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Genomic Exploration of Insulin-like Proteins in *Arthrospira platensis* SPKY1

Yuvarani K, Kathireshan AK*

Department of Microbiology, School of life sciences, Vels University, Pallavaram, Chennai, Tamilnadu, India

*Corresponding author: Kathireshan AK, Department of Microbiology, School of Life Sciences, Vels University, Pallavaram, Chennai, Tamil Nadu, India.

Abstract

The prevalence of diabetes, especially type 2 diabetes (T2DM), has reached epidemic levels globally, significantly impacting populations across various countries, including India. This study aimed to explore the potential of *A. platensis* SPKY1 in producing insulin as a cost-effective and sustainable alternative to traditional insulin sources. Water samples were collected from the Ennore estuary in Chennai, India, and *A. platensis* SPKY1 was isolated and identified. Growth studies revealed *A. platensis* SPKY1 as the most potent species in terms of growth rate and biomass accumulation. Screening for insulin presence among isolated strains showed *A. platensis* SPKY1 to exhibit insulin production of 15 µg/g of dry biomass. This finding was further confirmed through protein estimation, SDS-PAGE, 2D gel electrophoresis, and MALDI, revealing a 6 kDa band similar to previous insulin identification studies. Whole genome sequencing of *A. platensis* SPKY1 confirmed the presence of genes encoding insulin-like proteins, with eight insulinase family proteins identified. HPLC analysis confirmed the presence of insulin in *A. platensis* SPKY1, with a retention time of 6.79 minutes. This study underscores the potential of *A. platensis* SPKY1 as a viable source for insulin production, offering advantages such as minimal cost, easy availability, and no reported side effects. Further research and development in this direction could contribute to addressing the growing demand for insulin, particularly in regions with a high prevalence of diabetes, providing a breakthrough in insulin production and accessibility.

Introduction

Diabetes emerged as a significant global health concern in the 21st century, significantly contributing to mortality rates. Data from the International Diabetes Federation (IDF) revealed substantial diabetes prevalence in key countries, including China with 116.4 million, India with 77.0 million, and the United States of America with 31.0 million cases. This rise had reached epidemic levels, with 90% of cases being type II, and it notably affected younger populations [1]. The Global Burden of Disease study, conducted across 195 countries and territories, highlighted a significant surge in the incidence (11.6 million) and prevalence (264.8 million) of diabetes from 1990 to 2017 [2].

In India, the prevalence of diabetes has steadily risen over the past 30 years, significantly contributing to the global burden of the disease. A 2017 study by the Indian Council of Medical Research revealed that the prevalence of diabetes among all age groups increased by 64.3% from 1990 to 2016 [3]. According to the India State-Level Disease Burden Initiative Diabetes study, Tamil Nadu exhibited the highest prevalence in 2016, followed by Kerala, Delhi, Punjab, Goa, and Karnataka [4]. The largest national study conducted in 15 states of India reported diabetes

prevalence ranging from 4.3% to 13.6%, with higher rates in rural areas (5.8% to 15.5%) than urban areas (3.5% to 8.7%) [5]. Additionally, the prevalence of type 2 diabetes was higher among Asian Indians residing in Chennai (38%) compared to those in San Francisco and Chicago, USA (24%) [6]. Data from the population-based representative Center for Cardio-metabolic Risk Reduction in South Asia (CARRS) Study indicated that six out of ten adults in South Asian cities were either diagnosed with diabetes or had pre-diabetes [7]. This epidemic led to a growing demand for anti-diabetic medications, including insulin therapy.

Insulin therapy is essential in managing diabetes, as it is required by all individuals with type 1 diabetes and the majority of those with type 2 diabetes (T2DM) at some point during their treatment. Approximately 4 out of 10 patients with T2DM in India utilized only insulin or a combination of insulin and oral anti-diabetic medications (OADs) [8]. Certain insulin products in India were included in both national and state essential medicines lists (EMLs), allowing for their provision free of charge in public-sector health facilities [9]. However, a survey conducted in Delhi found that the mean availability of recombinant human and analogue insulin in the private sector was significantly lower, at 44.4% and 13.1%, respectively, which were much lower than the WHO recommendation of 80% [10]. Initially, insulin was derived from animals and developed in various formulations. To meet the demand, the production of recombinant human insulin and analogue products has been facilitated through the use of genetically modified microbes (GMOs) [11,12]. Indian individuals tended to prefer private healthcare sectors for diabetes management. However, all insulin and analogues were produced abroad by foreign companies and then imported into the Indian market, making them inaccessible to millions due to poor availability and unaffordable prices [13].

People with diabetes frequently preferred for complementary and alternative medicine (CAM) due to various reasons, including its lower cost, higher medication adherence, perceived lack of side effects, easy availability, and natural origins. A review highlighted alternative sources of insulin beyond the pancreas [14]. Studies showed that *Spirulina* supplementation could decrease blood sugar levels [15], HbA1c levels [16], glucose-6-phosphate activity [17], oxidative stress [18], lipid profiles [19], triglycerides, and cholesterol [20], amylase activity [21] body weight, and waist circumference [22]. Studies also reported that *Spirulina* supplementation increased insulin levels [23-26]. Thus, the objective of this study is to isolate and identify the nucleotide sequence encoding the insulin gene and produce insulin in *Spirulina sp.*

Materials and methods

Study Area

The Ennore estuary, located north of Chennai and extending beneath the Kathivakkam region, occupied a precise geographical area at Latitude 13.23N and Longitude 80.32E with a shallow depth of 2-5 meters. It received waters from the Kortalaiyar River, Buckingham Canal, and several small channels. Mangroves present nearby helped in the decomposition of organic matter through microbial processes.

