclensis* is shown in Fig. 4. The IR spectrum of acarbose displayed strong OH stretching (optimal at 3290 cm^{-1}) at wide peak ranging from 3000 to 3800 cm^{-1} . The C-H stretching appeared at 2875 cm^{-1} . The C=C weak stretching has appeared at a wide range of 1420 – 1650 cm^{-1} . The medium C-H bending and C-O stretching was displayed at 1364 and 1034 cm^{-1} , respectively. The IR spectrum of a purified compound of *A. enclensis* showed a strong OH stretching at 3272 cm^{-1} . The C-H stretching appeared at 2925 cm^{-1} , C=C stretching at 1639 cm^{-1} and C-H bending at 1359 cm^{-1} ranges of a peak. The C-O stretching was displayed at 1012 – 1144 cm^{-1} . From the above, it was found that most of the groups displayed correspond to the oligosaccharides. Acarbose is a pseudo-oligosaccharide compound, which contains N-H amide linkage, which usually ranges from 3360 to 3310 cm^{-1} , and in case of acarbose it is occupied by a wide range of OH groups [18]. The IR spectra of the purified compound of *A. enclensis* also showed the presence of an oligosaccharide compound.

3.4 Mass spectrometry (MS) and tandem mass spectrometry (MS/MS)

The purified compound was then identified by positive ion mode electrospray ionization mass spectrometry (ESI-MS) and tandem mass spectrometry (MS/

