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Sucrose reduces biofilm formation by *Klebsiella pneumoniae* through the PTS components ScrA and Crr

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ABSTRACT

The presence of sucrose at concentrations of 0.5–5% can either increase bacterial biofilms (*Streptococcus mutans* and *Escherichia coli*) or have no significant effect on biofilms (*Pseudomonas aeruginosa* and *Staphylococcus aureus*). However, our study revealed that 1 % sucrose reduced the biofilm formation by *Klebsiella pneumoniae* STU1. To explore the role of the phosphoenolpyruvate-dependent-carbohydrate: phosphotransferase system (PTS) in regulating this process, the *scrA* gene, which encodes the sucrose-specific EIIBC of the PTS, was deleted in *K. pneumoniae* to create a *scrA* mutant (Δ*scrA*). Thereafter, we observed that the biofilm formation and type 3 fimbriae production were not affected by sucrose in the Δ*scrA* while sucrose reduced these processes in the wild type. Furthermore, we discovered that Crr, the glucose-specific EIIA of PTS, was the primary but not the sole EIIA of ScrA in *K. pneumoniae* by sucrose fermentation test. In addition, deficiency of Crr reduced the biofilm formation in *K. pneumoniae*. Our proposed model suggests that, through the action of Crr in the absence of sucrose, the transcription of the *mrk* operon, which produces type 3 fimbriae, was increased, thereby influencing biofilm formation by *K. pneumoniae* and bacterial number in the gut of nematode. This observation differs from the regulation of polysaccharide and biofilm by sucrose in other bacteria. Our findings extend the understanding of the effects of sucrose on biofilm formation.

1. Introduction

Sucrose, the main compound in table sugar, is widely used and primarily produced from sugar beet and sugar cane [1]. Numerous studies have focused on the relationship between sucrose and bacteria, particularly *Streptococcus mutans*, which is known to cause tooth decay, and *Streptococcus gordonii*, a common colonizer of the oral cavity. Rezaei et al. found that *S. mutans* biofilm formation increased nearly fourfold in the presence of 1 % sucrose concentration [2–4]. Sucrose consumption also led to increased abundance of streptococci, specifically *S. gordonii*, *Streptococcus parasanguinis*, and *Streptococcus sanguinis*, on the enamel surface [5]. In *Enterococcus faecalis* reference strain and four clinical strains, biofilm formation increased with 0.5 % and 1 % sucrose [6]. However, sucrose had no significant effect on the bacterial biofilm formation in 61 clinical isolates of *Staphylococcus aureus* [7]. In addition to gram-positive bacteria, *Escherichia coli* O157:H7 biofilm increased with 20 mM (0.68 %) sucrose due to elevated bacterial lipopolysaccharide (LPS) production [8]. *Pseudomonas aeruginosa* biofilm formation increased at a very high concentration of sucrose (11.7 %), but showed no effect at concentrations from 1 % to 5 % (Bouffartigues et al., 2014). *Yersinia pestis* biofilm formation was not influenced by 2 % sucrose, but slightly increased with 6 % sucrose [9]. However, the impact of sucrose on the biofilm of *Klebsiella pneumoniae* remains unclear.

Klebsiella pneumoniae, a member of the Enterobacteriaceae family, is

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commonly found in natural surface waters and soils [10]. This bacterium is known to cause opportunistic infections such as pneumonia, urinary tract infections, bacteremia, and pyogenic liver abscesses [11,12]. In addition, *K. pneumoniae* is a part of the gut microbiota in humans and animals, including birds and earthworms [13,14]. Furthermore, *K. pneumoniae* has the ability to form biofilms on both abiotic surfaces and human tissue [15]. Biofilm formation on medical devices increases the risk of infection, and *K. pneumoniae* is commonly associated with infections related to indwelling medical devices, such as catheter-associated urinary tract infections, catheter-related bloodstream infections, and ventilator-associated pneumonia [16,17]. *K. pneumoniae* produces fimbriae, which are hair-like protein appendages that extend from the bacterial surface and facilitate bacterial adhesion to both abiotic and biotic surfaces. This adhesion is the initial step in biofilm formation [15]. Numerous putative fimbrial-gene clusters are present on the *K. pneumoniae* genome, but only a select few of these are expressed under laboratory growth conditions [18]. Type 1 and type 3 fimbriae are the predominant and commonly found adhesive structures in *K. pneumoniae*. Type 3 fimbriae are produced by many members of the Enterobacteriaceae [19]. Type 1 fimbriae are crucial for *K. pneumoniae* in causing urinary tract infections, while type 3 fimbriae aid in the attachment of *K. pneumoniae* to the extracellular matrix, binding to human endothelial and bladder cells. Type 3 fimbriae also promote biofilm formation on both biotic and abiotic surfaces [20]. The components of type 3 fimbriae are encoded by the genes in the *mrkABCDF* operon. The *mrkA* gene encodes the major subunits of the type 3 fimbrial shaft [15], and the transcriptional activity of the *mrkABCDF* operon can be regulated by cyclic 3′5′-adenosine monophosphate (cAMP) and ci-di-GMP, which are second messengers in bacterial cells [21–23]. MrkH is a transcriptional activator of the *mrk* operon through direct binding to the upstream region of the *mrkA* promoter [15].

The level of cAMP can be modulated by Crr, which is the glucose-specific EIIA ($EIIA^{Glc}$) of the phosphoenolpyruvate:carbohydrate phosphotransferase system (PTS). Bacteria possess multiple PTSs to transport and phosphorylate various sugars as needed. Generally, the PTS comprises enzyme I (EI), histidine-containing phosphocarrier protein (HPr), and enzyme II (EII) complexes. While EI and HPr are common to all PTSs, the EII complexes consist of EIIA, EIIB, and EIIC (sometimes EIID) proteins/domains, which are specific to different sugars and vary in each PTS. In most PTSs, EIIAs transfer the phosphoryl group from HPr to their corresponding EIIB in the cytoplasm. Subsequently, the phosphorylated EIIB transfers the phosphoryl group to the sugar (carbohydrate) bound to the corresponding EIIC in the cytoplasmic membrane. The gene *crr* encodes the glucose-specific EIIA, which transfers the phosphoryl group to the EIIB domain of PtsG in *E. coli* to facilitate glucose uptake [24,25]. In addition, *K. pneumoniae* utilizes sucrose-specific EIIBC (formerly known as $EnzymeII^{Scr}$), encoded by the *scrA* gene, for sucrose uptake. Sprenger et al. demonstrated that both ScrA and Crr are necessary for sucrose uptake by using the *E. coli crr* mutant and the *scrA* gene from *K. pneumoniae* in a plasmid [26]. However, the role of the *crr* gene from *K. pneumoniae* in sucrose uptake has not been investigated.

In this study, we examined the impact of sucrose on *K. pneumoniae* biofilm formation. Our findings revealed a unique response of *K. pneumoniae* to sucrose, distinguishing it from other bacteria. Furthermore, we investigated the roles of *scrA* and *crr* in biofilm development when *K. pneumoniae* was cultivated in a sucrose-rich environment. In addition, our study highlighted the significance of the *crr* gene, which, unlike in *E. coli*, was found to be important but not essential when combined with ScrA in *K. pneumoniae*.

2. Materials and methods

2.1. Bacterial strains and incubation conditions

The bacterial strains and plasmids utilized in this study are detailed in Table 1. *K. pneumoniae* STU1 is a laboratory-maintained strain that was acquired from National Taiwan University (Taipei, Taiwan) and its capsular type is K5. *K. pneumoniae* clinical strains (10495, 42200, 38507, and 93687) were isolated from clinical specimens at Tzu Chi Hospital (Hualien, Taiwan) and subsequently transferred to Tzu Chi University (Hualien, Taiwan) through an official transfer. Patient information was separated from the bacterial strain. STU1 and its mutant derivatives were routinely grown in Luria-Bertani (LB) medium (1 % NaCl, 0.5 % yeast extract, and 1 % tryptone) at 37 °C. For specific purposes, the bacteria were cultured in M9 minimal medium (1 × M9 salt, 2 mM magnesium sulfate, and 0.1 mM calcium chloride) with 1 % sucrose (M9-sucrose) at 37 °C. One liter of 1 × M9 salt solution contained 12.8 g disodium phosphate heptahydrate, 3 g potassium dihydrogen phosphate, 0.5 g sodium chloride, and 1 g ammonium chloride. Bacteria carrying pBSK-Km:ZsGreen were incubated in LB supplemented with 50 μg/mL kanamycin in routine culture.