Water collection

Water samples of about 2 liters were collected from the Ennore estuary using clean plastic containers at a depth of approximately 10 cm in three different collection areas. Initially, the water temperature and pH were measured using a centigrade thermometer and a digital pH meter, respectively. Subsequently, the water samples were preserved in 5% neutralized formalin and stored in a refrigerator for further investigations.

Isolation and identification

Algae were isolated from the water samples through serial dilution, and these samples were streaked on petri plates containing agar and Zarrouk medium. Colonies of algae that grew were isolated and sub-cultured onto fresh agar plates. These colonies were then inoculated into freshly prepared liquid culture medium. *Arthrospira sp.* present in the water samples were identified based on their morphological characteristics such as color of scum, fibrous nature, floating or deposited filaments, presence of heterocysts, and shape, using both low and high power objectives of the compound microscope. The prominence of a species in the *Arthrospira* community can be assessed quantitatively by frequency (F). Frequency is the percentage of sites at which the species occurred and calculated by the formula:

$$\text{Frequency (F)} = \frac{\text{No. of water samples in which a particular Arthrospira occurred}}{\text{Total No. of water samples examined}} \times 100$$

Unialgal cultures

To ensure that different species of organisms did not exist in the isolated cultures, the mother cultures originating from single algal colonies were used for further investigations. However, these seed cultures still contained bacteria, leading to a risk of contamination and potential loss of the prepared mother cultures. To obtain bacterial-free cultures of *Arthrospira sp*, small portions of algae were immersed in chlorinated water (25mg/100ml) for 5 minutes [27]. Subsequently, algae samples were centrifuged at 10,000 rpm at room temperature for 5 minutes, and the supernatant was discarded. The algae pellet was then washed twice with distilled water to remove excess chlorine, and finally, the pellet was inoculated into freshly prepared culture medium. All further experiments were conducted using these cultures, which served as the seed cultures for the biomass production of *Arthrospira sp.*

Estimation of cell population by direct microscopic method

Conn's direct microscopic count method involves counting the number of cells in several microscopic fields to estimate the algae cell population. First, 0.01ml of culture was added to a glass slide covering an area of about 1 square centimeter, and it was spread uniformly. The glass slide was then observed with a cover glass using a microscope [28].

Biomass estimation

The harvesting process involved centrifuging 100ml of cultures at 5,000rpm at room temperature. After centrifugation, the supernatant was carefully discarded. The resulting pellet was washed twice by centrifugation with distilled water to remove any salts present to the pellet. Subsequently, the pellet was dried at room temperature, and the dry weight of the algal biomass was calculated in terms of grams per liter (g/l) [29].

Protein Estimation

10mg of dried algal powder was combined with 10ml of hot trichloroacetic acid (TCA) (55ºC) in a test tube and left to incubate at room temperature for 5 minutes. After centrifugation, the supernatant was removed, and the pellet was left overnight at room temperature with 1N NaOH. Subsequently, 4ml of alkaline reagent was added to 0.1ml of the pellet to dissolve the TCA-precipitated proteins. The solution was left at room temperature for 3 minutes. Following this, 0.5ml of Folin's reagent was added to all test tubes and mixed for 5 minutes. 1N NaOH served as the blank solution. Various concentrations (10-100mg) of bovine serum albumin were used as standards. After 30 minutes of incubation at room temperature, the absorbance of all test tubes was measured. The protein content in 10mg of algae was determined using a standard curve [30].

SDS-PAGE

A 12% SDS-PAGE gel mixture was prepared and run, followed by staining with Coomassie Blue. The composition of the separating gel (10 ml) was as follows: MilliQ water (3.3 mL), 30% acrylamide (4 mL), 1.5M Tris pH 8.8 (2.5 mL), 10% SDS (0.1 mL), 10% APS (0.1 mL), and TEMED (0.004 mL). The composition of the stacking gel (4 ml) was as follows: MilliQ water (2.7 mL), 30% Acrylamide (0.67 mL), 1M Tris-pH 6.8 (0.5 mL), 10% SDS (0.04 mL), 10% APS (0.04 mL), and TEMED (0.004 mL). The gel was run with 10 µl of samples at 150 V and 260 A for 2 hours before staining with Coomassie Brilliant Blue G250 [31].