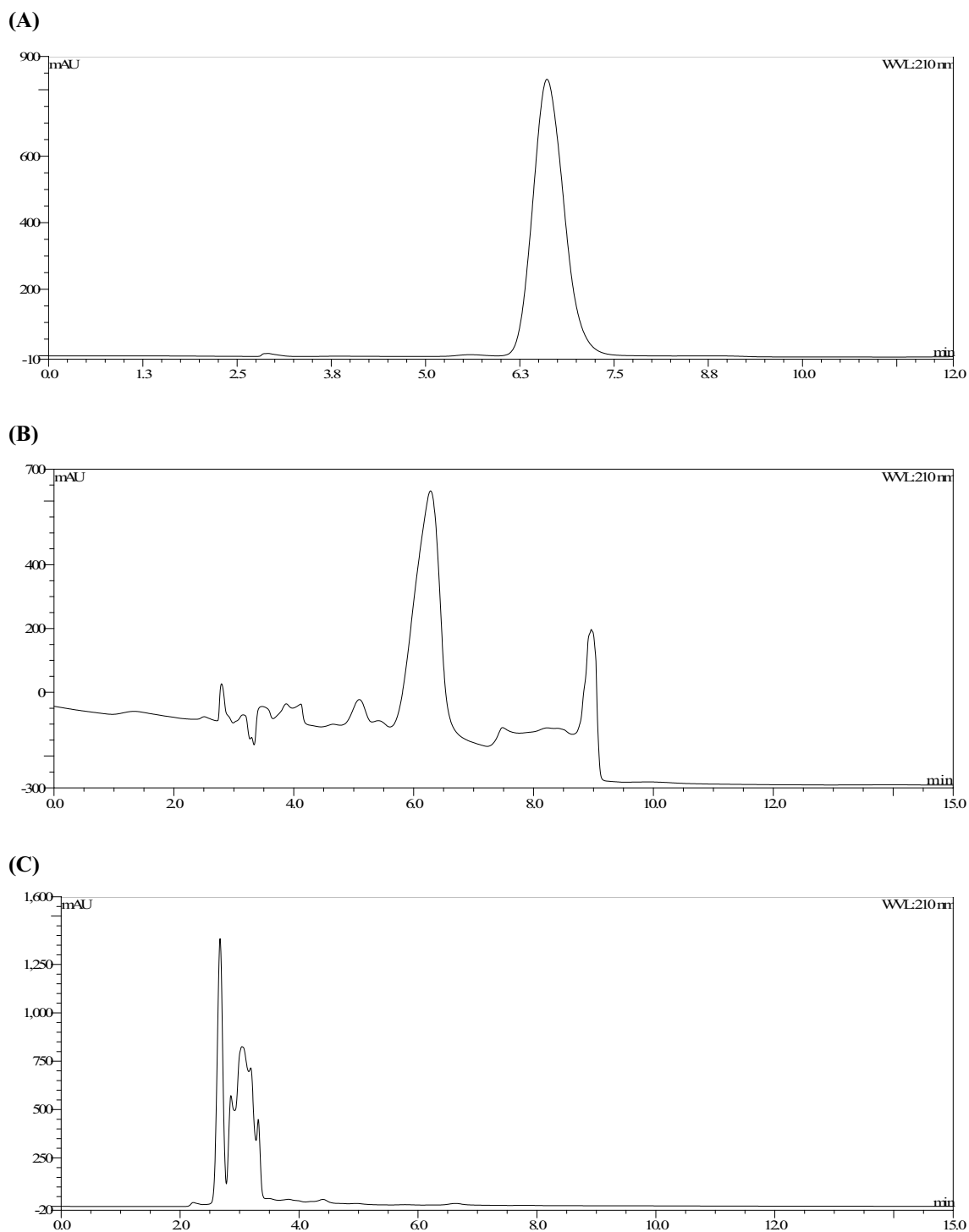
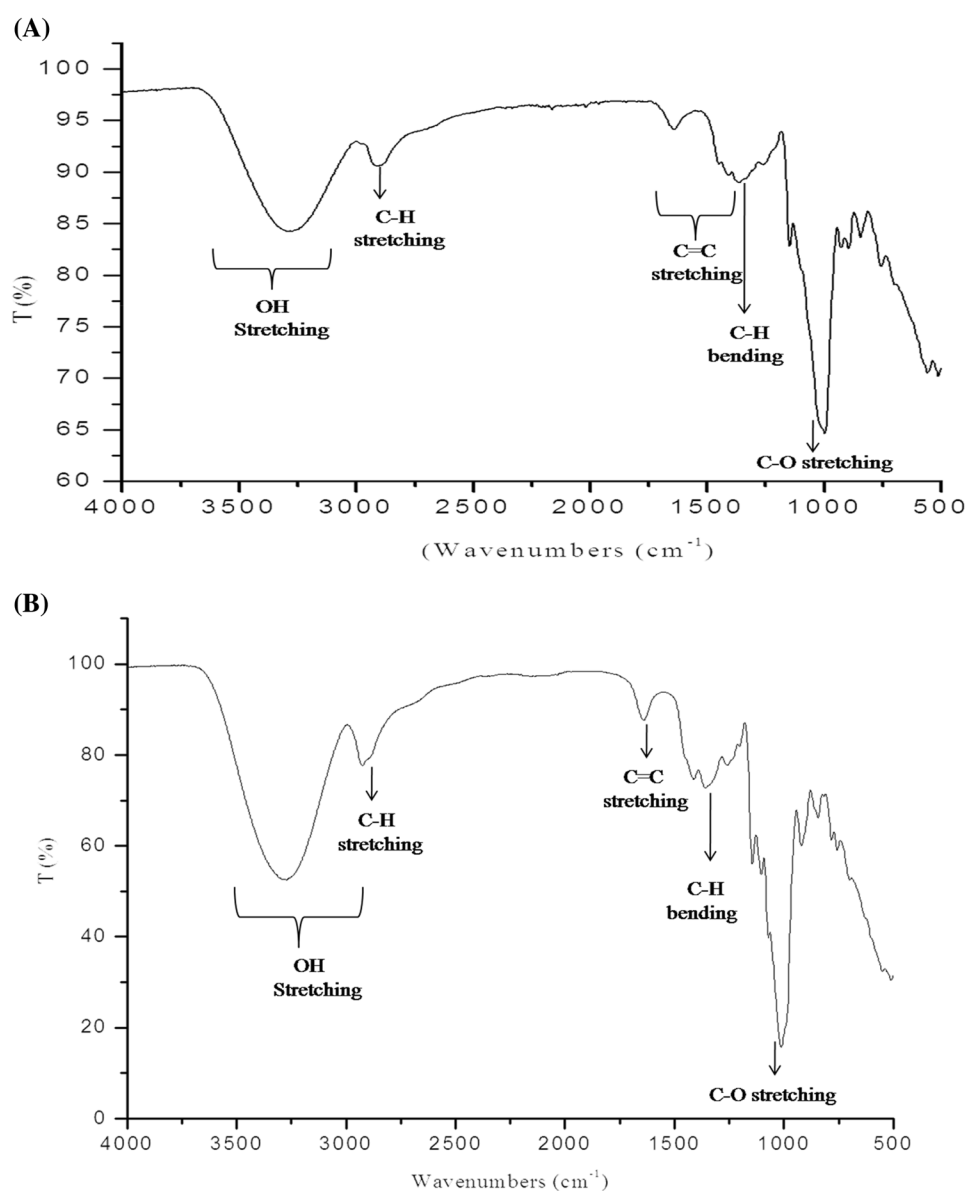


