Inhibition spectrum, purification and kinetic study of alpha-amylase inhibitor from *Murraya koenigii* endophytic actinobacterium (*Streptomyces koyangensis* strain B025)

Diabetes mellitus type II, the chronic endocrine disorder, affects metabolism of carbohydrates, proteins, fats, electrolytes and water. This type of diabetes can only be controlled by maintaining low levels of blood glucose and through a healthy lifestyle[1]. The currently available medicines, viz. biguanides, sulphonylureas and thiazolidinediones, pose risks of secondary failure and prominent side-effects. Therefore, it is imperative to go for the alternative approach, a strong antidiabetic activity, without any spin-off results.

Ayurvedic literature suggests that plant extracts have always been used to treat diabetes, owing to low-cost, easy availability and fewer consequences[2]. *Murraya koenigii* (family Rutaceae) is a small, deciduous shrub that possesses notable pharmacological effects like antidiabetic, antidiarrhoeal, antimicrobial, antioxidant, antiulcer, cardiovascular activities as well as cytotoxic, cholesterol reducing and phagocytic potential[3]. Several studies confirmed the inhibitory effects of *M. koenigii* leaves on diabetic animal models as well[4–6].

Nearly all plants retain a microbiota and some microbial colonizers reside in the inner plant tissues as endophytes. It is a well-demonstrated fact that compounds held by the host plant, are also released by a few of their resident endophytes. Endophytic actinobacteria from the well-known Indian medicinal plant *Azadirachta indica* have been isolated and identified for their antidiabetic activity[7]. With the potential isolates obtained from medicinal plants, it will be possible to produce α-amylase inhibitory compounds in greater amounts and with better quality. Based on this hypothesis, we aimed at isolating and identifying endophytic actinobacteria from *M. koenigii* and characterizing the bioactive compounds.

The actinobacterium was isolated from root tissues of a randomly selected healthy *M. koenigii* shrub from Ludhiana (Punjab) on starch casein agar (SCA) medium. The isolate was identified according to *Bergey's Manual of Systematic Bacteriology*[8].

To carry out molecular identification, DNA was isolated from actinobacterium and amplified with 16S rRNA specific primers (8F and 1492R). Thereafter, bi-directional Sanger DNA sequencing reaction was performed (Primers: 704F and 907R). The 16S rDNA sequence of 1492 base pairs was fed into BLAST alignment search tool of NCBI GenBank database. Ten most closely related sequences were aligned using multiple alignment software-3 program Clustal W. The phylogenetic tree was developed using MEGA5.

Well-grown slant culture was inoculated into 500 ml SCB production medium. This was followed by incubation at 28 ± 2°C for 7 days at 200 rpm. After fermentation, the broth was centrifuged (10,000 rpm, 15 min) at 10°C. The supernatant was extracted twice with ethyl acetate : methanol (4 : 1) and lyophilized using a freeze dryer at –130°C. Different concentrations of ethyl acetate extract were made in 10% dimethyl sulphoxide (DMSO) by weighing the crude obtained after lyophilization.

The α-amylase inhibitory reaction was initiated by adding ethyl acetate extract (250 µl, 10–1000 µg/ml in 10% DMSO) to α-amylase (250 µl, 0.05 mg/ml dissolved in 0.02 M sodium phosphate buffer). After 10 min of incubation (25°C), 1% starch solution (250 µl) was poured to start the reaction. After incubation (10 min, 25°C), 500 µl of dinitrosalicylate reagent (DNS) was added. Following this, the reaction mixture was held in a water bath (100 degrees, 5 min). The mixture was then cooled and diluted to 5 ml with distilled water. Absorbance of the mixture was read at 540 nm. Acarbose acted as a standard for comparison[9].

Total phenol content was assayed according to the Folin–Ciocalteau method[10] by mixing ethyl acetate extract (0.1 ml, 10–1000 µg/ml), distilled water (1.9 ml), Folin–Ciocalteau reagent (1 ml) and Na_2CO_3 (1 ml of 100 g/l) in a tube. The mixture was incubated thereafter (2 h, 25°C) and absorbance measured at 765 nm. Standard curves of catechol and gallic acid were prepared using the same reagents and conditions.