2D- Gel electrophoresis

Two-dimensional gel electrophoresis of processed proteins (100 µg) was conducted using the following steps: Firstly, 13 cm IPG strips with a pH range of 3-10 (GE Healthcare, Uppsala, Sweden) were employed in the first dimension. The proteins underwent focusing for a total of 40,000 Vhs at a constant temperature of 20°C under a linear voltage ramp after passive IPG rehydration. The isoelectric focusing (IEF) conditions were as follows: 100 V step-n-hold for 1 hour, 300 V step-n-hold for 2 hours, 1000 V gradient for 1 hour, 5000 V gradient for 3 hours, and 5000 V step-n-hold for 7 hours. Following the IEF, each IPG strip was sequentially incubated in equilibration buffer containing 2% DTT (dithiothreitol) and then in another buffer with 2.5% iodoacetamide replacing DTT. Finally, the second dimension polyacrylamide gel electrophoresis (PAGE) (12.5%) was carried out in an SE600 apparatus (GE Healthcare, Uppsala, Sweden) initially at 1 W/gel for 1 hour, followed by 13 W/gel for 3 hours.

Protein Identified through MALDI

The process of in-gel trypsin digestion was performed for all the selected spots from the 2D gels following the previously described protocol [32]. The extracted peptides were subsequently analyzed via mass spectrometry using a SHIMADZU MALDI 7090 (TOF/TOF MS) instrument. The acquisition parameters were set as follows: the lyophilized samples were first diluted in 8 µL of 50:50 (ACN:Water with 0.1% FA). These samples were then vortexed and approximately 0.7 µL in volume was spotted onto a stainless-steel target plate. The CHCA matrix, prepared in a concentration of 50/50 (ACN/Water with 0.1% TFA), was then spotted above the sample spot and air-dried. The analysis was conducted in Reflectro Mode with a mass range of 1-5000 Da.

Whole genome sequencing and Insulin gene prediction

DNA was extracted from the algal powder, and the purified DNA was analyzed for quality using a Qubit fluorometer. Whole Genome Sequencing was conducted using Next Generation Sequencing technology. Library preparation was performed using a specific kit from Illumina, and adapters were added to facilitate sequencing. The quality of the prepared library was assessed using a Bioanalyzer, followed by deep sequencing on the Illumina platform with a minimum of 150X2 paired-end reads. Demultiplexing and FASTQ conversion were carried out, and the raw data were evaluated using an in-house quality control module.

The assembly of the genome was generated from the clean data using masurca version 4.1.0. Repeat classification and masking were performed using Repeat Modeler Version 2.0.1 and Repeat Masker version 4.1.1, respectively. Gene prediction was conducted using AUGUSTUS (version 3.4.0). Protein sequences obtained from the gene prediction were utilized for homology search (BLAST) using the AAA59172.1 insulin sequence from Homo sapiens.

Biomass Harvesting

The biomass was harvested on the 20th day from the inoculum using the filter paper method, and the biomass slurry was

washed twice with distilled water by centrifugation (3000 rpm) to remove excess salts. The filtered biomass was then dried at room temperature for 24 hours. After drying, the dried algal flakes were ground in a mixer to obtain fine algal powder, which was stored at 4°C in plastic containers for further analysis.

Extraction of Insulin

To extract insulin from the biomass, 5g of biomass was ground with a homogenized solution consisting of 2 mL of distilled water (DW), 10 mL of 95% ethanol, and 0.72 mL of concentrated sulphuric acid. The mixture was thoroughly mixed for 20 minutes. Then, an additional solution of 13 mL distilled water and 40 mL of 95% ethanol was added, and the pH was adjusted to 1.7 using sulphuric acid. The resulting suspension was filtered using Whatman paper (no. 1), and the pH was readjusted to 3.0 using ammonium hydroxide. To this suspension, a solvent system consisting of 150 mL of 95% ethanol and 200 mL of diethyl ether was added, and the mixture was stored at 4°C for 12 hours. After 12 hours, the supernatant was removed by centrifuging at 3,000×g for 10 minutes. The obtained pellet was washed with an equal ratio of acetone and diethyl ether. The pellet was then dissolved in 25% ethanol, and the pH was adjusted to 8.5. Subsequently, 100 µL of 1 M zinc chloride was added to this solution, and the mixture was incubated at 25°C for 18 hours to precipitate insulin. After 18 hours, the precipitate was collected by centrifugation [33].

HPLC for Insulin

The precipitated insulin sample was dissolved in 500 µL of 0.1% trifluoroacetic acid (TFA) containing 0.1 M EDTA and injected into a C-18 Phenomenex column (250×4.6 mm, 5 µm). The mobile phase consisted of a gradient from 30% to 80% acetonitrile in 0.1% trifluoroacetic acid. The flow rate was set at 1 mL/min, and detection was carried out at 214 nm. Human insulin (50 µg of protein in 500 µL) served as the standard for comparing retention times [34].

Statistical analysis

The experiments conducted in triplicates in this study were subjected to various statistical analyses using SPSS software (Version 25). The mean, standard error mean and P value significance were estimated and the results were inferred for logical interpretations.

Results

The frequency of occurrence of various *Arthrospira sp* was assessed across all water samples. Among the *Arthospira sp. A.platensis* exhibited the highest occurrence rates, with frequencies of 94.3%, 87.2% and 92.4% in collection site 1-3 respectively. Additionally, *A.maxima* emerged as the second most prevalent species with frequencies of 85.6%, 71.1%, and 58. 8% in collection site 1-3 respectively. *A.fusifomis* with a frequency of 61.0%, 48.2% and 44.2% and *A.indica* was found to be the least frequent, with frequencies of 7.3%, 6.7%, and 10.1%, respectively.