Fig. 3 HPLC chromatogram of acarbose (A), purified compound of *A. enclensis* (B), and unfermented media (C)

MS) analysis. The ESI-MS of acarbose was done which showed a peak at m/z 646.2542 $[M + H]^+$ signal is shown in (Fig. 5a). In full scan of an active compound of *A.*

enclensis showed a peak at m/z 646.2545 $[M + H]^+$ signal is shown in (Fig. 5b). The tandem mass spectrometry (MS/MS) spectrum of parental compounds of both

Fig. 4 FTIR spectrum of standard acarbose (A) and purified compound of *A. enclensis* (B)



standard acarbose and peak of m/z 646.2545 of *A. enclensis* was done (Fig. 5c, d). The fragmentation pattern of both the parental compounds was found to be similar. The annotation of peaks in the spectrum and the integration of spectrum were done by using a web tool CFM-ID. The CFM-ID provides the method for the efficiently identified metabolites in a spectrum for a known chemical structure generated by MS/MS [2]. The signals at 466 m/z and 304 m/z correspond to the loss of one to two glucose units from the molecule. This indicates the dissociation of glycosidic bond. The fragment ion 304 m/z , 268 m/z and 286 m/z were matched with acarviosine unit. The fragment 146 m/z indicates the cleavage at N-H (amide) linkage of acarviosine moiety. The 146 and 128 fragment ions were matched with cyclitol

ring of an acarviosine molecule. From the above, it was observed that the fragments produced upon ionization were of dissociation products from a glycosidic bond and from an amide bond. By comparison of the spectrum of acarbose to the active compound of *A. enclensis*, it was matched highly with the acarbose. From spectrum analysis it was observed that the purified compound showed the matches with amide linkage of acarviosine moiety and glycosidic linkage to one glucose moiety, which is a characteristic of C_7N aminocyclitol class compounds.

3.5 Alpha-glucosidase inhibition

We investigated the inhibitory effect of purified compound from *A. enclensis* and standard acarbose on

