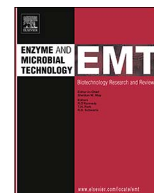
Enzyme and Microbial Technology 108 (2018) 21–25

Contents lists available at ScienceDirect

Enzyme and Microbial Technology

journal homepage: www.elsevier.com/locate/enzmictec



Short Communication

Displaying a recombinant protein on flocs self-produced by *Escherichia coli* through fused expression with elongation factor Ts

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ARTICLE INFO

Keywords:
Floc engineering
Escherichia coli
Protein display
tsf

ABSTRACT

The utility of engineering flocculation is wildly recognized in applied and environmental microbiology. We previously reported self-produced flocculation of *Escherichia coli* cells by overexpressing the native *bcsB* gene that encodes a component of the cellulose synthesis pathway. Further experiments clarified that the spontaneous *E. coli* flocs were proteinous, and elongation factor Ts (Tsf) was the main component. In this study, we demonstrated successful expression of a fusion protein consisting of Tsf and green fluorescence protein (GFP) on *E. coli* flocs. Interestingly, the percentage of Tsf-GFP in total floc protein reached approximately 15% (w/w). The proposed design of a fusion protein with Tsf enables displaying a recombinant target protein on the floc structure.

1. Introduction

Flocculation is an aggregation phenomenon of bacterial cells in the form of flocs or flakes. Numerous microorganisms have been found to show floc-forming capabilities [1] that can be applied to wastewater treatment. In activated sludge, the components of flocs typically include polysaccharides, polynucleotides, and proteins [2].

Escherichia coli is a typical laboratory microbe that lacks the floc-forming ability. Many researches have attempted to induce floc formation of *E. coli* by adding artificial flocculants consisting of inorganic compounds including aluminum and cationic polymers such as chitosan [3–5]. We have previously reported self-produced flocculation of *E. coli* cells by overexpressing the native *bcsB* gene [6] that encodes a component of transmembrane cellulose synthase [7]. Further experiments clarified the spontaneous *E. coli* flocs were proteinous, and elongation factor Ts (Tsf) had the highest score among floc proteins in nano-liquid chromatography-tandem mass spectrometry (LC–MS/MS) analysis [8]. Tsf is known to promote the release of GDP by forming an intermediate complex with another elongation factor, Tu, which is involved in the elongation cycle of protein biosynthesis [9]. These results promoted us to evaluate Tsf for displaying a heterologous protein on the proteinous structure of self-produced *E. coli* flocs.

In this study, we designed a fusion protein consisting of Tsf and green fluorescence protein (GFP), which was expressed during the process of *E. coli* floc formation. Observations of GFP revealed localization of Tsf protein within the floc structure. Furthermore, quantitative evaluation based on western blotting was conducted to determine the amount of fusion protein throughout the floc structure.

2. Materials and methods

2.1. Bacterial strains and media

The bacterial strains and plasmids used in this study are listed in Table 1. *E. coli* K-12 strain BW25113 was obtained from the National BioResource Project (National Institute of Genetics (NIG), Mishima, Japan) [10]. The pNTR-SD-*bcsB* plasmid [11] was provided by NIG and the transformant was named BW25113/*bcsB* that forms spontaneous flocs [8]. ASKA-*tsf-gfp* and pCA24N plasmids were also provided by NIG [12]. To express GFP, the pCA24N-*gfp* plasmid was constructed from pCA24N by adjusting the *gfp* gene in frame [12]. BW25113/*bcsB* cells were transformed with pCA24N-*gfp* and ASKA-*tsf-gfp* plasmids and the resultant strains were named BW25113/*bcsB*/*gfp* and BW25113/*bcsB*/*tsf-gfp*, respectively.

E. coli cells were cultured in lysogeny broth (LB) medium (10 g/L Hipolypeptone, 5 g/L Bacto-yeast extract and 10 g/L NaCl). The culture medium for strains harboring the plasmids contained with 50 mg/L ampicillin or 25 mg/L chloramphenicol. All test cultures were precultured in LB medium for 14 h at 37 °C and then inoculated into L-tubes containing fresh LB medium with 2 g/L glucose (LB-G) giving an optical density of 660 nm (OD_{660}) = 0.01.

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http://dx.doi.org/10.1016/j.enzmictec.2017.08.010
Received 25 April 2017; Received in revised form 23 August 2017; Accepted 24 August 2017
Available online 01 September 2017
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Table 1
E. coli strains and plasmids used in this study.

Strains or plamisds	Note	Reference
Strains		
BW25113	Host strain of Keio collection	[10]
Plasmids		
pNTR-SD-*bcsB*	pNTR-SD carrying *bcsB* under P_{lac} control, Amp^r	[11]
pCA24N	Cm^r	[12]
pCA24N-*gfp*	pCA24N carrying *gfp* under $P_{T5\text{-}lac}$ control, Cm^r	This study
ASKA-*tsf-gfp*	pCA24N carrying *tsf* and *gfp* under $P_{T5\text{-}lac}$ control, Cm^r	[12]

2.2. Flocculation of each E. coli strain

For floc formation, *E. coli* cells were cultured in 10 mL LB-G medium at 37 °C in an L-tube with shaking at 45 strokes/min. The recombinant strains were cultured in medium containing isopropyl β-D-1-thiogalactopyranoside (1 mM), ampicillin (50 mg/L) and chloramphenicol (25 mg/L). After 24 h of culture, the L-tubes containing the floc and cell suspension were left to stand at room temperature for 15 min and the resultant precipitates were resuspended in a 0.9% (w/v) NaCl solution. The resultant flocs were transferred into glass bottles and photographed using a digital camera under irradiation by fluorescent light or black light (peak wavelength; 365 nm) lamp to confirm GFP expression. For quantitative assays, the resultant flocs were washed four times with the 0.9% (w/v) NaCl solution to remove loosely adsorbed cells on the flocs. The amount of protein in the flocs was measured by a Pierce™ BCA protein Assay Kit (Thermo Fisher Scientific Inc., UK) and the value was used as an index of floc amount as described previously [13]. The number of *E. coli* cells included in the floc structure was estimated by measuring ATP as described elsewhere [13]. ATP measurement was conducted using an ATP Colorimetric/Fluorometric Assay Kit (BioVision Inc., Milpitas, CA) according to the manufacturer's procedure.