Table 1 shows the growth studies of four different species of uni-algal cultures over a 20-day period. Across all species, there is a significant increase in cell density and biomass production. *A.platensis* consistently exhibits the highest cell density and biomass production among the four species at each time point. This suggests that *A.platensis* is the most potent species in terms of growth rate and biomass accumulation over the observed period. *A.maxima, A.indica, and A.fusiformis* also shows increasing trends in cell density and biomass production, but at lower levels compared to *A.platensis.*

Species	5th day (Mean ± SEM)		10th day (Mean ± SEM)		15th day (Mean ± SEM)		20th day (Mean ± SEM)		P*	P#
	Cells/ml)	Biomass(mg/l)	Cells/ml)	Biomass(g/l)	Cells/ml)	Biomass(g/l)	Cells/ml)	Biomass(g/l)		
A.maxima	122± 5	0.15± 0.004	133± 8	0.32± 0.012	227± 6	0.71± 0.040	700± 12	1.12± 0.030	0.002	0.002
A.indica	170± 8	0.09± 0.006	200± 5	0.28± 0.015	240± 7	0.83± 0.025	580± 6	1.02± 0.032	0.001	0.008
A.platensis	203± 12	0.19 ± 0.008	410± 4	0.63± 0.017	606± 5	1.05± 0.050	1380± 17	1.48± 0.021	0.001	0.001
A.fusifomis	300± 5	0.12± 0.002	407± 3	0.59± 0.010	501± 6	0.96± 0. 018	750± 14	1.23± 0.019	0.001	0.001
Each value is a mean of 4 individual analyses with standard error of mean. *- Significance ($p<0.05$) in cell count at different intervals. #- Significance ($p<0.05$) in biomass production at different intervals										

Table 1: Growth studies of unialgal cultures of *Arthospira sp*.

Protein estimation

The protein content of isolated *Arthrospira sp*. were studied in the lab condition and observed data presented in the Table 2. Among the four *Arthrospira sp* tested on protein content *A.platensis* showing the highest mean total protein content, followed by *A.maxima* and *A.indica*. *A.fusiformis* displayed a slightly lower mean total protein content compared to the other species. Since the P value is less than 0.05 there is a significantly different in protein content among the tested *Arthrospira sp*.

S.No	Species	Total Protein content µg/g (Mean ± SD)	p*	SDS (6 kDa band)	2D (6 kDa band)	Insulin
1	*A.maxima*	7.5 ± 0.4		Negative	Negative	Negative
2	A.*indica*	7.0 ± 0.2		Positive	Negative	Negative
3	A.*platensis*	7.9 ± 0.5	0.043	Positive	Positive	Positive
4	A.*fusifomis*	6.7 ± 0.2		Negative	Negative	Negative
*- Significance ($p<0.05$) in total protein content among the test *Arthrospira sp*.						

Table 2: Screening the presence of insulin among the isolated *Arthrospira sp*.