Table 1
Bacterial strains and the plasmids used in this study.

Strain	Relevant genotype and phenotype	Reference or source
K. pneumoniae		
STU1	Laboratory-maintained strain, K5, Amp^r	[23]
Δ*scrA*	deletion of *scrA* gene in STU1, Amp^r	This study
Δ*crr*	deletion of *crr* gene in STU1, Amp^r	[23]
Δ*crr*Δ*scrA*	deletion of *scrA* gene in Δ*crr*, Amp^r	This study
Clinical stains	isolated from clinical specimens at Tzu Chi Hospital (Hualien, Taiwan)	This study
10495		
42200		
38507		
93687		
Plasmid		
pBSK-Km: ZsGreen	pBSK carries the ZsGreen gene to produce green fluorescence and kanamycin resistance gene, Km^r	[29]
pBSK::Gm	pBSK derivative: the gentamicin resistance gene was inserted at the ScaI site of pBSK, Gm^r	[27]
pBSK:*scrAB*	pBSK::Gm carries *scrAB*	This study
pBAD33	P_{BAD} promoter, pACYC184 *ori*, Cm^r	[28]
pBAD33:*crr*	pBAD33 carried *crr*	[28]

2.2. Biofilm quantification

The biofilm quantification procedure was conducted following the method in the previous study [27]. In brief, the overnight bacterial culture was diluted in fresh LB (or LB with 1 % sucrose) to an optical density at 600 nm (OD_{600}) of 0.1 in a Falcon polystyrene tube (A tube) and a Falcon 50 mL conical centrifuge tube (B tube), and then incubated at 37 °C for 24 h. After measuring the OD_{600} value of the bacterial suspension in the B tube, the suspension in the A tube was discarded. Next, the A tube was rinsed twice with water and then treated with 0.1 % crystal violet. After standing for 20 min at room temperature, the solution in the A tube was discarded, and the tube was rinsed with water twice. Following air-drying, 95 % ethanol was added into the A tube. The absorbance of the ethanol solution containing the crystal violet was measured at 590 nm (OD_{590}). The specific biofilm was defined as the OD_{590} in the A tube divided by the OD_{600} in the B tube.

2.3. Construction of scrA mutant

The *scrA* gene was deleted from *K. pneumoniae* STU1 or Δ*crr* mutant to create Δ*scrA* or Δ*crr*Δ*scrA* respectively using unmarked mutagenesis, as described in the previous study [28]. In brief, approximately 900-bp flanking DNA regions of the *scrA* gene were amplified via PCR and then ligated into the suicide vector, $pK18^{R6K}$. The resulting plasmid, $pK18^{R6K}$::scrAup:scrAdown, was transferred from *E. coli* S17-1 into *K. pneumoniae* STU1 through conjugation. The transconjugants were

positively selected for kanamycin and ampicillin resistance and subsequently negatively selected using the *sacB* effect. The *scrA* mutant was confirmed through PCR using several sets of primers, followed by sequencing. The method is also described in detail in the supplementary material.

2.4. Bacterial growth curve

The OD_{600} of overnight bacterial culture was adjusted to be one ($OD_{600} = 1$). Subsequently, after centrifuging 1 mL of bacterial culture at 6000×*g* for 10 min, the supernatant was discarded. The bacterial pellet was then suspended in 100 mL of fresh medium and incubated at 37 °C and 220 rpm. The optical density of bacterial suspension was recorded every hour.

2.5. Sucrose fermentation

The OD_{600} of overnight culture was adjusted to be one ($OD_{600} = 1$) with LB. Then, 100 μL of the bacterial culture was centrifuged at 17000×*g* for 3 min, and the supernatant was discarded. Subsequently, After the bacterial pellet was suspended with 150 μL of fresh LB, phenol red and sucrose (or without sucrose) were added. Finally, water was added to the bacterial suspension to make the total volume 300 μL, and then it was incubated at 37 °C. The final concentrations of phenol red and sucrose were 0.05 % and 1 %, respectively. Phenol red was used as a pH indicator, turning yellow as the bacterial suspension became acidic.

2.6. Observation of bacteria in the gut of Caenorhabditis elegans N2

The *C. elegans* N2 (wild type) was propagated on nematode growth medium (NGM) plates seeded with *E. coli* OP50. Afterward, the worms were fed *K. pneumoniae* carrying pBSK-Km:ZsGreen, which can produce fluorescence, on NGM plates with or without 1 % sucrose for one day. After being washed three times with 0.9 % NaCl, the worms were paralyzed with 200 mM sodium azide for 5–10 min. The nematodes were observed using an upright fluorescence microscope Nikon Ni-E (Nikon, Japan). To quantify the fluorescence levels in worms, 20 worms in a well of microplate (PerkinElmer, USA) were measured at 505 nm after excitation at 492 nm using a Varioskan Flash microplate reader (Thermo Fisher Scientific, USA). The method used to observe the bacteria in the nematode intestine by a fluorescence microscope followed the procedure in the previous study [29]. The method is also described in detail in the supplementary material.

2.7. Colony forming unit assay

K. pneumoniae WT or △*scrA* were incubated in LB overnight before being seeded on NGM plates with or without sucrose. After the *C. elegans* N2 were fed *E. coli* OP50 for two days, the worms were transferred to NGM plates with or without sucrose and fed *K. pneumoniae* WT or △*scrA* for three days. Fifty worms were transferred to a tube containing 100 μL phosphate-buffered saline (PBS) on ice. After the worms were washed three times using 1 mL PBS, they were homogenized. The homogenate was mixed with 900 μL LB and then serially diluted in LB. One hundred microliters of each dilution were plated on LB agar plate with 50 μg/mL ampicillin and then incubated overnight. Finally, the colonies were counted.

2.8. Western blotting

After the bacterial lysate was analyzed by 12 % SDS polyacrylamide gel (SDS-PAGE), the total bacterial proteins were transferred to a nitrocellulose membrane. The observation of bacterial proteins, MrkA and ManA, by Western blotting followed the method in the previous study [29]. The method is also described in detail in the supplementary material. ManA (mannose 6-phosphate isomerase) was identified as the loading control in the Western blotting. The Western blotting signals were detected using the Gel Catcher 2850 chemiluminescence camera system (CLUBIO in Taipei, Taiwan), and were quantified using ImageJ (National Institutes of Health). Quantification of MrkA was performed by comparing the intensity of the MrkA band to that of ManA in Western blotting.