The bioactive crude extract was collected by column chromatography. The column (5 cm × 60 cm) was packed with 300 g of silica gel (60–120 mesh). Dry loaded portion was eluted with a stepwise gradient of chloroform : ethanol fraction (95 : 5, 90 : 10, 85 : 15, 80 : 20, 75 : 25, 70 : 30, 65 : 35, 60 : 40 and 55 : 45). The separation of compounds was tracked as a yellow-coloured band that travelled down the column. The inhibitor enriched fractions were identified, pooled together, concentrated and termed as EAE^P.

The mode of inhibition was deduced according to Kazeem *et al.*[9]. In one set of

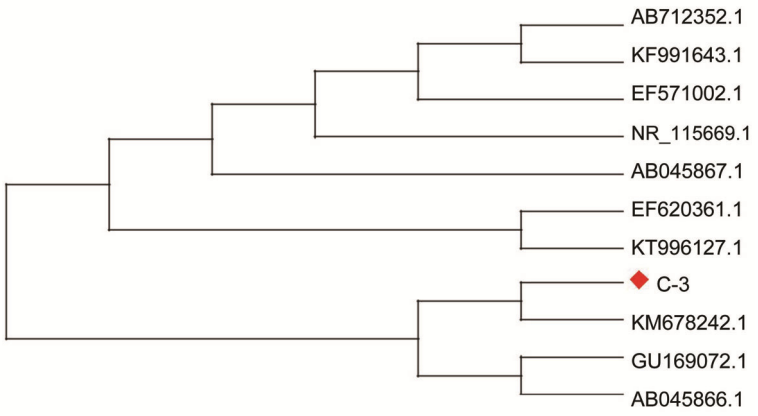

Figure 1. Phylogenetic tree of *Streptomyces koyangensis* strain B025 (C-3) and closest relatedness to species KM678242.1.

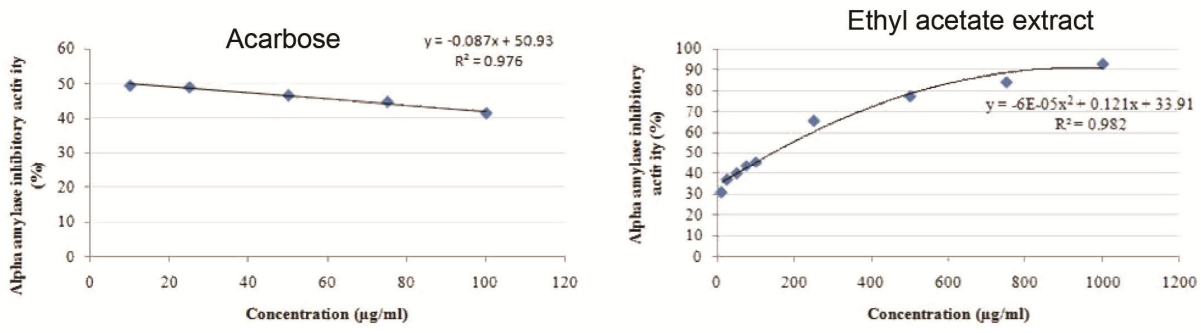

Figure 2. α-Amylase inhibitory activity of ethyl acetate extract in relation to commercial drug Acarbose.