SDS PAGE

Protein extracted from all isolated *Arthrospira sp*, were analysed in SDS PAGE and results shows that all the four *Arthrospira* strains shows different protein profiling. Previous studies shown that insulin were detected at 6kDa in SDS PAGE. Similar 6kDa band was observed in *A.indica* and *A.platensis* but it was not detected in *A.maxima* and *A.fusifomis* (Figure 1). To further conformation all strains were taken to 2D gel electrophoresis and MALDI.
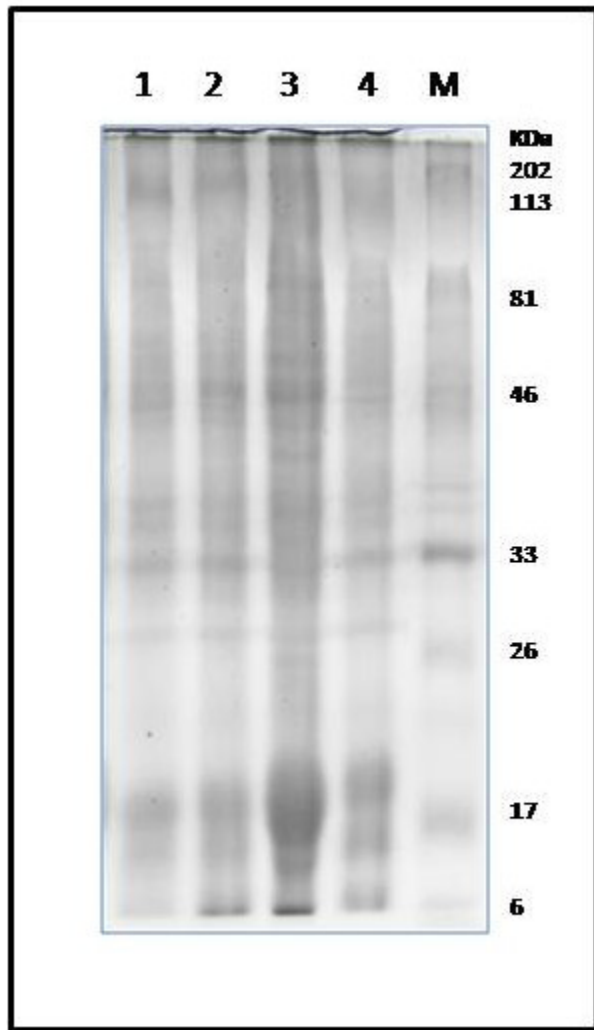
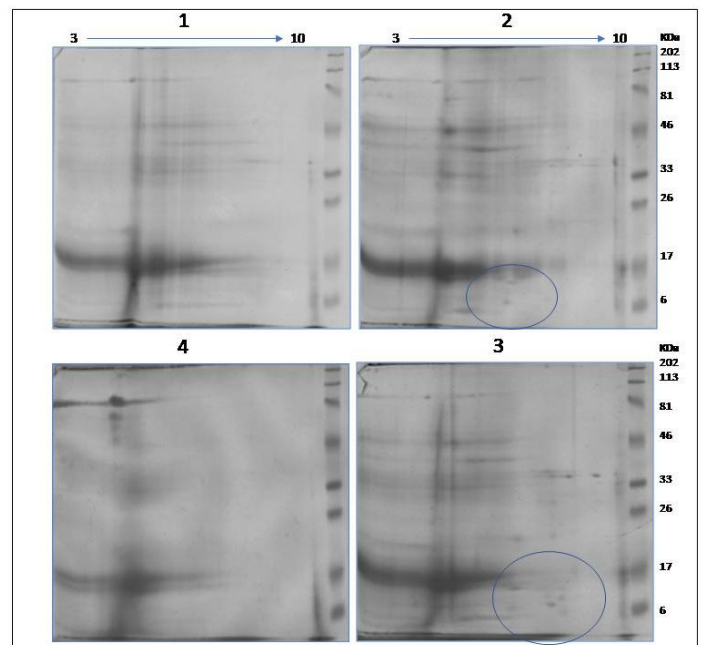1: *A.maxima*, 2: *A.indica*, 3: *A.platensis*, 4: *A.fusifomis*, M: *molecular ladder*

Figure 1: Protein profiling of the tested *Arthrospira sp* by SDS PAGE.

2D gel electrophoresis and MALDI

Extracted proteins from all tested *Arthrospira* strains were analysed by 2D gel electrophoresis and results shows that all the strains are different in their protein patterns. In contrast to SDS PAGE, 6kDa was observed only in *A.platensis* and 6kDa found in *A.indica* by SDS, now apperaed at 9.11kDa. Similar like SDS PAGE, the 6kDa band is also not observed in other two strains (*A.maxima* and *A.fusifomis*) by 2D gel electrophoresis (Figure 2).

Analysis of five significant spots including 6kDa in *A.platensis* were analyse by MALDI-TOF MS and subsequent searching of the PMF data against the databases to identify the proteins reveals the presence of phycocyanin beta chain (1), Histidine kinase (2), Hypothetical protein (3), HNH endonuclease (4), and Cyclic pyranopterin monophosphate synthase (Figure 3). The difficulty in searching for a good match of the protein in the databases might be due to the unavailability of a *Arthrospira* whole genome sequence.

1: *A.maxima*, 2: *A.indica*, 3: *A.platensis*, 4: *A.fusifomis*

Figure 2: Protein profiling of the tested *Arthrospira sp* by 2D gel electrophoresis.
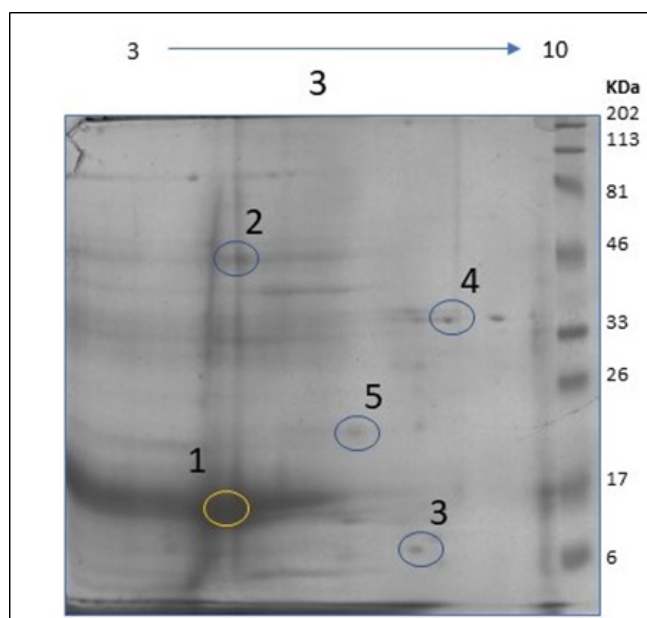
1: Phycocyanin beta chain, 2: Histidine kinase, 3: Hypothetical protein, 4: HNH endonuclease and 5: Cyclic pyranopterin monophosphate synthase.

Figure 3: Protein identification of *A. platensis* SPKY1 by MALDI.

