Glucose-Induced Biofilm Formation in *Bacillus thuringiensis* KPWP1 is Associated with Increased Cell Surface Hydrophobicity and Increased Production of Exopolymeric Substances

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Received: 9 February 2021 / Accepted: 29 October 2021 / Published online: 14 December 2021
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Abstract

*Bacillus thuringiensis* is an agriculturally and medically important bacteria as it produces insecticidal Cry proteins and can form biofilm on different plant surfaces. Previous studies reported that the ubiquitous carbon source glucose could induce restricted motility and fractal pattern formation in the growing colonies of pH, salt and arsenate tolerant *Bacillus thuringiensis* KPWP1. As bacteria are evolved with the ability to exhibit multicellular behavior and biofilm formation under limiting conditions for survival, the present study was focused on exploring the effect of glucose in biofilm formation by *Bacillus thuringiensis* KPWP1. A significant rise in biofilm loads was observed with increased glucose concentrations in growth media. Compared to control, six times more biofilm load was marked in presence of 2% of glucose. Interestingly, it was observed that the effect was glucose specific and also not due to any change in the sugar-induced physicochemical property of the growth media as the addition of galactose or arabinose could not induce any significant increase in KPWP1 biofilm load. Scanning electron-, confocal laser scanning-microscopic studies and biochemical tests revealed that increased concentrations of glucose could induce increased production of exopolymeric substances, increased number of densely-packed micro-colonies in KPWP1 biofilm and increased hydrophobicity and adherence properties in KPWP1 cells.

Introduction

Although bacteria are unicellular prokaryotes, they are evolved with the ability to adapt and survive under different growth and environmental conditions [1–3]. One of the most important mechanisms for their survival, particularly under harsh conditions, is the ability of bacteria to form biofilm on abiotic and biotic surfaces [4].

Biofilms are multicellular associations and critical components of the natural surroundings [4]. Bacterial are found in multiple habitats like in soil, aquatic systems, different parts of plants and animals [4–6]. Other than the natural habitats including human hosts (where bacteria are present as natural commensals and also as pathogens during infections), bacterial biofilms are also observed in different medical equipments and accessories [7]. This is alarming as biofilm formation gives an advantage to the pathogenic bacteria to survive in presence of antibiotics, thus favors the evolution of drug resistance in pathogenic bacteria.

The majority of microorganisms including bacteria have the potency to construct biofilm on a wide range of surfaces by producing the extracellular polymeric substances (EPS) [3, 4]. EPS are an intricate blend of biomolecules and polymers like proteins, polysaccharides, lipids and extracellular DNA (eDNA) secreted by microorganisms and also contributed by the dead and lysed cells present in biofilms [8–10].

Numerous factors including sources and abundance of nutrients, osmolality, temperature, and anaerobiosis were suggested to affect biofilm formation and EPS production [11]. Although glucose is a preferred carbon source for most living organisms, there are conflicting reports on the effect of glucose in bacterial biofilm formation. It is reported that exogenous glucose promotes biofilm formation and antibiotic resistance in *P. aeruginosa* and *Aeromonas hydrophila* [12, 13]. On the other hand, there is a report which suggests that glucose inhibits biofilm formation in *Bacillus subtilis* [14]. Although it was observed that glucose can inhibit biofilm formation by multiple species of *Enterobacteriaceae*, it was observed that glucose supplementation in growth media...
enhances biofilm formation in *E. faecalis*. Such divergent results indicate a bimodal fashion in the occurrence of glucose-mediated biofilm formation in enterococci [15].