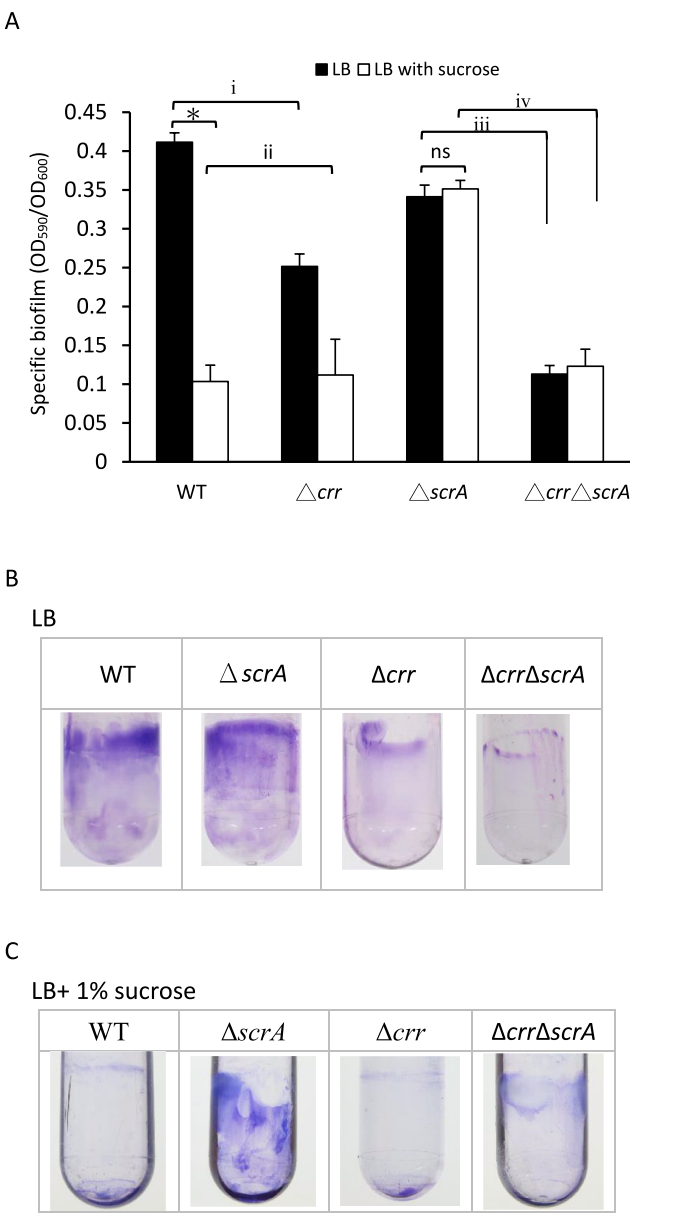


Fig. 1. The effect of sucrose on biofilm of *K. pneumoniae*. *K. pneumoniae* was incubated in LB (black bar) or LB with 1 % sucrose (white bar) before quantification of biofilm. (A) The specific biofilm, which is defined in the Methods section, is expressed as the mean ± SD from thrice experiments. Asterisk (*) represents $p < 0.05$. ns indicates no significance. (i) The comparison between WT and Δ*crr* incubated in LB showed a significant difference. (ii) The comparison between WT and Δ*crr*, both incubated in LB with sucrose, showed no significant difference. (iii) The comparison between Δ*scrA* and Δ*crr*Δ*scrA* incubated in LB showed a significant difference. (iv) The comparison between Δ*scrA* and Δ*crr*Δ*scrA*, both incubated in LB with sucrose, showed a significant difference. (B and C) The representative images of biofilms stained with crystal violet. WT: *K. pneumoniae* STU1 wild type, Δ*scrA*: *scrA* mutant, Δ*crr*: *crr* mutant, Δ*crr*Δ*scrA*: *crr* and *scrA* double-deleted mutant. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

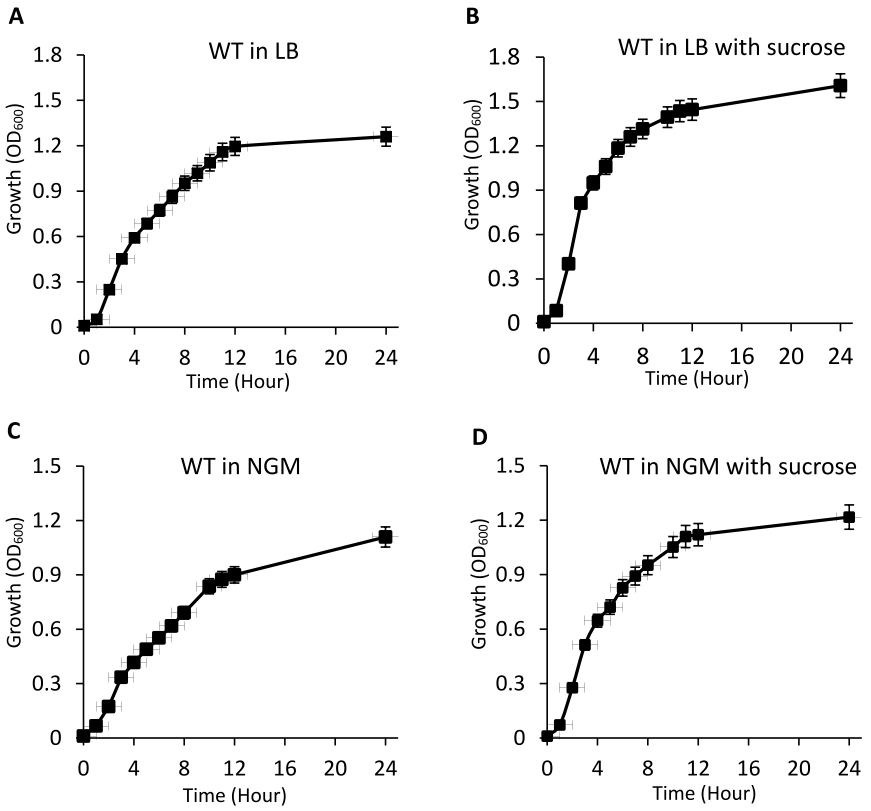


Fig. 2. The effect of sucrose on growth curve of *K. pneumoniae* STU1. *K. pneumoniae* STU1 was incubated in (A) LB, (B) LB with 1 % sucrose, (C) NGM, or (D) NGM with 1 % sucrose. The bacterial growth was monitored by measuring OD_{600} of the broth culture. The values were expressed as the mean ± SD from thrice experiments.

2.9. Reverse transcription quantitative real-time PCR (RT-qPCR)

The transcriptional levels of *mrkA*, *mrkH*, *crp*, *galF* and *recA* were quantified by RT-qPCR. RNA was reverse transcribed using a QuantiNova reverse transcription kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. To quantify the cDNAs from the transcripts in triplicate, either probe-based qPCR [fluorescein-labeled and dual-quenched probes (Integrated DNA Technologies, Coralville, IA, USA)] or the dye-based qPCR [the QuantiNova SYBR Green PCR kit (Qiagen, Hilden, Germany)] was performed. The data was normalized to that of 16S rRNA following the $2^{-\Delta\Delta CT}$ method, as previously described [28, 29]. The detailed method is also described in the supplementary

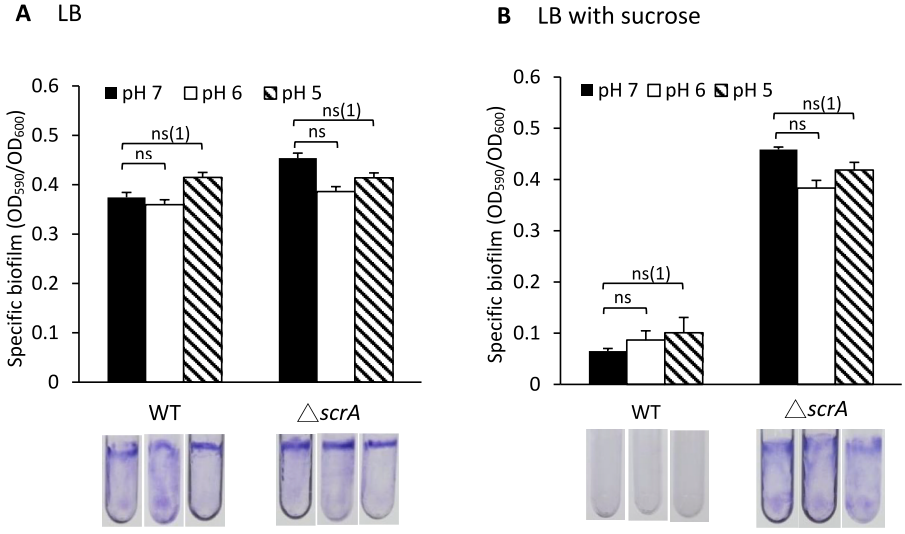


Fig. 3. The effect of pH **on biofilm of *K. pneumoniae*.** The pH of the medium was adjusted to 7, 6 or 5 before incubation. *K. pneumoniae* was incubated in pH-adjusted (a) LB or (b) LB with 1 % sucrose. The biofilm in the tube stained by crystal violet solution was shown and quantified. The values were expressed as the mean ± SD from thrice experiments. ns indicates no significance. (1) indicates the comparison between pH 7 and pH 5. WT: *K. pneumoniae* STU1 wild type, Δ*scrA*: *scrA* mutant. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

