Curcumin inhibits FtsZ assembly: an attractive mechanism for its antibacterial activity

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The assembly and stability of FtsZ protofilaments have been shown to play critical roles in bacterial cytokinesis. Recent evidence suggests that FtsZ may be considered as an important antibacterial drug target. Curcumin, a dietary polyphenolic compound, has been shown to have a potent antibacterial activity against a number of pathogenic bacteria including *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Enterococcus*. We found that curcumin induced filamentation in the *Bacillus subtilis* 168, suggesting that it inhibits bacterial cytokinesis. Further, curcumin strongly inhibited the formation of the cytokinetic Z-ring in *B. subtilis* 168 without detectably affecting the segregation and organization of the nucleoids. Since the assembly dynamics of FtsZ protofilaments plays a major role in the formation and functioning of the Z-ring, we analysed the effects of curcumin on the assembly of FtsZ protofilaments. Curcumin inhibited the assembly of FtsZ protofilaments and also increased the GTPase activity of FtsZ. Electron microscopic analysis showed that curcumin reduced the bundling of FtsZ protofilaments *in vitro*. Further, curcumin was found to bind to FtsZ in *in vitro* with a dissociation constant of 7.3 ± 1.8 µM and the agent also perturbed the secondary structure of FtsZ. The results indicate that the perturbation of the GTPase activity of FtsZ assembly is lethal to bacteria and suggest that curcumin inhibits bacterial cell proliferation by inhibiting the assembly dynamics of FtsZ in the Z-ring.

Key words: antibacterial activity, curcumin, filamentation, FtsZ polymerization, GTPase activity, Z-ring.

INTRODUCTION

FtsZ, a prokaryotic homologue of eukaryotic cytoskeletal protein tubulin, polymerizes to form a Z-ring at the mid cell that orchestrates bacterial cell division [1–5]. FtsZ is shown to be essential for bacterial cell division and viability (reviewed in [5,6]). Previous studies have shown that the perturbation of FtsZ functions by natural compounds and chemical agents leads to inhibition of bacterial proliferation [7–12]. Most recently, totarol, a naturally occurring diterpenoid phenol, has been found to inhibit bacterial cytokinesis by perturbing Z-ring formation [13]. Totarol has been shown to inhibit the assembly and GTPase activity of *Mycobacterium tuberculosis* FtsZ, *in vitro*. Several studies have suggested that inhibitors of FtsZ assembly may be developed into antibacterial drugs, including drugs against the multidrug-resistant strains of pathogenic bacteria [8,10,13,14].

Curcumin, a naturally occurring polyphenolic compound, is extracted from the rhizomes of *Curcuma longa* (Figure 1). It has been used as an important dietary component for a long time. It exhibits various biological activities including anti-proliferative activity against various cancer cells, antioxidant activity, wound healing ability and antimicrobial activity [15–19]. Recently, it has been found that curcumin binds to tubulin and perturbs microtubule polymerization [19]. Curcumin possesses antibacterial property against a number of Gram-positive and Gram-negative bacteria [17]. It has been shown to kill several pathogenic Gram-positive bacteria such as *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Enterococcus* that cause infections such as skin diseases, pneumonia, meningitis and urinary tract infections in human beings. In addition, curcumin in combination with lactoferrin, N-acetylcysteine and pantoprazole has recently been shown to significantly reduce the symptoms caused by *Helicobacter pylori* infection in humans [20].

Further, curcumin is shown to have a potent phototoxic effect against several bacteria such as *Salmonella* serotype Typhimurium and *Escherichia coli* [21,22]. Curcumin also displays antigenotoxic activity against DNA-damaging agents such as mutagens, UV rays, γ-radiation etc. [23–26]. Curcumin inhibits SOS induction in *Salmonella* serotype Typhimurium and *E. coli* [24]. The DMSO extracts of turmeric are shown to protect DNA in *E. coli* cells against deleterious effects of X-rays [27].