Whole genome sequencing and Insulin gene prediction

A.platensis SPKY1, which exhibited positive results for insulin via MALDI, underwent whole genome sequencing and was subsequently submitted to NCBI with the Genbank ID: JAWMAM000000000.1. The genome of *A. platensis* comprises a single, circular chromosome with a size of 5.7 Mb and an average GC content of 54.5%. The genome of *A.platensis* SPKY1 was predicted to harbor 13,482 protein-coding genes, which can be annotated based on known functions or identified as hypothetical proteins in the UniProt/Swiss-Prot and non-redundant protein databases, respectively. Additionally, the genome sequence was found to contain 43 RNA genes, including 28 tRNA genes and genes encoding rRNA (5S, 16S, 23S).

Table 3 shows the amino acid sequences obtained from the gene prediction were utilized for homology search (BLAST) using the AAA59172.1 insulin sequence from Homo sapiens showed that 4 hypothetical proteins (six residues) shows 100% identity with one BLAST hit with human insulin. Result suggests only partial identity with human insulin and also indicate that four different 6 residues which are 100% matched with human insulin might be present at 6kDa at SDS PAGE, 2D gel, and MALDI reported as hypothetical protein (Figure 4a & b). *A.platensis* SPKY1 was also reported 8 insulinase family proteins which further the confirming the presence of insulin (Figure 5).

Genbank Accession No	Query Identical Seq	Subject Identical Seq	Protein ID	Protein Name
JAWMAM010001280	263 PDPAAA 268	19 PDPAAA 24	MDV7390887.1	Hypothetical protein
JAWMAM010008923	32 RREAED 37	55 RREAED 60	MDV7398747.1	Hypothetical protein
JAWMAM010007148	81 GGGPGA 86	69 GGGPGA 74	MDV7397142 .1	Hypothetical protein
JAWMAM010003156	105 PDPAAA 110	19 PDPAAA 24	MDV7393163.1	Hypothetical protein

Table 3: Insulin like sequence from *A.platensis* SPKY1 showing significant alignment with Human insulin (Acc No: AAA59172.1, 110aa).

263 PDPAAA 268

81 GGGPGA 86

Figure 4a: *A.platensis* SPKY1 insulin like antigen sequences producing significant alignments with human insulin.

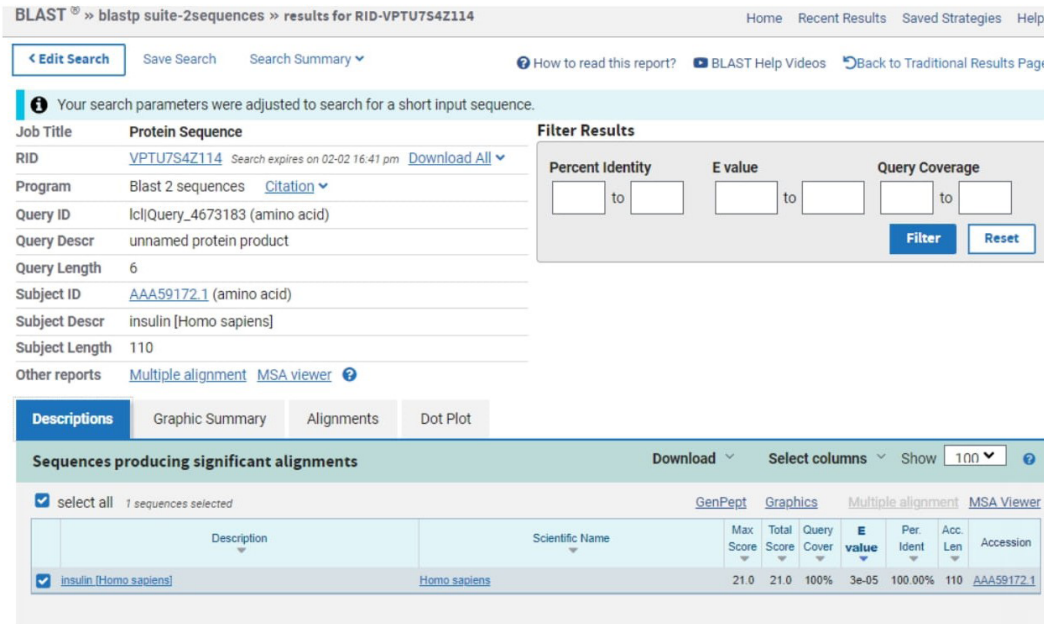**32 RREAED 37**
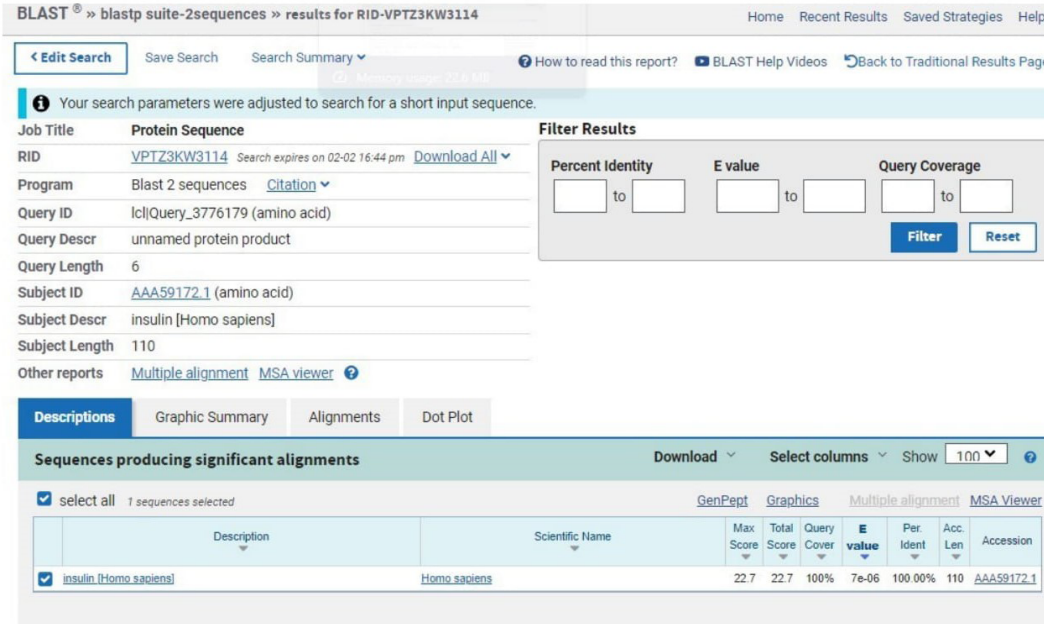
105 PDPAAA 110