Bacteria belonging to the *Bacillus cereus* group display a broad range of existence and ecological niches and consist of useful as well as pathogenic lines [16]. *Bacillus thuringiensis* belongs to the *Bacillus cereus* and is used as a biological pesticide as this microbe produces insecticidal toxins [17–19]. If biofilm formation of *B. thuringiensis* is encouraged onto the plant surface, the cells can adhere to the plant surface and ensure the insecticidal property. Therefore, studies on biofilm formation by *Bacillus thuringiensis* and other bacterial strains are increasingly recognized as an important area of research [17, 20–22]. Previous studies from our laboratory revealed that increased concentrations of glucose in growth media inhibit movement/swarming and induced fractal pattern formation in growing colonies of KPWP1 on semi-solid growth media. Interestingly, it was also observed that increased concentration of glucose in growth media resulted in a decreased number of flagella on the KPWP1 cell surface [23]. There are contrasting reports on the relation between swarming and biofilm formation in the bacterial world. On the one hand, it is generally observed that swarming motility and biofilm formation are inversely correlated [24–27] whereas, on the other hand, recent findings indicate that for some bacteria, there may be a positive correlation between bacterial swarming and biofilm formation [28, 29]. It is, therefore, important to know whether swarming and biofilm formation are inversely or positively correlated in *Bacillus thuringiensis* KPWP1 and also to know whether glucose can influence the biofilm formation by *Bacillus thuringiensis* KPWP1. In addition, such investigation is important to understand in general the effect of glucose on biofilm formation by *Bacillus cereus* group of bacteria including human pathogens.

The present study thus aimed to investigate the effect of glucose in biofilm formation of *Bacillus thuringiensis* KPWP1 and also to characterize the changes in EPS compositions, cell morphology, and surface hydrophobicity in KPWP1 biofilms due to the presence of differential concentrations of glucose in growth media.

**Materials and Methods**

**Bacterial Strain and Growth Characteristics**

Growth kinetics of *Bacillus thuringiensis* KPWP1, isolated from Kolkata port water [23], in presence of different concentrations of glucose was measured. For that, 5 mL of overnight grown KPWP1 culture (~ 10^7 cells/mL) was added to 20 mL of freshly prepared Nutrient broth (NB) media (0.5% peptone, 0.5% NaCl and 0.3% beef extract) in the absence and presence of 1% or 2% added glucose and incubated at 37 °C under shaking condition. Bacterial growth was monitored at different time points by measuring the optical densities of growing bacterial suspensions at 600 nm by using UV–Vis spectrophotometer (Shimadzu UV 2600). Bacterial growth kinetics characteristics were measured by plotting the OD values against time.

Biofilm formation by *Bacillus thuringiensis* KPWP1 was performed by using the method described by Pui et al. [30] with minor modifications. 10^6 number of KPWP1 cells in 1 mL of NB media were grown for 48 h in 24 well plates in the absence or presence of 1% and 2% of sugars (glucose or arabinose or galactose) at 37 °C under shaking condition (150 rpm). The planktonic cells were removed and the growth of planktonic cells was measured spectrophotometrically by measuring the turbidity/OD at 600 nm by using a UV–Vis spectrophotometer (Shimadzu UV 2600). After removal of planktonic cells, wells (containing the biofilm) of plates were gently washed with phosphate buffered saline (PBS) twice and incubated with 0.1% crystal violet (CV) for 15 min at room temperature. After incubation, the excess stain was removed by washing the wells with PBS. CV attached to the biofilm was extracted with 1 mL of 30% acetic acid. The biofilm load was calculated by measuring the optical density (O.D) of the extracted crystal violet at 600 nm using a microplate reader (BioTek ELx800).

**Scanning Electron Microscopy (SEM)**

To visualize the biofilm formed by KPWP1 cells, SEM was performed using the protocol described by Chakraborty et al. [31] with minor modifications. Biofilm was first formed onto the coverslip placed vertically in 24 well plates. The coverslips, containing KPWP1 biofilms, were washed with PBS and fixed with 200 μL of glutaraldehyde (2.5%) solution for 1 h at room temperature in dark. The coverslips were then re-suspended in 200 μL of 0.1% OsO4 and incubated at room temperature for 30 min. After repeated (three times) wash with PBS, cells were again washed with 30%, 50%, 70%, 90%, and 100% alcohol, respectively. Finally, 20 μL of 100% ethanol was added to the coverslips. The samples were then dried using desiccators and coated with a thin layer of conducting metal (gold–palladium) in a sputter coater (quorum technologies ltd.) The samples were observed under a field...
emission scanning electron microscope (Carl Zeiss) using smart SEM software.