Although curcumin is known for its antimicrobial and anticancer activity for a long time, its therapeutic potential is severely limited because of its poor bioavailability. Understanding the mechanism of the antibacterial activity of curcumin will greatly assist in designing potent analogues of curcumin, a natural remedy for several bacterial diseases. In the present study, curcumin has been found to induce filamentation in the *B. subtilis* 168, suggesting that it inhibits bacterial cytokinesis. FtsZ plays an important role in orchestrating bacterial cytokinesis. Curcumin binds to tubulin and inhibits microtubule polymerization [19]. Since FtsZ is the prokaryotic homologue of tubulin, we sought to determine the effects of curcumin on the assembly dynamics of FtsZ. We found that curcumin increased the GTPase activity of purified FtsZ and inhibited the assembly and bundling of FtsZ protofilaments *in vitro*. To the best of our knowledge, curcumin is the only inhibitor of FtsZ assembly found so far that increases the GTPase activity of FtsZ. We provide evidence suggesting that curcumin perturbs the Z-ring formation and inhibits bacterial cytokinesis by inhibiting FtsZ assembly.
concentration) of curcumin for against curcumin concentration. MIC (minimum inhibitory mined by plotting the percentage inhibition of cell proliferation and presence of different concentrations of curcumin. Appropriate mined by counting bacterial colonies as described earlier [13].

E. coli FtsZ was purchased from Bangalore Genei. All other chemicals used were of analytical grade. B. subtilis 168 (trpC2), E. coli K12 MG1655 and E. coli BL21 (DE3) are laboratory collection strains.

Methods
Curcumin stocks were prepared in DMSO and then diluted in aqueous buffer solution keeping the final concentration of DMSO below 1% (v/v) in all reaction milieu. The stability of curcumin in aqueous buffers changes with various pH conditions. Curcumin is highly unstable at neutral–basic pH conditions [28,29]. It has been found that curcumin is quite stable at pH 6.5. Therefore all in vitro experiments were performed at pH 6.5. It is also reported that ascorbic acid helps to maintain curcumin stability [30]; therefore we incubated the bacterial culture with curcumin supplemented with 1 mM ascorbic acid.

Determination of antibacterial activity of curcumin
The bacteria (B. subtilis 168, E. coli BL21 and E. coli K12 MG1655) were inoculated in LB (Luria–Bertani) broth (10 g/l casein enzymic hydrolysate, 5 g/l yeast extract and 5 g/l sodium chloride) containing 1 mM ascorbic acid without or with different concentrations of curcumin. The inhibition of bacterial growth was determined by monitoring by monitoring $D_{600}$, the attenuation at 600 nm, at different time (0, 30, 60, 90, 120, 150, 180, 240 and 300 min) intervals in the presence of different concentrations (20–120 µM) of curcumin. The $D_{600}$ of respective blanks having only curcumin was subtracted to give the final $D_{600}$. The percentage inhibition of bacterial growth was calculated by using the equation:

$$\text{% Inhibition} = \left[1 - \left(\frac{X_{D_{600}}}{C_{D_{600}}}\right)\right] \times 100$$

where $X_{D_{600}}$ represents the final $D_{600}$ value of $X$ µM curcumin-treated culture at a particular time, $C_{D_{600}}$ represents the $D_{600}$ of the control culture at the same time. The percentage inhibition of bacterial growth was determined 4 h after the addition of curcumin to the bacterial culture. The $IC_{50}$ of curcumin was determined by plotting the percentage inhibition of cell proliferation against curcumin concentration. MIC (minimum inhibitory concentration) of curcumin for B. subtilis 168 cells was determined by counting bacterial colonies as described earlier [13]. Briefly, B. subtilis 168 cells were grown for 4 h in the absence and presence of different concentrations of curcumin. Appropriate dilutions of bacterial culture were spread on to the LB agar plates and incubated for an additional 12 h at 37°C. The number of colony-forming units was calculated by counting the colonies on each plate. The concentration of curcumin at which no colony was detected in plates was considered as the MIC. The experiment was performed three times.