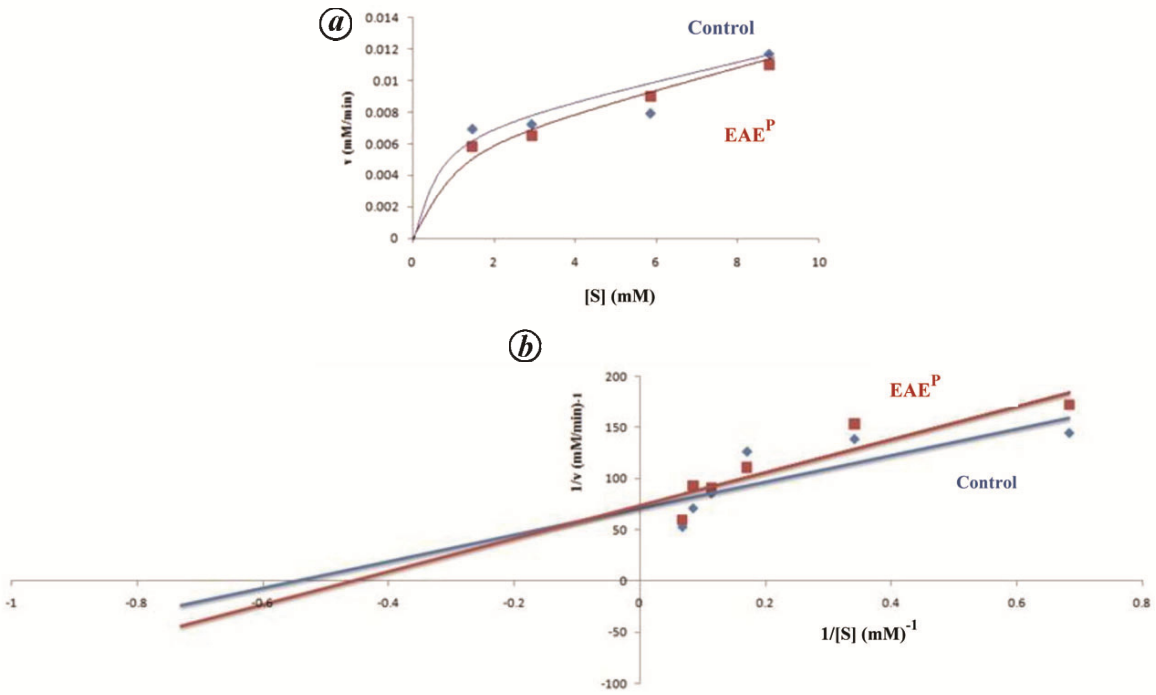

Figure 3. ***a***, Michaelis–Menten; ***b***, Lineweaver–Burk plots for near competitive inhibition exhibited by EAE^P (α-amylase).

tubes, EAE^P (250 µl, 2 mg/ml) was pre-incubated with α-amylase solution for 10 min at 25°C. In the second set of tubes, α-amylase solution (250 µl) was incubated with sodium phosphate buffer (250 µl). Varying concentrations (0.50–5.0 mg/ml) of starch solution (250 µl) were then added to both the sets followed by an incubation at 25°C for 10 min. Thereafter, 500 µl of DNS was added and then incubated at boiling temperature for 5 min in a hot water bath. Reaction velocities were calculated by determining the released reducing sugars calorimetrically and comparing with a maltose standard curve.

Lineweaver–Burk plot was constructed by plotting $1/v$ (inverse of reaction velocity) against $1/[S]$ (inverse of substrate concentration). The plot was analysed using Michaelis–Menten kinetics[11]. The 50% inhibitory concentrations were calculated from linear/polynomial regression analysis.

The isolate exhibited whitish-grey aerial and substrate hyphae with brown diffusible pigments. Under the compound microscope, it was observed to have chain-spore morphology. Results of 16S rDNA analysis indicated that the isolate (C-3) had 99% similarity with *Streptomyces koyangensis*, strain B025 (accession number: KM678242.1) (Figure 1). This is for the first time that an isolate of *S. koyangensis* has been reported from the Indian medicinal shrub *M. koenigii*.