**Confocal Laser Scanning Microscopy of Biofilm (CLSM)**

Confocal laser scanning microscopy (CLSM) was performed to measure the three-dimensional structure and related topological parameters of KPWP1 biofilms. For that, KPWP1 biofilms were formed on the coverslip using the same method as described in method Sect. 2.3. The coverslips containing the biofilms were washed with PBS (two times) followed by staining with 0.001% acridine orange and then incubated in dark for 10 min. The coverslips were washed with PBS again to remove the excess stain and observed under the confocal microscope (Axio Observer Microscope, Version Z.1; Carl Zeiss, Germany).

The mean thickness (μm), volume (μm³), skewness, kurtosis and bio-volume (μm³/μm²) of KPWP1 biofilms were quantified from the confocal stacks using the Zen software. The bio-volume is defined as the volume of the biomass (μm³) divided by the surface area of the substratum (μm²). The skewness determines the porosity in the biofilm which helps the cells to get access to the nutrient. The kurtosis value determines the adherence property of the bacteria [32].

**Microbial Adhesion to the Hydrocarbons (MATH Assay)**

To measure the hydrophobicity of the cell surface of bacteria grown under different conditions, MATH assay was performed using hydrophobic hydrocarbon n-hexadecane (Sigma, purity > 99%) [33]. For that, KPWP1 cells were grown in Nutrient Broth in presence of different concentrations of glucose. The optical densities of cell suspensions were adjusted to 0.6 by diluting the suspensions with PBS (A₀). Next, 1.5 mL of adjusted cell suspensions were thoroughly mixed with 0.5 mL of n-hexadecane for 30 min at room temperature. The mixtures were then allowed to settle for 30 min and the separated aqueous layers were collected and the O.D of the collected aqueous layers was measured at 600 nm (A₁). The percentage hydrophobicity was calculated by the formula; Percentage hydrophobicity = [1 – A₁/A₀] × 100.

**Live-Dead Staining of Biofilm**

The presence of live and dead cells in KPWP1 biofilm was measured by staining the biofilm (formed on coverslips) with the live/dead BACLIGHT bacterial viability kit (Invitrogen, Paisley, UK) [34]. The SYTO 9 dye and Propidium iodide (PI) stained the live and dead cells, respectively. The images of stained live and dead cells were captured by using a fluorescence microscope (Olympus Provis ax70). Intensities of SYTO 9 and PI in the stained images were measured (by using ZEISS ZEN Lite software 3.4) to compare the relative abundance of live and dead cells in KPWP1 biofilms grown in absence and presence of 1% and 2% glucose in growth media.

**Quantification of Carbohydrate, Protein and eDNA in Exopolymeric Substances (EPS) of KPWP1 Biofilms**

The carbohydrate, protein and eDNA present in KPWP1 biofilms were measured. For that, the biofilms formed in 24 well plates were isolated by scraping the biofilms and suspending in PBS. The anthrone method was used for the quantification of the carbohydrate content of EPS [35]. In brief, 160 μL of anthrone reagent (0.125% anthrone [wt/vol] in 94.5% [vol/vol] H₂SO₄) was mixed with 80 μL of the biofilm sample and incubated at 100 °C for 14 min and cooled at 4 °C for 5 min. The absorbance at 625 nm was measured using a microplate reader (Hidex Plate Chameleon).