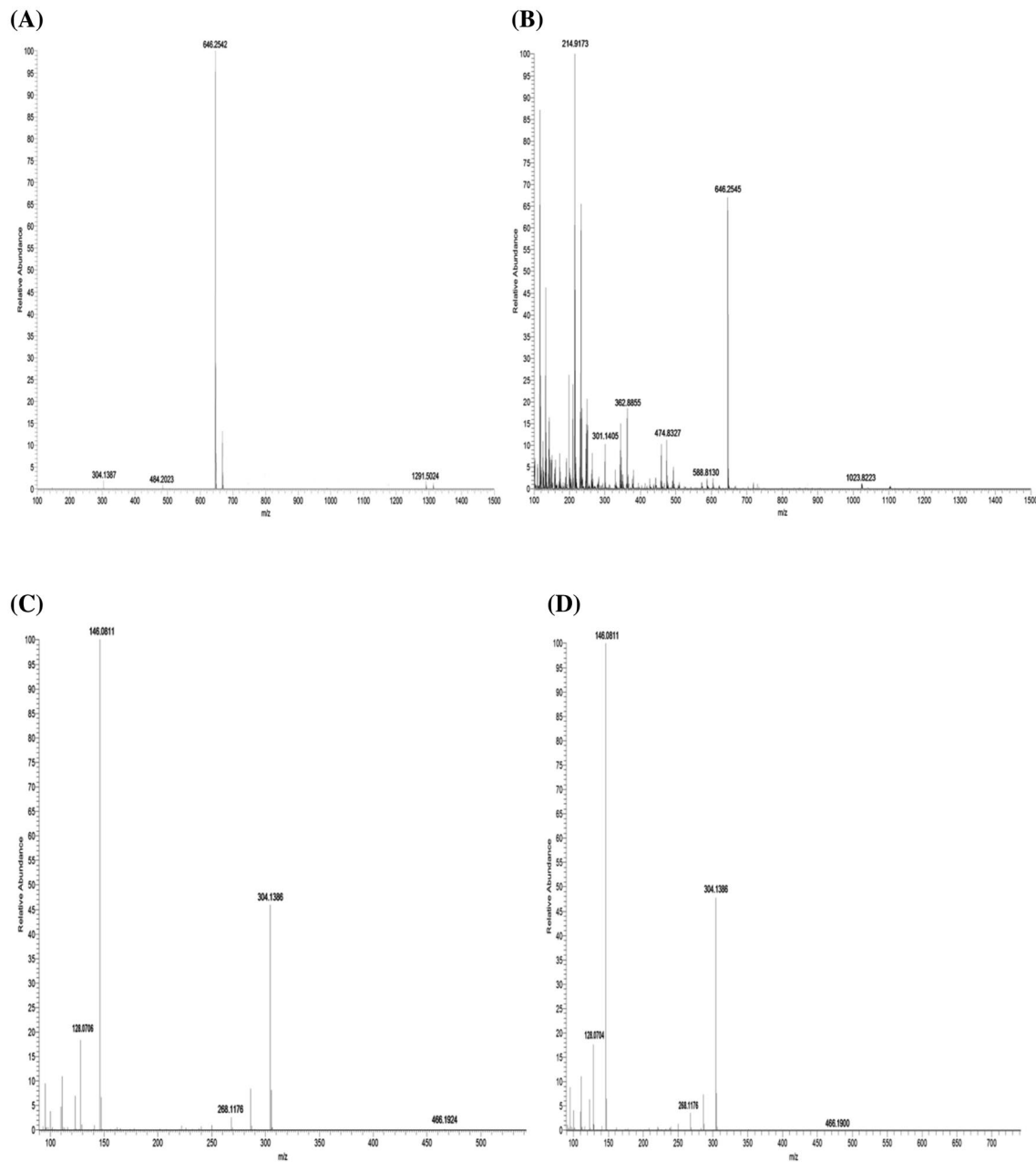


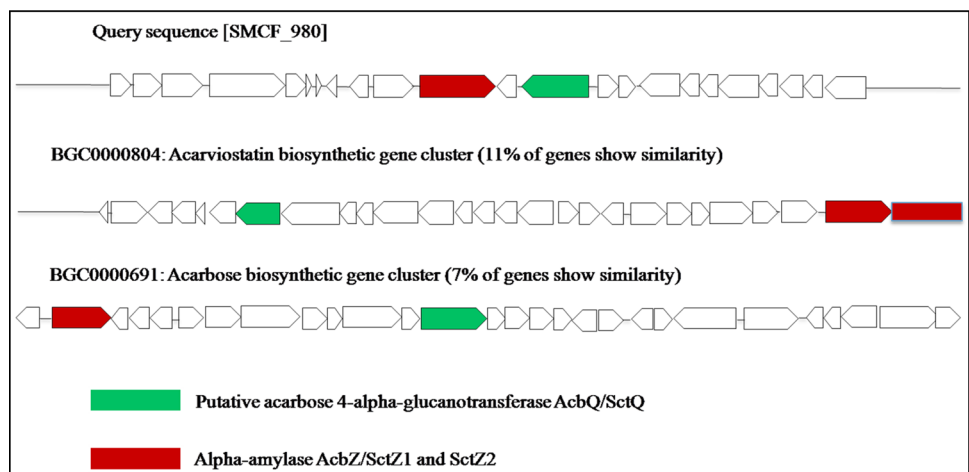
Fig. 5 **A** ESI-MS of standard acarbose. **B** ESI-MS of purified compound of *A. enclensis*. **C** MS/MS of standard acarbose. **D** MS/MS of parent compound m/z 646.2545 of *A. enclensis*