α-amylase is the major enzyme responsible for conversion of starch into simple sugars. The inhibitors of this enzyme prevent intestinal glucose absorption, thus reducing post-prandial blood glucose levels, which is the major cause of diabetic nephropathy and retinopathy[12]. Ethyl acetate extract from *S. koyangensis* strain B025 displayed a dose-dependent increase in inhibitory activity against α-amylase (Figure 2). The activity of Acarbose was almost constant at the various concentrations tested (IC_{50} = 10.64 ± 1.33 µg/ml).

Out of the 10 fractions obtained by column chromatography, 8 displayed inhibition against α-amylase *in vitro*. The inhibition percentages exhibited by these

fractions were 90.59 ± 1.36, 30.31 ± 0.87, 31.70 ± 0.40, 55.40 ± 0.12, 52.96 ± 0.53, 20.90 ± 0.46, 23.34 ± 0.25 and 6.27 ± 0.38.

As observed in Figure 3, near competitive inhibition was exerted by the purified extract (EAE^P) on commercially available α-amylase. The K_m for α-amylase control was 1.81 mM, whereas in the presence of inhibitor, an increase was observed (2.22 mM). As evident in case of competitive inhibitions, the V_{max} values were constant (0.011 mM/min) even after the introduction of inhibitor into the reaction mixture. Likewise, Rey *et al.*[13] studied the mode of antidiabetic activity by crude extracts of *P. peruviana* fruit. Their results revealed that it was able to inhibit maltase in a competitive manner. The K_m (1.110) in control, changed to 7.087 on addition of the crude extract, whereas V_{max} remained constant (0.009 mM/min). However, no such kinetic study is available pertaining to α-amylase inhibitory activity of endophytic actinobacteria of *M. koenigii*.

The antidiabetic activity is believed to be due to the presence of measurable amount of phenolics, which summed up to 32.36 ± 3.31 and 2.21 ± 1.57 mg/g of the extract in terms of the catechol and gallic acid equivalents. The presence of phenol moieties has previously been associated with the inhibition of carbohydrate hydrolysing enzymes[14].

This study suggested that endophytic actinobacteria from *M. koenigii* possess significant antidiabetic potential which might help in preventing or slowing down the progress of the disease. It is important to consider that Acarbose, the commercial drug used to treat type 2 diabetes mellitus is also produced industrially by the actinomycete, i.e. *Actinoplanes* sp.[15]. Further study on the ethyl acetate extract may help develop chemical entities for clinical use.

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Reactivation of minor scars to major landslides – a satellite-based analysis of Kotropi landslide (13 August 2017) in Himachal Pradesh, India

On 13 August 2017, a massive landslide occurred close to the village of Kotropi (near Kotropi bus stop) in Mandi district, Himachal Pradesh, India. It occurred on National Highway 154, the road between Mandi and Pathankot. Media reports suggest that a section of the slope totally collapsed and two buses of the Himachal State Transport Corporation along with few other vehicles were buried under the debris. News reports also suggest that there have been 46 fatalities from the incident. Around 300 m of the highway has been completely buried under debris, thus disrupting communication on an important route[1].

Rugged topography, deformed rock formations and steep slopes make the Himalayas a highly landslide-prone mountain belt. The landslides in this region are recorded to be triggered by rainfall and earthquakes[2,3]. With both the triggering parameters, it is seen that the pre-existing condition of the slope has an important control on the occurrence of new landslides. Scars of pre-existing minor landslides make a particular slope more vulnerable to a major landslide event.

Satellite images are useful for rapid damage and morphological assessment of landslides in the Himalaya[4,5]. Post-event Resourcesat-2 LISS-IV Mx and Cartosat-2S data were acquired by ISRO on 15 and 16 August 2017 over the landslide-affected area through emergency payload programming. Analysis of Cartosat-2S image shows the occurrence of a large landslide in the area near Kotropi bus stop (Figure 1). The landslide zone is a near a thrusted contact (Main Central Thrust) between Shali group (dolomite and brick red shale) in the north and Siwalik group (sandstone and shale) in the south. The landslide is a 'debris-flow' type with a rotational failure mechanism[6]. It has long runout zone, which clearly suggests that heavy rainfall