The Bradford method was used to measure the protein content in EPS. In brief, 400 μL of the filtered sample was blended tenderly with 100 μL of Bradford reagent in 96 well plates. After 5 min of blending, 200 μL of the blended sample was used to measure the absorbance at 595 nm by using a microplate reader [36].

For eDNA quantification, KPWP1 biofilms were grown in 24 well plates. The planktonic cells were discarded after 48 h of incubation and the wells were washed with 0.9% NaCl solution two times. After washing, 1 mL of 0.9% NaCl was added to the wells and dispersed properly. The mixture was vortexed vigorously for 1 min and centrifuged at 5500×g at 4 °C for 10 min [37]. The supernatant was collected and the eDNA content was measured using Nanodrop 1000 spectrophotometer (Thermo scientific Asheville, NC, USA).

**Quantification of Lipid and Glycoconjugates in EPS In Situ**

The quantification of lipid moieties glycoconjugates present KPWP1 biofilms was done in situ by staining method using Nile red and Concanavalin A (ConA), respectively.

Nile red stain lipid and lipid conjugates such as triglycerides phospholipids and neutral lipid droplets. The biofilm onto the coverslips was stained with the dye (2.5 mg/L) followed by incubation for 15 min in dark at room temperature. Following incubation, the samples were washed with PBS and observed under a confocal laser scanning microscope with the excitation wavelength of 530 nm and emission at 580 nm [38].

Similarly, Concanavalin A (Con A) was used to stain the glycoconjugates part of biofilm flocs in situ. The samples
were stained with 500 mg/L of the dye. The staining procedure was the same as above but the incubation time, in this case, was 15 min followed by PBS wash. The samples were observed under a confocal laser scanning microscope with the excitation wavelength of 485 nm and emission at 530 nm [39].

Statistical Analysis

GraphPad Prism 6 was used to analyze the data. All the analyses were carried out in triplicates and stated as mean ± standard error. Two-tailed Student’s t test was employed to determine significant differences between means. Asterisks indicate statistically significant differences (*P < 0.05; **P < 0.01; ***P < 0.001).

Results

Effect of Glucose on Growth and Biofilm Formation of Bacillus thuringiensis KPWP1

Initially, the characteristic (kinetics) of KPWP1 growth in presence of different added glucose concentrations in NB was determined (Fig S2). It was observed that 1% and 2% added glucose increased bacterial growth compared to the control condition where no glucose was added in NB medium.

Interestingly, a significant rise in biofilm load was observed with the increase in glucose concentrations in growth media. An increment of 11.45 and 18.36-folds in biofilm load was marked in 1% and 2% of glucose supplemented nutrient broth respectively, with respect to control (Nutrient broth without added glucose) (Fig. 1a), while 1% and 2% glucose could induce 1.33 and 1.35 folds of planktonic cell growth (Fig. 1b). The results indicate that KPWP1 was able to form better biofilm in presence of glucose. Moreover, the observed increase in biofilm formation was found to be glucose specific and not due to any change in the physicochemical property (e.g., osmolarity) of growth media as the addition of increased concentrations of galactose or arabinose could not induce any significant biofilm formation as compared to glucose (Fig. 1a), although galactose and arabinose did not show any growth inhibitory effect on KPWP1 cells (Fig. 1b).

We further explored the effect of glucose on Bacillus thuringiensis KPWP1 biofilm at the levels of cell morphology and the presence of EPS by scanning electron

![Fig.1](image)