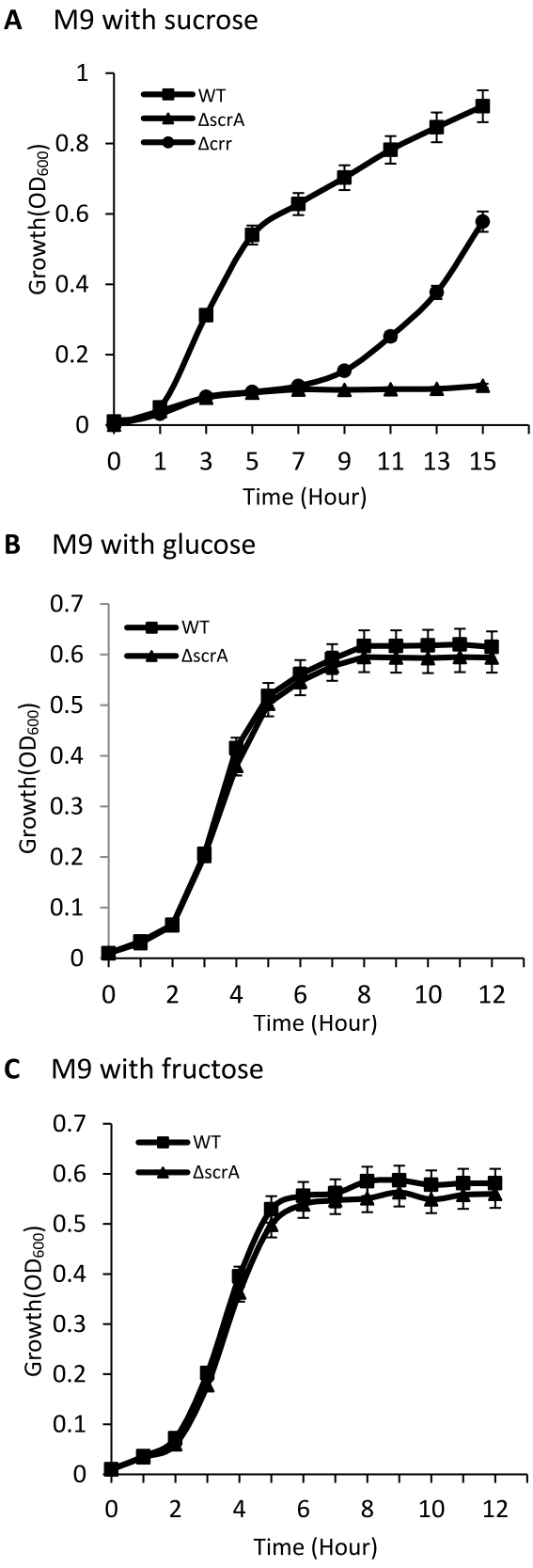


Fig. 4. ScrA is specific to sucrose uptake in *K. pneumoniae*. *K. pneumoniae* was incubated in the M9 minimal medium containing 1 % (a) sucrose, (b) glucose, or (c) fructose as sole carbon source. The bacterial growth was monitored by measuring OD_{600} of the broth culture. The values were expressed as the mean ± SD from thrice experiments. WT: *K. pneumoniae* STU1 wild type (square), Δ*scrA*: *scrA* mutant (triangle), Δ*crr*: *crr* mutant (circle).

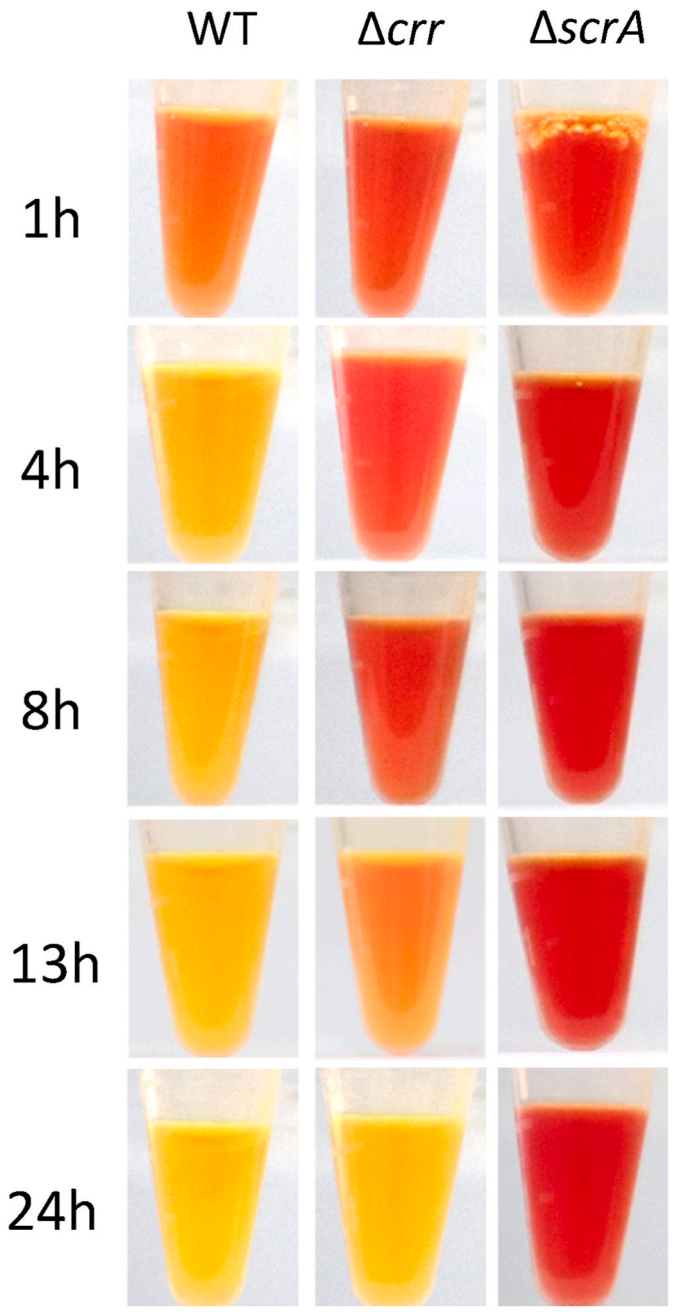
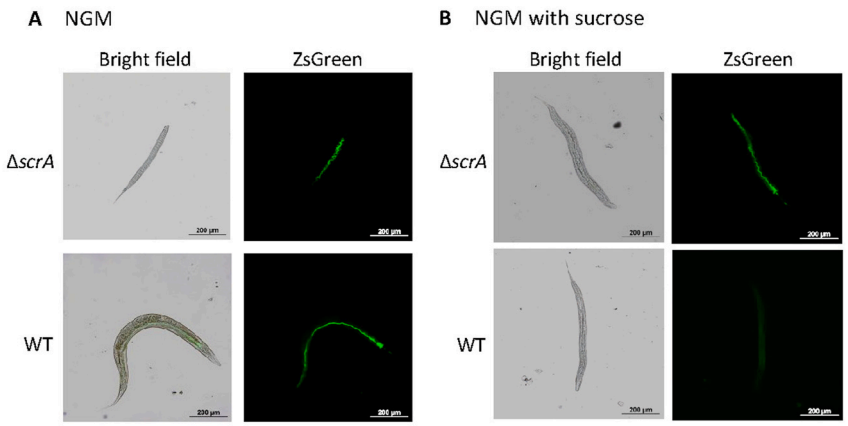


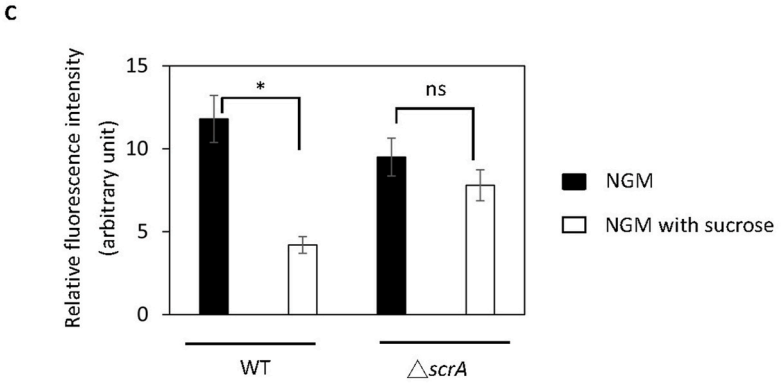
Fig. 5. Crr is the major EIIA of ScrA in the sucrose fermentation by *K. pneumoniae*. Bacteria were incubated in LB with 1 % sucrose and phenol red. The red medium turned yellow when the pH of the medium decreased due to sucrose fermentation. WT: *K. pneumoniae* STU1 wild type, Δcrr: *crr* mutant, ΔscrA: *scrA* mutant. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

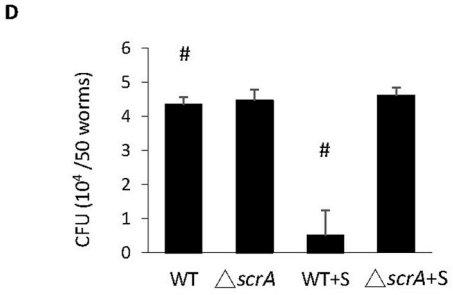
material. The housekeeping gene, *recA*, encoding recombinase A was used as a reference to compare the gene expression of *mrkA*, *mrkH*, *crp* or *galF*. All primers and probes are listed in the Supplementary Table S1.