Table 2 Inhibitory concentration values (IC_{50}) of acarbose and partially purified compound from *A. enclensis* against alpha-glucosidase

Compound	IC_{50} value	Maximum inhibitory activity (%)
Acarbose	$200 \pm 0.012 \mu\text{g/ml SD}$	91.11
<i>A. enclensis</i> purified compound	$500 \pm 0.142 \mu\text{g/ml SD}$	75.42

alpha-glucosidase (Table 2). We found that standard acarbose showed the inhibitory activity with an IC_{50} value of $200 \pm 0.012 \mu\text{g/ml}$ while the purified compound from *A. enclensis* showed the activity at an IC_{50} value of $500 \pm 0.142 \mu\text{g/ml}$. The maximum inhibition was observed 91.11% for acarbose and 75.42% for compound isolated from *A. enclensis*.

Fig. 6 Anti-SMASH analysis of draft genome of *A. enclensis* showing inhibitor cluster



3.6 Anti-SMASH analysis of draft genome of *A. enclensis*

The anti-SMASH helps in the alignment of a query gene cluster with the known gene cluster from a database and to identify biosynthetic gene cluster [3, 13, 16]. We observed that the cluster 21 from scaffold 00004 from gene SMCF_980 showed the putative gene cluster similarity with acarviostatin biosynthetic gene cluster (11%) and acarbose bio-synthetic gene cluster (7%) from the location of 48801–67650 nucleotides with Cluster-Finder probability of 0.8674. In both gene clusters, it matches with acarbose 4-alpha-glucanotransferase (AcbQ/SctQ) and alpha-amylase (AcbZ/SctZ1 and SctZ2) genes (Fig. 6). The acarviostatin and acarbose both is C₇N aminocyclitol compound and acarviostatin act as alpha-amylase inhibitor and acarbose as alpha-glucosidase and alpha-amylase inhibitor. The putative gene cluster of acarviostatin biosynthesis (Sct) exhibited similarity with gac-gene cluster of *Streptomyces glaucescens* GLA.O (GenBank accession AM409314, [17]) and with acb-gene cluster of *Actinoplanes* sp. SE50/110 (GenBank accession Y18523, [21]) for acarbose synthesis. Some steps of the putative intracellular synthetic pathway of acarviostatin are similar to the biosynthetic pathway of acarbose [8], suggesting that the formation of core cyclitol structure of both acarviostatin and acarbose shares some common steps.

Later on, we compare the protein encoding similarity of gene cluster of *A. enclensis* with acb-gene cluster of *Actinoplanes* sp. SE50/110 with reference gene cluster of *S. glaucescens* (Table 3). We found that, some putative gene

products of *A. enclensis* showed similarity with acarbose gene products of *Actinoplanes* sp. SE50/110. We observed that alpha-amylase matches 54% with AcbZ, ABC transporter ATP binding protein matches 32% with AcbW, 4-alpha-glucanotransferase matches 46% with AcbQ, dTDP-glucose 4,6-dehydratase matches 44% with AcbB, alpha-amylase matches 48% with AcbE and ABC transporter permease and ABC transporter binding protein matches 55%, 54% and 45% with AcbG, AcbF and AcbH respectively.