**Fig. 1** Growth and biofilm formation by *Bacillus thuringiensis* KPWP1. Biofilm formation a and planktonic growth b of KPWP1 in NB after 48 h of incubation in presence of glucose, arabinose and galactose. Scanning Electron Micrograph (SEM) of biofilm of KPWP1 that developed on a glass coverslip after 48-h period in nutrient broth with different percentage of glucose (c). Representative CLSM images of biofilm grown in presence of different percentages of glucose of KPWP1 (d). The experiments were repeated three times and the data in Figs. 1a and 1b represent mean ± SE. Asterisks indicate statistically significant differences when compared to control (*P < 0.05; **P < 0.01; ***P < 0.001; two-tailed Student’s t test)
microscopy (SEM). The SEM images of KPWP1 cells in biofilms grown in absence of any added glucose revealed that monolayer of a few spores adhering to the coverslips (Fig. 1c). Interestingly, the outcome due to the addition of 1% glucose in growth media presents a contrasting picture. At the lower magnification, it was observed that the heterogeneous cell population of KPWP1 adhering to the substratum forming multiple layers with void spaces which most probably help in nutrient circulation and excretion of the waste products. The higher magnification of the images revealed that the heterogeneous cell populations consisting of the densely packed region of elongated cells, normal healthy cells and spores embedded in EPS of KPWP1 biofilm. The effect of 2% glucose on KPWP1 biofilm is also captivating as images revealed the presence of densely packed 3-D structure of KPWP1 cells along with the void spaces with more EPS compare to the 1% glucose and the higher magnification gives an exact view of the cell morphology and it also indicated the heterogeneous cells trapped in EPS.

Similar results were also observed when KPWP1 biofilms were examined using confocal laser scanning microscopy. Figure 1d shows representative CLSM images of KPWP1 biofilms, in which the bacterial micro-colonies at 1% and 2% of glucose-containing media were observed to be denser and more aggregated than that of the control condition.

**Topological Parameters of KPWP1 Biofilm**

In order to characterize the topological parameters of KPWP1 biofilm, confocal laser scanning microscopy was done on KPWP1 biofilm formed on glass coverslips. The bio-volume, mean thickness, biomass, kurtosis and skewness of the 48-h grown biofilms were analyzed to evaluate changes in the biofilm structure with increased glucose concentrations in growth media. The mean thickness, bio-volume and biomass of the bacterial micro-colonies of the 48-h grown KPWP1 biofilms increased gradually as the glucose concentrations were increased in growth media. The thickness of the biofilm in 1% and 2% glucose supplemented media was found to be 1.43 and 1.86 times more than that of control (Fig. 2a) and the volume increased by 1.48- and 1.82-folds, respectively (Fig. 2b). The biomass of the KPWP1 biofilm was also observed to be increased by 1.61 and 2.06-folds in presence of 1% and 2% glucose in growth media (Fig S3).

The skewness and kurtosis value are the two statistical parameters that measure porosity in biofilm and adherence of the bacteria to the surface, respectively. The lesser the

![Fig. 2](image-url)

**Fig. 2** Topological features of biofilms formed by *Bacillus thuringiensis* KPWP1 in absence and presence of 1% and 2% Glucose after 48 h of incubation: **a** Thickness, **b** Volume, **c** Skewness and **d** Kurtosis. The experiments were repeated three times and the data represent mean values±SE of 3 biological replicates. Asterisks indicate statistically significant differences when compared to control (**P < 0.05; **P < 0.01; ***P < 0.001; two-tailed Student’s t test)
skewness value more is the porosity that enables the bacteria to access more nutrients. The effect of increased glucose concentrations in growth media on skewness was observed to be gradual (Fig. 2c), with the lowest skewness value in presence of 2% glucose which implies the presence of more porosity in the formed biofilm. The highest kurtosis value (Fig. 2d) was noticed in 2% glucose supplemented media states that the adherence property of bacteria increases as glucose is added to the media.

Glucose Induced Change in Cell Surface Hydrophobicity of KPWP1 Cells

Cell surface hydrophobicity plays a major role in biofilm formation by bacteria on different biotic and abiotic surfaces. Cell surface hydrophobicity (CSH) of KPWP1 grown under different conditions was assessed using the microbial adhesion assay for hydrocarbons (MATH). High CSH enables microorganisms to attach to the hydrophobic surface and form a biofilm. The CSH changes as the cell surface macromolecules change with response to environmental stimulus and nutrient variation. The results (Fig. 3) depict that the addition of 1% and 2% glucose in nutrient media increased the percentage hydrophobicity of KPWP1 cells by 3.77 and 4.85 folds, respectively, compared to the hydrophobicity of cells grown in absence of any added glucose.