Visualization of the morphology of bacteria
B. subtilis 168 was grown in the absence or presence of different concentrations of curcumin for 4 h in LB broth supplemented with 1 mM ascorbic acid. The cells were fixed in 2.8% formaldehyde and 0.04% glutaraldehyde at 25°C. The bacterial cells were finally collected, washed and resuspended in PBS (pH 7.4). The cell morphology was examined under a DIC (differential interference contrast) microscope (Nikon ECLIPSE TE2000-U) at x40 magnification. The images were captured using a Cool SNAP-Pro camera. The cell length was measured using Image-Pro Plus software (Media Cybernetics, Silver Spring, MD, U.S.A.).

Immunofluorescence microscopy
The immunostaining of the Z-ring was performed using FtsZ antibody as described earlier [11]. Briefly, the B. subtilis 168 cells were grown for 4 h in LB broth containing 1 mM ascorbic acid without or with different concentrations of curcumin (25 and 50 µM). The cells were fixed in 2.8% formaldehyde and 0.04% glutaraldehyde for 45 min at 25°C. The cells were permeabilized and then incubated with a polyclonal FtsZ antibody developed in rabbit against E. coli FtsZ and Cy3 (indocarbocyanine)-conjugated goat anti-rabbit secondary antibody. The nucleoids were stained with 1 µg/ml DAPI. The cells were observed under a fluorescence microscope (Nikon ECLIPSE TE2000-U) at x40 magnification. The images were analysed using Image-Pro Plus software.

Purification of FtsZ
Recombinant E. coli FtsZ was overexpressed and purified from E. coli BL21 strain as described previously [31]. The purity of the protein was analysed by SDS/10% PAGE and found to be >98%. The concentration of purified FtsZ was determined by the method of Bradford [32] using BSA as a standard. The protein was aliquoted and stored at −80°C.

Light scattering assay
FtsZ (12.5 µM) was incubated without or with different concentrations (10–40 µM) of curcumin in 25 mM Pipes buffer (pH 6.5) at 25°C for 10 min. Then, 50 mM KCl, 10 mM MgCl2 and 1 mM GTP were added to the reaction mixture. The kinetics of the assembly of FtsZ was monitored by 90° light scattering (550 nm) at 37°C using a JASCO 6500 spectrophotometer equipped with a constant temperature water circulating bath. A 0.3 cm path length cuvette was used for all the measurements. The appropriate blank was subtracted from all experimental data.

Electron microscopic analysis
FtsZ (12 µM) was incubated in 25 mM Pipes buffer (pH 6.5) without or with different concentrations (10–50 µM) of curcumin at 25°C for 10 min. Then, 50 mM KCl, 5 mM MgCl2 and 1 mM GTP were added to reaction mixtures and polymerization was carried out at 37°C. After 15 min of polymerization, the protein polymers were transferred to Formvar-carbon-coated copper grids (300 meshes) for 45 s. The sample-containing grids were stained with 1% uranyl acetate for 35 s, air-dried and observed.

Figure 1 Structure of curcumin [1,7-bis-(4-hydroxy-3-methoxy-phenyl)hepta-1,6-diene-3,5-dione]
under a transmission electron microscope (FEI TECNAI G² 12) [31].

Measurement of GTPase activity of FtsZ

The amount of P<sub>i</sub> released during the assembly of FtsZ was measured using a standard Malachite Green/ammonium molybdate assay as described earlier [33–35]. Briefly, FtsZ (12 µM) was incubated without or with different concentrations (5, 10, 20 and 30 µM) of curcumin in 25 mM Pipes buffer (pH 6.5) containing 50 mM KCl and 5 mM MgCl<sub>2</sub> on ice for 10 min. Then, 1 mM GTP was added to the reaction mixtures and incubated at 37 °C to start the hydrolysis reaction. After 5 min of incubation, 40 µL samples were taken out from the reaction mixtures, and hydrolysis reaction was quenched by addition of 10% (v/v) 7 M HClO<sub>4</sub>. The samples were kept in ice for 2 min and then all the samples were incubated at 25 °C for 10 min. A 900 µL portion of freshly prepared filtered Malachite Green/ammonium molybdate solution was added to the samples, and incubated at 25 °C for 30 min in the dark. The P<sub>i</sub> release was determined by measuring the absorbance at 650 nm. The background A<sub>0</sub> was subtracted from all the readings. A phosphate standard curve was prepared using sodium phosphate. All the solutions were prepared in deionized water.