2.10. Statistical analysis

The results of biofilm quantification, bacterial growth, and quantification of the band intensity from Western blotting were expressed as the mean±standard deviation (SD) from three independent tests. The data were analyzed using a student's t-test, and the significant difference of the data was considered at $p < 0.05$.







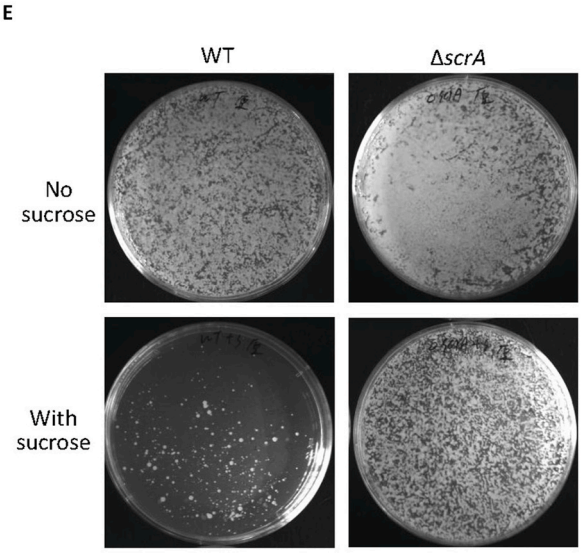


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Fig. 6. Sucrose reduced the numbers of *K. pneumoniae* in *C. elegans* via ScrA. *K. pneumoniae* carrying the fluorescence gene (in the pBSK-Km:ZsGreen plasmid) was used to feed *C. elegans* on (A) NGM or (B) NGM with 1 % sucrose. Representative images of bacteria with pBSK-Km:ZsGreen in the gut of nematodes were from thrice experiments. WT: *K. pneumoniae* STU1 wild type, Δ*scrA*: *scrA* mutant. (C) The fluorescence levels of 20 worms that had been fed bacteria with pBSK-Km:ZsGreen were quantified and then expressed as the average arbitrary units±SD from three independent experiments. Asterisk (*) represents $p < 0.05$. ns indicates no significance. (D) The numbers of WT and Δ*scrA* in the nematodes from the NGM (No sucrose) and NGM with sucrose (With sucrose) were counted by colony-forming unit assay. WT and Δ*scrA* indicate that bacteria from nematode on NGM. WT + S and Δ*scrA* + S indicate that bacteria from nematode on NGM with sucrose. The symbol # indicates that the comparison between WT and WT + S showed a significant difference. The representative images of colonies in colony-forming unit assay were shown in (E).

3. Results

3.1. Sucrose decreases biofilm formation by K. pneumoniae

To study the effects of sucrose on biofilm formation by *K. pneumoniae* STU1 (wild type, abbreviated as WT), we measured the biofilm formed in Luria-Bertani (LB) medium with and without sucrose (1 %). The results demonstrated that sucrose reduced the biofilm of WT (Fig. 1). Besides STU1, we examined the biofilm formation of four clinical *K. pneumoniae* strains. Like STU1, their biofilms in LB with sucrose were reduced compared to those in LB alone (Fig. S1). However, sucrose did not decrease, and in fact slightly enhanced, bacterial growth of WT (Fig. 2A and B). Therefore, the decrease in biofilm formation of WT due to sucrose is not caused by reduced growth. Since sucrose fermentation leads to a decrease in the pH of medium, we investigated the effects of pH on the biofilm. The WT was cultured in LB medium with pH levels of 7, 6, or 5. After incubation, the WT biofilms in pH 6 and 5 media did not show significant differences compared to those in the pH 7 medium (Fig. 3A). Even when the WT was cultured in LB medium with an additional 1 % sucrose and adjusted to pH levels of 6 or 5, the amount of WT biofilm also did not show significant differences, compared to that in LB-sucrose medium with pH of 7 (Fig. 3B). Furthermore, we observed that sucrose also reduced WT biofilm formation even at pH levels of 7, 6, and 5 (Fig. 3). Therefore, we hypothesized that the reduction in LB biofilm due to sucrose is related to sucrose uptake and not to the acidic products from sucrose fermentation.

3.2. The biofilm of the scrA mutant is not decreased by sucrose

Since the gene *scrA* encodes the protein for sucrose transport in other bacteria, the *scrA* gene in *K. pneumoniae* STU1 was deleted to create the *scrA* mutant (Δ*scrA*). To test whether *scrA* is specific for sucrose utilization in *K. pneumoniae* STU1, we incubated both WT and Δ*scrA* in M9 minimal medium with 1 % sucrose as the sole carbon source (M9-sucrose). We observed that Δ*scrA* showed a growth defect in M9-sucrose compared to WT (Fig. 4A). However, the growth curves of WT and Δ*scrA* in M9 minimal medium containing 1 % glucose or fructose as the sole carbon source (M9-glucose or M9-fructose) were similar (Fig. 4B and C). In addition, to detect acid products after sucrose fermentation, we added 0.05 % phenol red in LB with 1 % sucrose (LB-sucrose-phenol red). After 4 h of incubation, WT turned yellow while Δ*scrA* remained red for more than 24 h (Fig. 5), showing the *scrA* is essential for sucrose fermentation.

Since these results indicate that *scrA* is specific for sucrose uptake in *K. pneumoniae* STU1, we investigated whether *scrA* is involved in the reduction of biofilm by sucrose. The biofilm produced by Δ*scrA* in LB with and without sucrose was quantified. The results showed that, unlike WT, the biofilms of Δ*scrA* in LB and LB with sucrose are not significantly different (Fig. 1). However, the complementation of *scrA* restored the phenotype of Δ*scrA* when incubated in LB with sucrose, making it similar to the WT (Fig. S2A), suggesting that sucrose decreased biofilm formation via ScrA in *K. pneumoniae*. Furthermore, like WT, the biofilm of Δ*scrA* was not affected by pH in the medium (LB and LB with sucrose) (Fig. 3).

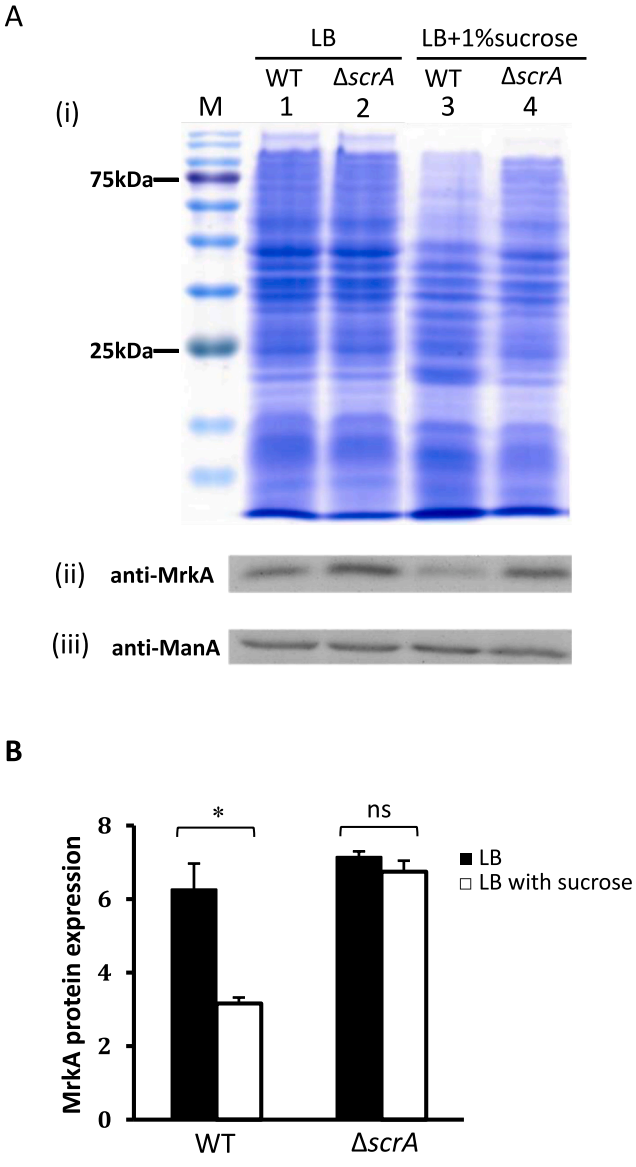

Fig. 7. Western blotting analysis of MrkA and ManA in *K. pneumoniae*. (a) Bacteria were incubated in LB (lane 1 and 2) or LB with 1 % sucrose (lane 3 and 4). The total bacterial cell proteins were analyzed by (i) SDS-PAGE followed by Coomassie blue staining and by Western blotting using (ii) anti-MrkA antibody and (iii) anti-ManA antibody. The molecular weights of MrkA and ManA are approximately 21 kDa and 36 kDa respectively. ManA was detected as loading control. M indicates protein marker. Representative images are from thrice experiments. (b) The intensity of the MrkA bands in Western blotting was quantified by Image J and compared to that of ManA. The quantification results were expressed as the mean ± SD from thrice experiments. Black bar: LB. White bar: LB with 1 % sucrose. WT: *K. pneumoniae* STU1 wild type (lane 1 and 3). Δ*scrA*: *scrA* mutant (lane 2 and 4). Asterisk (*) represents $p < 0.05$. ns indicates no significance. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.3. Reduction of *K. pneumoniae* number in the nematode gut by sucrose is dependent on ScrA