Distribution of Live and Dead Cells in KPWP1 Biofilms

As it is reported that dead cells play important role in the adherence of the bacteria while forming biofilm [34], imaging of live/dead cells in KPWP1 biofilm, grown in the absence and presence of added glucose was performed. Both live and dead bacteria embraced the biofilm of Bacillus thuringiensis KPWP1 were observed when they were allowed to develop on 18 mm coverslips in 24 well plates. By using the BACLIGHT live/dead viability probe (molecular probes), we observed that cell death is manifested in the microcolonies within the KPWP1 biofilms (Fig. 4). The overlapped images of live and dead cells (probed with SYTO 9 and PI, respectively) depict that the dead cells (indicate by red) are present at the bottom while the live cells (indicate by green) are present at the top of the biofilm. Interestingly, it was observed that although with the increase of glucose concentrations from 1 to 2% caused increase in number of live cells (represented by the intensity of SYTO 9) in KPWP1 biofilm (Fig S4a), the abundance of dead cells (represented by the intensity of PI) also significantly increased in presence of 2% glucose as compared to 1% in growth media (Fig S4b), which indicates that the dead cells might help the live cells to adhere to the substratum during biofilm formation. It is to be mentioned that cell death inside microcolonies is an imperative physiological process that plays a part in the subsequent differentiation and dispersal of a subpopulation of surviving biofilm cells [34].

Identification and Estimation of EPS in KPWP1 Biofilm

Exopolymeric substances in the bacterial biofilm are generally made of polysaccharides, proteins, glycolipids and eDNA.

The total polysaccharide was determined biochemically by Anthrone method. The carbohydrate content in KPWP1 biofilms was found to be 8.45 and 31.70-fold more in the presence of 1% and 2% glucose in growth media (Fig. 5a). Bradford reagent is used to quantify the protein content in the EPS matrix. The results suggest that the total protein content was 9.8 and 36.24-fold more in biofilms when KPWP1 was grown in presence of 1% and 2% glucose as compared to the control condition (Fig. 5b). eDNA was quantified by spectrophotometric analysis. Notably, eDNA contents in KPWP1 biofilm was found to be 1.9 and 5.01 folds more in 1% and 2% glucose with respect to the control condition with no added glucose in the growth medium (Fig. 5c).

Nile red was used for in-situ detection and determination of the relative abundance of lipids residues present in the EPS matrix of KPWP1 Biofilms. The lipophilic stain Nile red shows the presence of lipid moieties or exceedingly hydrophobic areas within biofilms (supplementary Fig S5). The intensity for Nile red was 1.74 and 4.60 times more in the EPS matrix of 1% and 2% glucose with respect to the control condition with no added glucose (Fig. 5d).

In order to check the relative presence and abundance of carbohydrate residues within KPWP1 biofilms in-situ,
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The EPS matrix was stained with Con A (supplementary Fig S6). The observed intensities for Con A bound with glycosyl residues are 2.8 and 3.1 folds (supplementary Fig S6) more in the EPS matrix of biofilms grown in presence of 1% and 2% glucose, respectively as compared to the control condition.

**Discussion**

Biofilm development is a multiplex procedure that is influenced by many factors, which include growth condition, stress, surface attributes and host defense mechanism (for
pathogenic bacteria) [5, 7, 11]. Bacterial biofilms are captivating areas to study because of their wide varieties in nature, importance in infection and therapeutics, and use in bioremediation and industries [40, 41]. Advances in molecular and biochemical methods have helped in improving our comprehension of biofilm structure and functions [42]. Exploiting naturally occurring- and in-situ biofilm development by various bacteria are promising ways for future advances in the agricultural field, in bioremediation and in understanding the mechanism of infection and drug resistance [40, 41].