2.3. Microscopic observation of flocs

Flocs were observed under an inverted Eclipse Ti microscope with NIS-Elements Advanced Research software (Nikon Corp., Japan). Prior to analysis, the flocs were fixed with 2.5% (v/v) glutaraldehyde for 1 h. After washing, DNA of *E. coli* cells in the floc structure was stained with 20 mg/L propidium iodide (PI) by incubation for 30 min in the dark. The samples were observed under sequential excitation at wavelengths of 488 and 561 nm.

2.4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and western blotting analyses of flocs

A floc sample (5 μL) was subjected to SDS-PAGE. For western blotting, proteins were transferred from the gel to a membrane sheet of Hybond P (GE Healthcare Ltd., UK) using the semi-dry transfer method. Hybridization was conducted using an anti-GFP primary antibody (Medical & Biological Laboratories Co., Japan) and an ECL Western Blotting Starter Kit (GE Healthcare Ltd.) according to the manufacturers' protocols. Hybridization signals were detected using a ChemiDoc imaging system (Bio-Rad Laboratories Inc., Hercules, CA).

The protein bands were analyzed by densitometry (Image J software; NIH, Bethesda, MD) as an index of target protein expression. The amounts of GFP and Tsf-GFP were determined using purified GFP and Tsf-GFP as standards. Purification of GFP and Tsf-GFP was conducted using a Bio-scale Mini Profinity IMAC cartridge and Profinia protein purification system (Bio-Rad Laboratories Inc.) after ultrasonication of *E. coli* cells expressing each protein. Finally, the percentages of GFP and Tsf-GFP were determined based on total floc protein determined by the BCA method.

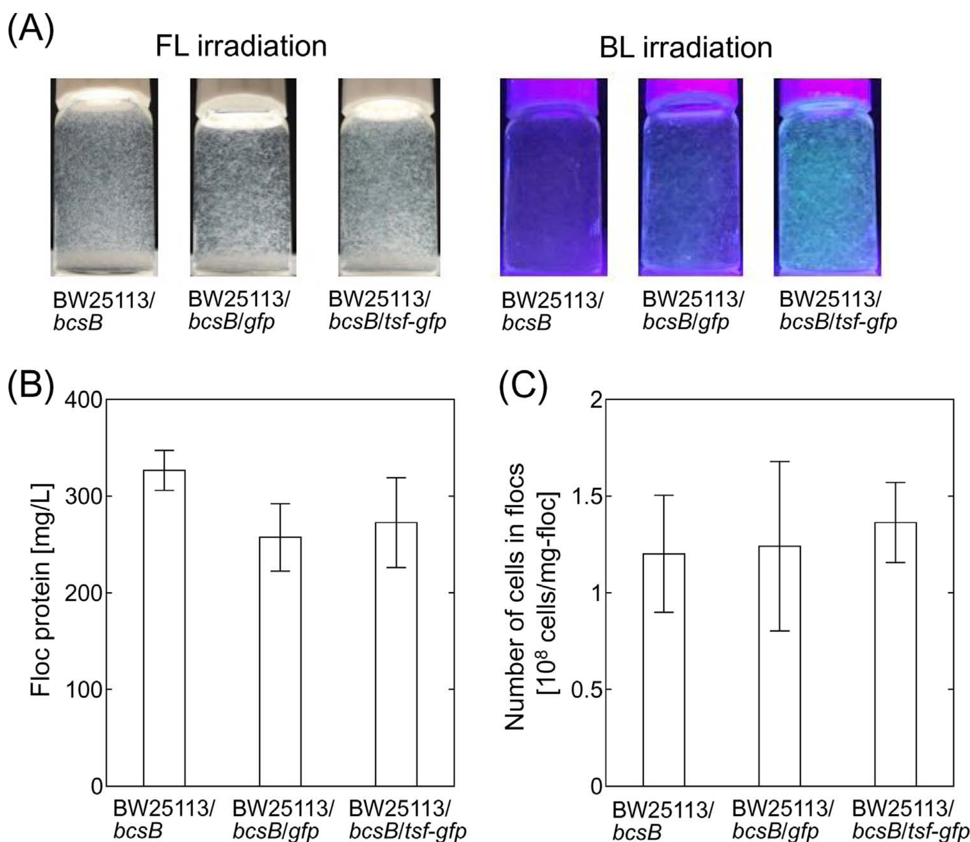

Fig. 1. Floc formation of *E. coli* BW25113/*bcsB*, BW25113/*bcsB/gfp* and BW25113/*bcsB/tsf-gfp* strains. (A) Image of *E. coli* floc suspensions irradiated under fluorescent light (FL) or black light (BL) lamp. The flocs were harvested at 24 h of culture. (B) Floc protein concentration of each *E. coli* strain. (C) Cell number of each *E. coli* strain included in flocs. In graphs (B) and (C), data were obtained from more than three independent experiments. Vertical bars indicate standard deviation.

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