Since we observed that sucrose reduces the biofilm of *K. pneumoniae* on abiotic surfaces, we explored whether sucrose reduces the number of *K. pneumoniae* in the nematodes. To do this, the plasmid pBSK-Km:ZsGreen, which contains green fluorescent gene [29], was introduced into both WT and Δ*scrA* strains. These bacteria were then fed to *Caenorhabditis elegans* on both nematode growth medium (NGM) and NGM with 1 % sucrose (NGM-sucrose), and the fluorescence emitted from the worms was observed after incubation for one day. The results showed that the fluorescence intensity emitted from the worms fed with WT with pBSK-Km:ZsGreen on NGM-sucrose was lower, compared to that on NGM (Fig. 6A, B, C), indicating that sucrose reduced the amount of *K. pneumoniae* in the nematode intestine. To further investigate the effect of sucrose on bacterial growth on NGM, the WT strain was incubated in NGM broth with and without sucrose (NGM-sucrose and NGM, respectively). The results showed that the growth of WT in NGM was not affected by the presence of sucrose (Fig. 2C and D). This implies that sucrose did not reduce bacterial number on NGM before entry the nematode. However, the fluorescence intensity emitted from the worms fed with the Δ*scrA* with pBSK-Km:ZsGreen strain on NGM-sucrose was not reduced, compared to that on NGM. (Fig. 6A, B,C). We further examined the effect of sucrose on the number of *K. pneumoniae* in the worms using colony-forming unit assay. After the worms were fed either WT or Δ*scrA*, the bacterial numbers in the nematodes were counted. The results showed that the number of WT bacteria in the nematodes was reduced when the worms were fed WT on NGM with sucrose, compared to when they were fed WT on NGM alone (Fig. 6D and E). However, the number of Δ*scrA* bacteria in the nematodes was not affected by sucrose (Fig. 6D and E). These results suggest that ScrA is involved in the reduction of *K. pneumoniae* numbers in the worms in the presence of sucrose.

3.4. Sucrose reduces the expression of fimbriae through ScrA

Type 3 fimbriae facilitate bacterial attachment to surfaces. To investigate the effect of sucrose on the type 3 fimbrial shaft protein, MrkA, Western blotting and RT-qPCR was performed. The results showed that the amount of MrkA protein and *mrkA* transcript in WT incubated in LB with sucrose was lower, compared to those in LB alone, indicating that sucrose reduced the expression of MrkA in *K. pneumoniae* (Fig. 7 and Fig. S3A). In addition, the amount of MrkA protein and *mrkA* transcript in the Δ*scrA* strain was not significantly different when incubated in LB or LB with sucrose (Fig. 7 and Fig. S3A). This suggests that ScrA is involved in the regulation of MrkA production in *K. pneumoniae* by sucrose. Besides, MrkH is a transcriptional activator of *mrkABCDF* and auto-activates *mrkH* expression [15]. To examine whether sucrose regulates the transcription of *mrkH*, the amount of *mrkH* mRNA in bacteria incubated in LB or LB with 1 % sucrose was quantified by RT-qPCR. The results showed no significant difference between WT in LB and WT in LB with sucrose (Fig. S3B), indicating that sucrose did not affect the transcription of *mrkH*.

3.5. The cognate EIIAs of ScrA include, but are not limited to, the Crr

It has been reported that ScrA relies on the Crr protein, also known as Enzyme IIIA, for the transportation and phosphorylation of sucrose. However, this was demonstrated using an *E. coli crr* mutant and the *scrA* gene of *K. pneumoniae* [26]. To investigate the role of Crr in sucrose uptake by *K. pneumoniae*, the bacterial growth curve in M9-sucrose and the sucrose fermentation of the *K. pneumoniae crr* mutant (Δ*crr*) were observed. Initially, the Δ*crr* mutant showed a delay in growth in M9-sucrose, but after 9 h, it began to grow rapidly, like being in the exponential phase (Fig. 4A). In the sucrose fermentation test, the Δ*crr* mutant did not produce acid from sucrose after incubation for 8 h in LB-sucrose-phenol red, but it did so after 13 h. In contrast, the wild-type strain fermented sucrose after 4 h (Fig. 5). These results indicate that Crr plays an important role in sucrose uptake by *K. pneumoniae*, particularly during the exponential phase. Therefore, Crr is likely the major cognate EIIA of ScrA for *K. pneumoniae* during this phase. However, it is possible that there are minor EIIAs that compensate for the role of Crr in sucrose uptake by ScrA.

To investigate the impact of Crr on biofilm formation by *K. pneumoniae*, the biofilm of Δ*crr* incubated in LB was quantified. The results revealed a reduction in biofilm in Δ*crr*, compared to WT in LB (Fig. 1). The complementation of *crr* restored the phenotype of Δ*crr* when incubated in LB with sucrose, making it similar to the WT (Fig. S2B). Furthermore, the decreased biofilm was also observed in the *crr* and *scrA* double-deleted mutant (Δ*crr*Δ*scrA*) incubated in LB, compared to Δ*scrA* mutant in LB (Fig. 1). These results indicate that Crr promotes biofilm formation by *K. pneumoniae* in LB. However, in LB with 1 % sucrose, the biofilms of WT and Δ*crr* did not show significant differences (Fig. 1), indicating that Crr did not regulate biofilm formation when *K. pneumoniae* was treated with sucrose. The results also suggest that Crr in a sucrose-rich environment does not affect the production of type 3 fimbriae, an important factor in biofilm formation by *K. pneumoniae*.

3.6. Sucrose regulates the transcriptional levels of crp via ScrA

Crr positively modulates the activity of adenylyl cyclase (AC), which elevates the intracellular cAMP level in *K. pneumoniae* [23] and *E. coli* [24]. Furthermore, the Crp-cAMP complex positively autoregulates the transcription of the *crp* gene [24,30]. To examine whether *crp* transcription is regulated by sucrose, the amount of *crp* mRNA in bacteria incubated in LB or LB with 1 % sucrose was quantified by RT-qPCR. The results showed that the *crp* transcript in WT was reduced by sucrose. However, the effect of sucrose on *crp* transcription was abolished in Δ*scrA* (Fig. S3C), suggesting that sucrose reduced *crp* expression via ScrA.

3.7. The capsular gene, galF, is controlled by sucrose

The effects of capsular polysaccharide (CPS) on the biofilm of *K. pneumoniae* are varied and controversial. The results of previous studies often depended on the studied genes [15]. The gene *galF* in *K. pneumoniae* is responsible for the translocation and surface assembly of CPS and is negatively regulated by CRP [31,32]. Additionally, Crr has been reported to negatively regulate *galF* [28]. Therefore, we examined the effect of sucrose on the transcription of *galF*. After measuring the *galF* transcript in bacteria incubated in LB or LB with 1 % sucrose by RT-qPCR, the results showed that the transcriptional level of *galF* in WT was elevated by sucrose, but not in Δ*scrA* (Fig. S3D). We speculate that sucrose increases the *K. pneumoniae* capsule via ScrA.

4. Discussion

Previous studies have shown that a 1 % concentration of sucrose had no effect on some bacteria (staphylococci, *P. aeruginosa*, *Y. pestis*) or increased biofilm formation in others (streptococci, enterococci, *E. coli*) [2,5–9,33]. However, in this study, sucrose decreased *Klebsiella* biofilm formation (Fig. 1) without reducing bacterial abundance (Fig. 2B). These findings demonstrate the diverse responses of bacteria to sucrose. In addition, we discovered that ScrA plays a key role in sucrose-regulating biofilm formation by *K. pneumoniae* (Fig. 1). The *K. pneumoniae scrA* mutant did not uptake sucrose (Figs. 4A and 5) and did not reduce biofilm formation (Fig. 1). We also found that the shaft protein of type 3 fimbriae, MrkA, is negatively regulated by ScrA in the presence of sucrose (Fig. 7), indicating that ScrA in the presence of sucrose plays a negative role in type 3 fimbriae and bacterial attachment to the surface, thereby reducing biofilm formation by *K. pneumoniae*.