Regarding the regulation of biofilm formation, it was reported that in the case of Bacillus subtilis, glucose repressed biofilm development through the catabolic control protein CcpA [43]. On the other hand, contrasting reports showed that the addition of glucose and NaCl in Tryptic Soy broth (TSB) favored more biofilm formation in Bacillus cereus cells compared to the addition of glucose or glycerol or NaCl in growth media [44]. Interestingly, a recent study reported that glucose can induce better biofilm formation and biofilm-mediated antibiotic resistance in Pseudomonas aeruginosa [12]. Many studies suggest that the biofilm develops in restricted supplemental of nutrients or the presence of any sort of stress i.e. antibiotic, high salt, or a corrosive substance like acid [7]. It is also reported that such non-favorable conditions affect adherence, biofilm arrangement, and synthesis of EPS in bacteria [3, 4]. Thus, an increase in adherence properties of bacterial cells and biofilm forming ability in presence of salt and/or glucose is a concern as not only the human hosts but also different medical equipment and accessories might be the potential sites for biofilm formation and evolution of antibiotic resistant bacteria.

On the other hand, recent studies revealed the biofilm-forming abilities of the Bacillus thuringiensis isolates in vitro and on plant surfaces [2]. Therefore, B. thuringiensis biofilms on plant surfaces can help the plant by protecting it from the pathogens like agricultural pests. It is also reported that Bacillus thuringiensis and other Bacilli spores have a higher hydrophobicity, conferring a higher adhesive potential to diverse materials [45, 46]. Interesting findings in recent times revealed that Bacillus thuringiensis cells undergo differentiation and represent a mixed population of heterogenous cells under stressed conditions [2].

A previous study from our laboratory revealed that the addition of higher concentrations of glucose (1%) in growth media could induce restricted swarming motility due to impaired flagellation in Bacillus thuringiensis KPWP1 on nutrient agar plates [23]. This finding had raised the question of whether the restricted motility in presence of glucose can trigger the biofilm-forming ability in Bacillus thuringiensis KPWP1 cells? The present study was therefore to explore whether restricted motility induced by glucose can regulate the adherence property, production of EPS components, thus biofilm formation ability in Bacillus thuringiensis KPWP1. The results from the present study indicate that the presence of added glucose in nutrient-rich media induces biofilm formation (Fig. 1a) by KPWP1 as a result of an increase in EPS production (Fig. 5), cell surface hydrophobicity (Fig. 3) and increased adherence property mediated by increased cell surface hydrophobicity and differential distribution of live and dead cells. Such induced biofilm formation is glucose specific as the addition of galactose or arabinose in growth media could not induce any significant biofilm formation by KPWP1(Fig. 1a).

The increased biofilm formation, in presence of glucose, was manifested by the increased thickness (Fig. 2a), bio-volume (Fig. 2b), and biomass (supplementary Fig S3). Moreover, it was observed that the skewness (Fig. 2c) which reflects the intracellular void spaces and the kurtosis values (Fig. 2d) which expresses the adherence of microscopic organisms in KPWP1 biofilm also increased with the increased glucose concentrations in growth media.

It is reported that EPS add to the mass and 3-D structure of the biofilm framework [47]. The present study revealed that the EPS components i.e. polysaccharides, proteins, eDNA and lipids increased (Fig. 5a, 5b, 5c and 5d) in KPWP1 biofilms with the increase in glucose concentrations in growth media. In the 48-h grown biofilms, EPS contents—sugar, proteins, lipid and eDNA were altogether higher in the presence of higher concentrations of glucose than that of control condition and supports the observed increase in the physical appearance of EPS in SEM images of KPWP1 biofilms grown in different glucose concentrations (Fig. 1c). Furthermore, it is also indicative that the EPS formed in presence of 1 and 2% glucose helps in the adherence of the bacteria to the surface (Fig. 3). Collectively, the present study uncovered the effect of glucose on the biofilm arrangement of KPWP1 cells. Glucose prompts the adherence property of the Bacillus thuringiensis KPWP1 cells and induces more EPS production resulting in more biofilm formation. It is thus possible that the human pathogens of Bacillus cereus group of bacteria might be able to form better biofilm in presence of glucose in human hosts and also on the surfaces of different medical devices.