Figure 4b: *A.platensis* SPKY1 insulin like antigen sequences producing significant alignments with human insulin.

Figure 5: Eight insulinase family proteins from *A.platensis* SPKY1.

HPLC for Insulin

Insulin isolated from *A.platensis* was analysed using HPLC and insulin was detected at the retention time of 6.79min respectively and similarly chromatogram was also observed when analysed with human insulin as standard. On the 20th day following inoculation it was observed that *A.platensis* containing 15.89μg/g of dry biomass (Figure 6).
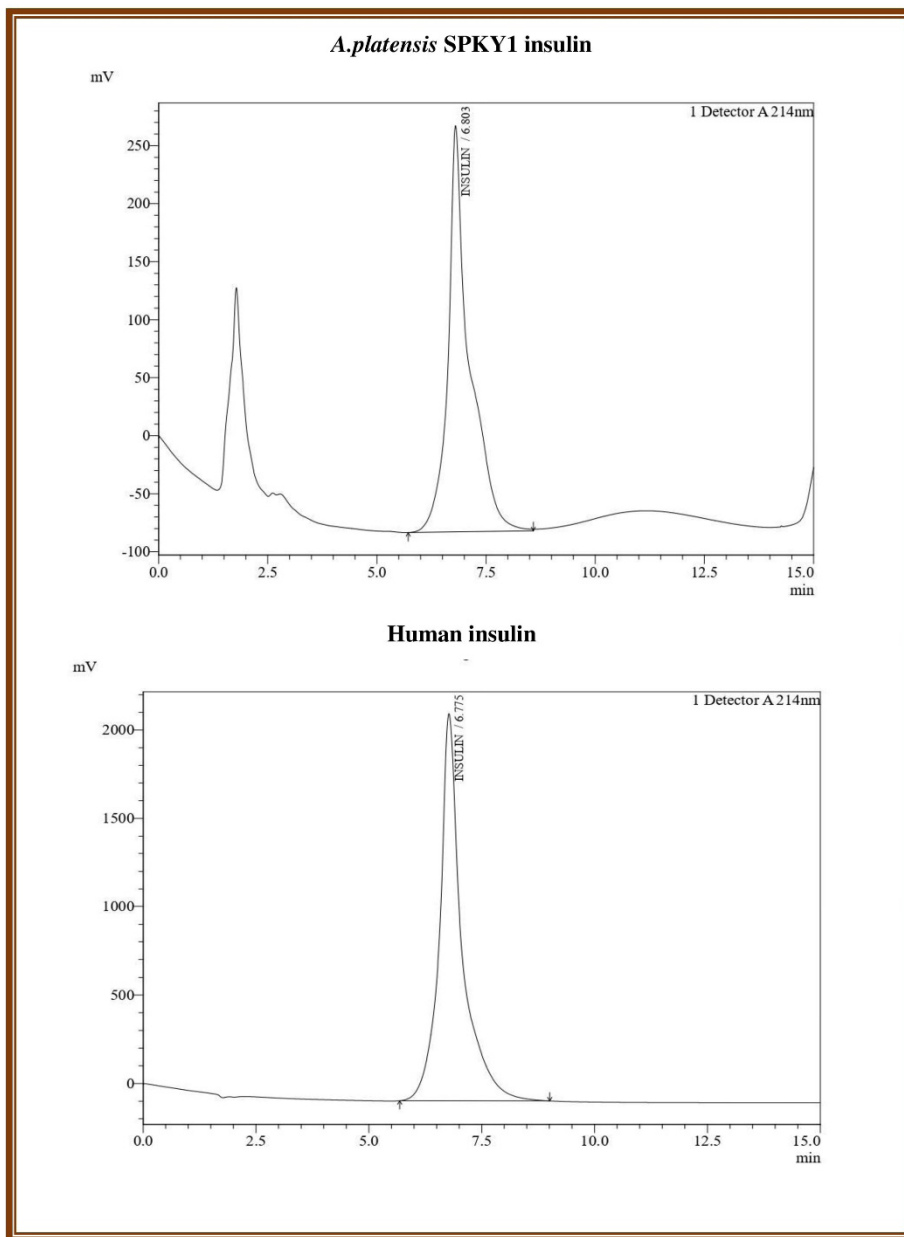
Figure 6: HPLC analysis of *A. platensis* SPKY1 insulin and human insulin.