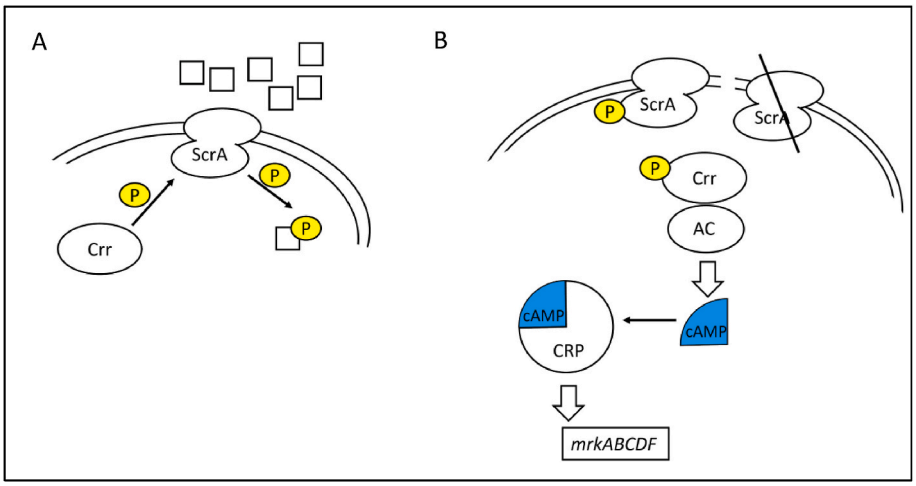

Fig. 8. The schematic diagram to explain the role of ScrA in sucrose-reducing biofilm by *K. pneumoniae* STU1. This regulatory model is proposed based on combination of the previous and present studies (detail in the text). (a) In the sucrose-rich environment, *K. pneumoniae* transports the sucrose (white square) into the bacterial cell by EIIC domain of ScrA which is located on the membrane. The intracellular sucrose is phosphorylated by EIIB domain of ScrA. The phosphoryl group (p in yellow circle) from ScrA to sucrose is received from Crr (The Crr phosphorylates the ScrA). (b) In the sucrose-poor environment or *scrA* mutant, the phosphoryl group stays on the Crr, leading to activating the AC to produce cAMP (blue sector). The binding of CRP with cAMP results in activating transcription of *mrk* operon (*mrkABCDF*) which encodes the type 3 fimbrial proteins in *K. pneumoniae*. Therefore, sucrose does not directly reduce the biofilm formation by *K. pneumoniae*. On the contrary, type 3 fimbrial synthesis is increased in the sucrose-poor environment. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Furthermore, sucrose via ScrA also led to reduced bacterial number in the gut of nematodes (Fig. 6).

The spatial interaction between ScrA and the upstream DNA region of *mrk* in the chromosome is believed to be challenging due to the membrane protein nature of ScrA [34]. Nevertheless, the presence of sucrose in the environment was found to inhibit the production of the MrkA protein (Fig. 7), leading us to propose a model to elucidate how sucrose diminishes the biofilm of *K. pneumoniae*. In this model, Crr is suggested to act as an intermediary between ScrA and *mrkA* (Fig. 8). As per previous research, Crr positively modulates the activity of adenylyl cyclase (AC), which elevates the intracellular cAMP level in *K. pneumoniae* [23]. The increased cAMP level activates the transcriptional factor, cAMP receptor protein (CRP), through direct interaction [35]. Subsequently, the CRP-cAMP complex triggers the transcription of *mrkA*, leading to heightened type 3 fimbriae production and facilitating biofilm formation [23]. Given that Crr is the primary EIIA of ScrA (Figs. 4 and 5), we speculate that the level of cAMP is influenced by sucrose through Crr. In an environment containing 1 % sucrose, the sucrose is transported across the membrane by the EIIC domain of ScrA and then phosphorylated intracellularly by the EIIB domain of ScrA, which itself has been phosphorylated by Crr [25] (Fig. 8A). In an LB medium without sucrose, the phosphoryl groups of ScrA and Crr are unable to be transferred to sucrose. Consequently, the phosphorylated Crr activates AC to generate cAMP [24], thereby triggering the transcription of *mrk* operon through the CRP-cAMP complex [23] (Fig. 8B). As a result, sucrose deficiency or *scrA* deletion leads to the sustained phosphorylation of Crr in LB, leading to increased *mrkA* RNA transcripts (Fig. S3A) and subsequent biofilm formation (Fig. 1). In conclusion, sucrose does not directly decrease biofilm formation by *K. pneumoniae*; rather, the production of type 3 fimbriae is activated in an environment without sucrose, thereby increasing biofilm formation by *K. pneumoniae*.

In addition to ScrA (sucrose-specific EIIBC), Crr also serves as the cognate EIIA of PtsG, which is the glucose-specific EIIBC in Enterobacteriaceae [24]. As a result, the composition of LB and NGM does not contain glucose, allowing us to observe the effect of sucrose on the biofilm of *K. pneumoniae* STU1 in this study. Furthermore, in a previous study, it was observed that 1 % glucose inhibited the production of type 3 fimbriae and biofilm formation in *K. pneumoniae* STU1 and 49 *K. pneumoniae* clinical isolates [29]. Therefore, it is speculated that the roles of Crr in a sucrose-poor environment (Fig. 8A) and in a glucose-poor environment are similar, both promoting the synthesis of type 3 fimbriae.

K. pneumoniae has been reported to be present in 9–21 % of food samples, including raw food, ready-to-eat food, street foods, and drinks [36,37]. Sucrose is common in food and can be degraded by sucrase to produce glucose and fructose in the intestine [38]. It can be speculated that *K. pneumoniae* decreases the production of type 3 fimbriae and biofilm formation in sucrose-rich or glucose-rich environments in the intestine. Although the benefits to *K. pneumoniae* are not clear when the biofilm is decreased in the presence of sucrose (or glucose) [29], we speculate that this behavior can promote the subpopulation of bacteria to move from the old niche to a new one in a rich environment. For *K. pneumoniae*, reducing biofilm in a rich environment also helps avoid competition for limited space with other bacteria.

CRediT authorship contribution statement

Yu-Tze Horng: Writing – review & editing, Writing – original draft, Visualization, Data curation, Conceptualization. **Chih-Ching Chien:** Resources. **Novaria Sari Dewi Panjaitan:** Writing – review & editing, Methodology, Investigation. **Shih-Wen Tseng:** Methodology, Investigation. **Hsueh-Wen Chen:** Methodology, Investigation. **Hung-Chi Yang:** Resources. **Yih-Yuan Chen:** Resources. **Po-Chi Soo:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Data availability

All data generated or analyzed in this study are included in the published article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biofilm.2025.100269.