4 Conclusion

A alpha-glucosidase inhibitory compound was isolated and purified from a novel marine bacteria *A. enclensis*. Inhibitory compound is identified based on HPLC, FTIR and tandem mass spectrometry (MS/MS) analysis. It was observation that, isolated compound showed a similarity with a C₇N aminocyclitol like molecule. Further, we found that inhibitory compound showed the presence of acarviosine like ring in the compound from the MS/MS analysis and was annotated and confirmed by CFM-ID, and the purified compound of *A. enclensis* matches with the acarbose molecule. The draft genome of *A. enclensis* showed the biosynthetic gene cluster similarity with acarviostatin and acarbose. Both acarviostatin and acarbose are C₇N aminocyclitol compounds and are known to possess glycosidases inhibition activities. From present study, we concluded that the *A. enclensis* has a tendency to produce C₇N aminocyclitol like molecule which showed inhibitory activity towards alpha-glucosidase

Table 3 Identities and similarities of proteins encoded by *A. enclensis*

aa length	Gene product <i>Arthrobacter enclensis</i> (Identity/e-value to an Acb-protein)	aa length	Identity/e-value to an Acb-protein from <i>Actinoplanes sp. SE50/110</i>	aa length	Identity/e-value to an Acb-protein from <i>Streptomyces glaucescens</i>
582	Diguanylate cyclase/PAS Domain S-box-containing protein/diguanylate cyclase (GGDEF) domain-containing protein (28%/2e-13)	402	Hypothetical protein (100%/0.0)	713	GGDEF domain-containing protein (31%/3e-09)
1036	Alpha-amylase (54%/0.0)	1103	Alpha-amylase AcbZ (100%/0.0)	1111	Alpha-amylase GacZ2 (52%/0.0)
264	No homolog encoded by the acb-gene cluster	267	ABC transporter permease protein AcbY (100%/0.0)	266	ABC transporter permease protein GacY (56%/2e-100)
267	No homolog encoded by the acb-gene cluster	267	ABC transporter permease protein AcbX (100%/0.0)	269	ABC transporter permease protein GacX (50%/2e-78)
357	ABC transporter/lipooligosaccharide transport system ATP-binding protein (32%/2e-33)	357	ABC transporter ATP-binding protein AcbW (100%/0.0)	342	ABC transporter ATP-binding protein GacW (64%/5e-147)
456	4-aminobutyrate-2-oxoglutarate transaminase (30%/5e-47)	453	dTDP-4-keto-6-deoxy-glucose 4-aminotransferase AcbV (100%/0.0)	430	dTDP-4-keto-6-deoxy-glucose 4-aminotransferase GacV (75%/0.0)
293	Glycosyl transferase family 2 (34%/0.94)	492	Putative kinase (1-epi-valienol-7-phosphate 1-kinase) AcbU (100%/0.0)	489	Putative kinase (1-epi-valienol-7-phosphate-1-kinase) GacU (43%/2e-85)
795	Beta-galactosidase (32%/8.2)				
303	LysR family transcriptional regulator (30%/4.2)	709	Glycosyltransferase AcbS (100%/0.0)	699	Glycosyltransferase GacS (59%/0.0)
468	Glucose-1-phosphate adenyllyltransferase (32%/3e-44)	363	Putative 1-epi-valienol-1,7-bisphosphate-1-adenyltransferase AcbR (100%/0.0)	377	Putative 1-epi-valienol-1,7-bisphosphate-1-adenyltransferase GacR (72%/5e-175)
219	ADP-ribose pyrophosphatase (29%/6e-08)	195	Putative NUDIX hydrolase AcbP (100%/7e-144)	208	ADP-ribose pyrophosphatase (30%/3e-09)
419	Beta-glucosidase (25%/4.7)	734	Glycosyltransferase AcbI (100%/0.0)	420	Glycosyltransferase (47%/0.0)
272	Haloacid dehalogenase (39%/2e-35)	283	putative hydrolase AcbJ (100%/0.0)	1028	putative glycosyltransferase GacI (56%/2e-80)
740	4-alpha-glucanotransferase (46%/3e-180)	698	Putative acarbose 4-alpha-glucanotransferase AcbQ (100%/0.0)	699	Putative acarbose 4-alpha-glucanotransferase GacQ (59%/0.0)
373	Sugar or nucleoside kinase, ribokinase family (36%/0.001)	299	Acarbose 7-kinase AcbK (100%/0.0)	306	Acarbose-7-kinase GacK (48%/7e-81)
335	ROK family transcriptional regulator/glucokinase (35%/0.007)	359	2-epi-5-epi-valiolone 7-kinase AcbM (100%/0.0)	364	C7-cyclitol-7-kinase GacM (29%/4e-17)
336	Alcohol dehydrogenase(31%/3e-09)	366	Cyclitol dehydrogenase AcbL (100%/0.0)	365	Alcohol dehydrogenase (29%/1e-07)
304	NAD(P)-dependent oxidoreductase (30%/1e-12)	251	Cyclitol oxidoreductase AcbN (100%/1e-180)	248	Ketoreductase/putative short-chain dehydrogenase/reductase SDR (36%/4e-13)
248	Xylose isomerase/Sugar phosphate isomerase/epimerase (28%/0.010)	270	2-epi-5-epi-valiolone-7-phosphate 2-epimerase AcbO (100%/0.0)	452	Hypothetical protein SGLAU_12830 (39%/0.58)
363	3-dehydroquinase synthase (34%/8e-30)	384	2-epi-5-epi-valiolone synthase AcbC (100%/0.0)	388	2-epi-5-epi-valiolone synthase GacC (40%/5e-56)
332	dTDP-glucose 4,6-dehydratase (44%/7e-93)	317	dTDP-glucose 4,6-dehydratase AcbB (100%/0.0)	330	dTDP-glucose 4,6-dehydratase (59%/1e-134)
290	Glucose-1-phosphate thymidyltransferase (57%/4e-118)	303	dTDP-glucose synthase AcbA (100%/0.0)	355	dTDP-glucose synthase GacA (43%/4e-49)
1036	Alpha-amylase (48%/0.0)	1038	Acarbose resistant alpha-amylase AcbE (100%/0.0)	1111	Alpha-amylase GacZ2 (45%/0.0)