The results of the present study signify that restricted motility and increase in cell surface hydrophobicity in Bacillus thuringiensis KPWP1 induced by glucose helps the bacterial cells to form biofilm, thus give a major premise to a more elaborate investigation of the in-vivo biofilm formation by this insecticidal bacterium Bacillus thuringiensis on plant surfaces, particularly in response to glucose.

In recent years, instead of focusing of creating genetically modified plants harbouring Bacillus thuringiensis cry genes or other bacterial genes, research is more focused on spraying Bacillus thuringiensis strains or other bacterial strains on plant surfaces as biocontrol agents and to study the
consequence of that [17, 48]. It is reported that only spraying *Bacillus thuringiensis* on plant surface may not benefit as the bacteria need to stay and adhere on the surface to execute insecticidal activities or to be consumed by the harmful pests and it was observed that the plant polysaccharides can modulated the extent of adherence of bacterial population on plant surface and insecticidal activities [49]. Therefore, a way should be there for better adherence of bacteria on the surface of different plants with different levels of polysaccharide contents. It is also reported that addition of Xylan and/or pectin helps in better adherence of *Bacillus thuringiensis* and biofilm formation on plant surfaces [49]. Based on our study and other reports, we have the opinion that spraying *Bacillus thuringiensis* grown in presence of glucose can be considered as an effective option as glucose is not as costly as xylan or pectin but it can ensure adherence of bacteria on plant surface. Also, an experiment conducted by us showed decrease of glucose concentrations slowly reduces or releasees the total loads from pre-formed (in presence of 2% glucose) biofilm (Supplementary Section, page 4), which has a special advantage as on the one hand we do not need to spray the bacterial suspension (in presence of higher concentrations of glucose) on plant surfaces with short intervals. On the other hand, during the time of harvest, spraying water will ensure decrease of glucose concentration, thus removal of bacterial biofilms to a greater extent and less harmful for human consumption.

Results of this investigation also open the area of investigation on the glucose mediated increased biofilm formation and drug resistance in the case of pathogenic bacteria, particularly *Bacillus cereus* group of organisms.

**Conclusions**

The present study revealed that the addition of glucose in growth media can induce biofilm formation by *Bacillus thuringiensis* KPWP1 and the glucose induced increase in biofilm formation was found to be associated with increased production of EPS components and increased cell surface hydrophobicity and adherence. Thus, the results of this study give a major premise to the future investigations on biofilm formation on plant and other surfaces by *Bacillus thuringiensis* and *Bacillus cereus* group of bacteria, particularly in response to glucose.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s00284-021-02699-z.

**Acknowledgements** All authors would like to acknowledge Mr. Ritarbrata Ghosh and Kashinath for their technical support of confocal laser scanning and scanning electron microscopy respectively. The authors also thank Mr. Sushant K Sinha for his help in doing the GOD-POD assay of glucose.

**Author Contributions** SJ and TKS conceived the study and designed the experiments. SJ, NKB, and AK performed the experiments and analysed the data. SJ and TKS have written the manuscript.

**Funding** The study was funded by IISER Kolkata.

**Availability of Data and Materials** In this study all data generated or analyzed are included in the article.

**Code Availability** Not applicable.

**Declarations**

**Conflict of interest** The authors declare that there is no conflict of interest.

**Ethical Approval** Not applicable.

**Consent to Participate** All authors had consent to participate in the study.

**Consent for Publication** All authors have given consent for publication.

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