References

[1] Bavyko O, Bondarchuk M. Trends in the development of the world food market. 2019.
[2] Rezaei T, Kamounah FS, Khodadadi E, Mehramouz B, Gholizadeh P, Yousefi L, Ganbarov K, Ghotaslou R, Yousefi M, Asgharzadeh M, Eslami H, Taghizadeh S, Pirzadeh T, Kafil HS. Comparing proteome changes involved in biofilm formation by *Streptococcus mutans* after exposure to sucrose and starch. Biotechnol Appl Biochem 2023;70:1320–31.
[3] Waldman LJ, Butera T, Boyd JD, Grady ME. Sucrose-mediated formation and adhesion strength of *Streptococcus mutans* biofilms on titanium. Biofilm 2023;6:100143.
[4] Jeong GJ, Khan F, Tabassum N, Kim YM. Alteration of oral microbial biofilms by sweeteners. Biofilm 2024;7:100171.
[5] Anderson AC, Rothballer M, Altenburger MJ, Woelber JP, Karygianni L, Lagkouvardos I, Hellwig E, Al-Ahmad A. In-vivo shift of the microbiota in oral biofilm in response to frequent sucrose consumption. Sci Rep 2018;8:14202.
[6] Kim MA, Rosa V, Min KS. Characterization of *Enterococcus faecalis* in different culture conditions. Sci Rep 2020;10:21867.
[7] Singh AK, Prakash P, Achra A, Singh GP, Das A, Singh RK. Standardization and classification of in vitro biofilm formation by clinical isolates of *Staphylococcus aureus*. J Global Infect Dis 2017;9:93–101.
[8] Yeom J, Lee Y, Park W. Effects of non-ionic solute stresses on biofilm formation and lipopolysaccharide production in *Escherichia coli* O157:H7. Res Microbiol 2012;163:258–67.
[9] Ren GX, Fan S, Guo XP, Chen S, Sun YC. Differential regulation of c-di-GMP metabolic enzymes by environmental signals modulates biofilm formation in *Yersinia pestis*. Front Microbiol 2016;7:821.
[10] Podschun R, Pietsch S, Holler C, Ullmann U. Incidence of *Klebsiella* species in surface waters and their expression of virulence factors. Appl Environ Microbiol 2001;67:3325–7.
[11] Zhang H, Zhang G, Yang Y, Zhang J, Li D, Duan S, Yang Q, Xu Y. Antimicrobial resistance comparison of *Klebsiella pneumoniae* pathogens isolated from intra-abdominal and urinary tract infections in different organs, hospital departments and regions of China between 2014 and 2017. J Microbiol Immunol Infect 2021;54:639–48.
[12] Zhang T, Huang X, Xu T, Li S, Cui M. Pyogenic liver abscess caused by extended-spectrum beta-lactamase-producing hypervirulent *Klebsiella pneumoniae* diagnosed by third-generation sequencing: a case report and literature review. J Int Med Res 2023;51:3000605231206296.
[13] Parthasarathi K, Ranganathan LS, Anandi V, Zeyer J. Diversity of microflora in the gut and casts of tropical composting earthworms reared on different substrates. J Environ Biol 2007;28:87–97.
[14] Stenkat J, Krautwald-Junghanns ME, Schmitz Ornes A, Eilers A, Schmidt V. Aerobic cloacal and pharyngeal bacterial flora in six species of free-living birds. J Appl Microbiol 2014;117:1564–71.
[15] Li Y, Ni M. Regulation of biofilm formation in *Klebsiella pneumoniae*. Front Microbiol 2023;14:1238482.
[16] Percival SL, Suleman L, Vuotto C, Donelli G. Healthcare-associated infections, medical devices and biofilms: risk, tolerance and control. J Med Microbiol 2015;64:323–34.
[17] Miller T, Lange D, Kizhakkedathu JN, Yu K, Felix D, Samejima S, Shackleton C, Malik RN, Sachdeva R, Walter M, Krassioukov AV. The microbiological burden of short-term catheter reuse in individuals with spinal cord injury: a prospective study. Biomedicines 2023;11.
[18] Clegg S, Murphy CN. Epidemiology and virulence of *Klebsiella pneumoniae*. Microbiol Spectr 2016;4.
[19] Ong CL, Beatson SA, Totsika M, Forestier C, McEwan AG, Schembri MA. Molecular analysis of type 3 fimbrial genes from Escherichia coli, *Klebsiella* and *Citrobacter* species. BMC Microbiol 2010;10:183.
[20] Schroll C, Barken KB, Krogfelt KA, Struve C. Role of type 1 and type 3 fimbriae in *Klebsiella pneumoniae* biofilm formation. BMC Microbiol 2010;10:179.
[21] Wilksch JJ, Yang J, Clements A, Gabbe JL, Short KR, Cao H, Cavaliere R, James CE, Whitchurch CB, Schembri MA, Chuah ML, Liang ZX, Wijburg OL, Jenney AW, Lithgow T, Strugnell RA. MrkH, a novel c-di-GMP-dependent transcriptional activator, controls *Klebsiella pneumoniae* biofilm formation by regulating type 3 fimbriae expression. PLoS Pathog 2011;7:e1002204.
[22] Schumacher MA, Zeng W. Structures of the activator of *K. pneumonia* biofilm formation, MrkH, indicates PilZ domains involved in c-di-GMP and DNA binding. Proc Natl Acad Sci USA 2016;113:10067–72.
[23] Panjaitan NSD, Horng YT, Cheng SW, Chung WT, Soo PC. EtcABC, a putative EII complex, regulates type 3 fimbriae via CRP-cAMP signaling in *Klebsiella pneumoniae*. Front Microbiol 2019;10:1558.
[24] Deutscher J, Francke C, Postma PW. How phosphotransferase system-related protein phosphorylation regulates carbohydrate metabolism in bacteria. Microbiol Mol Biol Rev 2006;70:939–1031.
[25] Deutscher J, Ake FM, Derkaoui M, Zebre AC, Cao TN, Bouraoui H, Kentache T, Mokhtari A, Milohanic E, Joyet P. The bacterial phosphoenolpyruvate: carbohydrate phosphotransferase system: regulation by protein phosphorylation and phosphorylation-dependent protein-protein interactions. Microbiol Mol Biol Rev 2014;78:231–56.
[26] Sprenger GA, Lengeler JW. Analysis of sucrose catabolism in *Klebsiella pneumoniae* and in Scr+ derivatives of *Escherichia coli* K12. J Gen Microbiol 1988;134:1635–44.
[27] Horng YT, Dewi Panjaitan NS, Chang HJ, Wei YH, Chien CC, Yang HC, Chang HY, Soo PC. A protein containing the DUF1471 domain regulates biofilm formation and capsule production in *Klebsiella pneumoniae*. J Microbiol Immunol Infect 2022;55:1246–54.
[28] Panjaitan NSD, Horng YT, Chien CC, Yang HC, You RI, Soo PC. The PTS components in *Klebsiella pneumoniae* affect bacterial capsular polysaccharide production and macrophage phagocytosis resistance. Microorganisms 2021;9.
[29] Horng YT, Panjaitan NSD, Tsai YJ, Su PW, Yang HC, Soo PC. The role of EII complex in the bacterial responses to the glucose-survey in clinical *Klebsiella pneumoniae* isolates. PLoS One 2023;18:e0289759.
[30] Ishizuka H, Hanamura A, Inada T, Aiba H. Mechanism of the down-regulation of cAMP receptor protein by glucose in *Escherichia coli*: role of autoregulation of the crp gene. EMBO J 1994;13:3077–82.
[31] Lin CT, Chen YC, Jinn TR, Wu CC, Hong YM, Wu WH. Role of the cAMP-dependent carbon catabolite repression in capsular polysaccharide biosynthesis in *Klebsiella pneumoniae*. PLoS One 2013;8:e54430.
[32] Peng D, Li X, Liu P, Zhou X, Luo M, Su K, Chen S, Zhang Z, He Q, Qiu J, Li Y. Transcriptional regulation of galF by RcsAB affects capsular polysaccharide formation in *Klebsiella pneumoniae* NTUH-K2044. Microbiol Res 2018;216:70–8.
[33] Bouffartigues E, Duchesne R, Bazire A, Simon M, Maillot O, Dufour A, Feuilloley M, Orange N, Chevalier S. Sucrose favors *Pseudomonas aeruginosa* pellicle production through the extracytoplasmic function sigma factor SigX. FEMS Microbiol Lett 2014;356:193–200.
[34] Ebner R, Lengeler JW. DNA sequence of the gene *scrA* encoding the sucrose transport protein Enzymell(Scr) of the phosphotransferase system from enteric bacteria: homology of the Enzymell(Scr) and Enzymell(Bgl) proteins. Mol Microbiol 1988;2:9–17.
[35] Green J, Stapleton MR, Smith LJ, Artymiuk PJ, Kahramanoglou C, Hunt DM, Buxton RS. Cyclic-AMP and bacterial cyclic-AMP receptor proteins revisited: adaptation for different ecological niches. Curr Opin Microbiol 2014;18:1–7.
[36] Hartantyo SHP, Chau ML, Koh TH, Yap M, Yi T, Cao DYH, GutiÉrrez RA, Ng LC. Foodborne *Klebsiella pneumoniae*: virulence potential, antibiotic resistance, and risks to food safety. J Food Protect 2020;83:1096–103.
[37] Budiarso T, Amarantini C, Pakpahan S. Biochemical identification and molecular characterization of *Klebsiella pneumoniae* isolated from street foods and drinks in Yogyakarta, Indonesia using 16S rRNA gene. Biodiv J Biol Div 2021;22.
[38] Neyrinck AM, Pachikian B, Taminiau B, Daube G, Frédérick R, Cani PD, Bindels LB, Delzenne NM. Intestinal sucrase as a novel target contributing to the regulation of glycemia by prebiotics. PLoS One 2016;11:e0160488.