Table 3 (continued)

aa length	Gene product <i>Arthrobacter enclensis</i> (Identity/e-value to an Acb-protein)	aa length	Identity/e-value to an Acb-protein from <i>Actinoplanes</i> sp. SE50/110	aa length	Identity/e-value to an Acb-protein from <i>Streptomyces glaucescens</i>
1036	alpha-amylase (26%/2e-29)	724	Acarviose transferase (ATase) AcbD (100%/0.0)		Sulfonate ABC transporter ATP-binding protein (27%/5e-38)
307	ABC transporter permease (55%/4e-103)	263	ABC transporter membrane protein AcbG (100%/0.0)	342	ABC transporter permease (30%/2e-35)
323	ABC transporter permease (54%/3e-98)	326	ABC transporter membrane protein AcbF (100%/0.0)	316	Putative integral membrane binding-protein-dependent transport protein (28%/4e-30)
436	Sugar ABC transporter substrate-binding protein (45%/6e-128)	433	ABC transporter binding protein AcbH (100%/0.0)	424	Sugar ABC transporter substrate-binding protein (26%/1e-23)
785	No homolog encoded by the acb-gene cluster	785	Putative galactocerebrosidase (100%/0.0)	475	Endo-1,4-beta-xylanase A (58%/2e-39)
354	No homolog encoded by the acb-gene cluster	363	Putative xylanase	475	Endo-1,4-beta-xylanase A (48%/3e-30)

enzyme and matches high similarity with acarbose. This is the first time, we are reporting the alpha-glucosidase inhibitory compound from a newly described marine bacterium *A. enclensis*.

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Author's contribution The author SGD and MSD designed the study and the author RBM performed the experiments and data collection and analysis and manuscript writing.

Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

Human and animal rights No human and animal experiments were performed in this study.

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