Discussion

Human consumption of *Spirulina* as food dates back to the Aztec civilization due to its high protein content and other essential nutrients, along with its safety for human consumption without any potential side effects [35,36]. Previous studies have demonstrated the positive impact of *Spirulina* on various non-communicable diseases, including improvement in lipid profile [37,38], potential anticancer effects [39], alleviation of arthritis symptoms [40], treatment of anemia [41], and management of diabetes [42].

According to Wang et al. [43], lifestyle changes are the primary need for controlling diabetes, but adherence to dietary restrictions and increased physical activity can be challenging for many patients, leading to the use of pharmacological agents such as insulin, which may have side effects limiting their acceptability and efficacy [44]. Studies have also indicated the positive effects of *Spirulina* as a functional food for individuals with diabetes [45,46]. A study conducted by Anwer et al. (2012) reported the presence of insulin-like protein in sixteen *Spirulina sp* collected from culture collections [34]. In this study, we isolated *Spirulina sp* from the natural source (Ennore estuary) to screen for the presence of insulin-like protein. Out of four tested strains, *A. platensis* SPKY1 showed positive insulin production of 15 μg/g of dry biomass, which is lower than the insulin content reported in previous studies [34]. A study confirmed the presence of insulin by ELISA method in *A. maxima* but in contrast the *A.maxima* isolated in this study from Ennore estuary shows negative [47].

Further confirmation of insulin in *A. platensis* SPKY1 was done by SDS-PAGE, 2DE, and MALDI, which showed similar band patterns (6 kDa) to previous studies [48-50]. However, other studies have reported insulin with molecular weights both less than 6kDa [51-53], and greater than 6 kDa from plant sources [54-56].

All of these studies reported the presence of insulin from plant sources. However, studies on *Spirulina* supplementation for reducing blood sugar did not identify the exact biomolecules responsible for this anti-diabetic property. Only two studies reported the presence of insulin in *Spirulina sp.*, but these studies not reported whole genome sequencing to identify the insulin gene sequence. In this study, the potentially positive strain *A. platensis* SPKY1 was subjected to whole genome sequencing and subsequently submitted to NCBI. The genome size of *A. platensis* SPKY1 was determined to be 5.7 Mb, which is slightly smaller compared to other strains such as *A. platensis* C1 (6.09 Mb) [57], *Arthrospira sp.* PCC 8005 (6.23 Mb) [58], and *A. platensis* NIES-39 (6.79 Mb) [59]. In *A. platensis* SPKY1, a total of 7731 genes coding for hypothetical proteins were observed. Gene prediction revealed four different hypothetical proteins with 6 amino acid

residues, which showed 100% identity with Human insulin. Similarly, a study conducted by Anwer et al. 2011 reported 3 residues (44 GER 46) similar to bovine and human insulin with 100% identity in *A. platensis* [34]. Furthermore, HPLC analysis of insulin extracted from *A. platensis* SPKY1 showed a retention time of 6.79 minutes and exhibited a similar chromatogram to standard human insulin. Another study reported the retention time of insulin from B. variegate (6.95 minutes) [60], Arabidopsis thaliana seeds (17.2 minutes) [61], and Vigna unguiculata (31.0 minutes) [62].

Insulin remains inaccessible to millions of people due to poor availability and unaffordable prices [11]. The significant price gap between production costs and actual prices raises ethical concerns about the affordability of essential medication for millions with diabetes, especially in low- and middle-income countries. While insulin has traditionally been produced using E. coli bacteria via rDNA technology [63], there is a growing need for alternative sources to reduce production costs.

Exploring alternative production methods, such as using yeast or other microorganisms, is being investigated to create a more cost-effective insulin supply. The study represents the first instance of reporting eight insulinase family proteins from *A. platensis* SPKY1, thereby confirming the presence of insulin. This presence of insulinase was not reported in previous studies. Insulinase family proteins, which play a role in breaking down insulin, were identified in the cyanobacterium, suggesting a potential involvement in insulin metabolism.

Conclusion

The genomic exploration of insulin from *A. plantensis* SPKY1 presents a promising tool for insulin production with potential advantages in terms of minimal cost, easy availability, and no side effects. *A. plantensis* SPKY1 is a sustainable and economically viable alternative for insulin synthesis. Further research and development in this direction may contribute to addressing the rising demand for insulin, particularly in regions with a high prevalence of diabetes, offering a potential breakthrough in insulin production.

Disclosure

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