Measurement of the dissociation constant of curcumin–FtsZ interaction

The increase in curcumin fluorescence upon binding to FtsZ was used to determine the binding of curcumin to FtsZ [19]. FtsZ (1 µM) was incubated with different concentrations of curcumin (1, 2, 3, 4, 5, 7 and 10 µM) in 25 mM Pipes buffer (pH 6.5) at 25 °C for 30 min in the dark. The fluorescence spectra of the samples were recorded using a λ<sub>ex</sub> of 425 nm. The fluorescence intensities at 495 nm were used for determining the binding constant. The dissociation constant (K<sub>d</sub>) of curcumin–FtsZ interaction was calculated by plotting L<sub>bound</sub>/L<sub>free</sub> against L<sub>bound</sub>, where L<sub>bound</sub> represents the bound curcumin concentration and L<sub>free</sub> represents the free curcumin concentration [36]. The L<sub>bound</sub> was calculated from the equation

$$L_{\text{bound}} = F + C/\Delta F_{\text{max}}$$

where $\Delta F_{\text{max}}$ is the fluorescence intensity of 1 µM fully bound curcumin. $\Delta F_{\text{max}}$ was calculated by plotting 1/fluorescence intensity (495 nm) of curcumin against 1/[FtsZ]. For this, 1 µM curcumin was incubated without or with different concentrations (1, 2, 4, 6, 8 and 12 µM) of FtsZ in 25 mM Pipes buffer (pH 6.5) at 25 °C for 30 min in the dark. $\Delta F_{\text{max}}$ was calculated by subtracting F<sub>0</sub> from F<sub>c</sub>, where F is the fluorescence intensity of the FtsZ–curcumin complex at a particular curcumin concentration and F<sub>0</sub> is the fluorescence intensity of only curcumin in the absence of FtsZ.

The fluorescence intensities were corrected for the inner filter effects using the following equation: $F_{c} = \Delta F \times \text{antilog}[\text{a} \times \text{log}(A_{\text{ex}} + A_{\text{em}})/2]$. $F_{c}$ represents the corrected fluorescence intensity.

Analysis of secondary structural changes

FtsZ (4 µM) was incubated without or with different concentrations of curcumin (20, 30 and 50 µM) in 25 mM phosphate buffer (pH 6.5) for 30 min at 25 °C. The far-UV CD spectrum was monitored over a wavelength range of 200–250 nm using a JASCO J-810 spectropolarimeter equipped with a Peltier temperature controller using a 0.1 cm path length quartz cuvette. An average of five scans was taken for each spectrum. Deconvolution and statistical analysis of the CD spectra were performed using CDNN, SSE software from Jasco and OriginPro 7.5 software respectively.

RESULTS

Effect of curcumin on the proliferation of B. subtilis 168 and E. coli K12 MG1655 cells

Curcumin inhibited the growth of B. subtilis 168 in a concentration-dependent fashion (Figure 2A). The IC<sub>50</sub> was found to be 17 ± 3 µM for B. subtilis 168 (Figure 2B). The MIC of curcumin for B. subtilis 168 cells was determined to be 100 µM. Similarly, curcumin also inhibited the growth of E. coli K12 MG1655 (see Supplementary Figure 1A at http://www.BiochemJ.org/bj/410/bj4100147add.htm). The IC<sub>50</sub> for E. coli K12 MG1655 was calculated to be 58 ± 5 µM, and 100 µM curcumin inhibited the growth of E. coli K12 MG1655 cells by 80% (Supplementary Figure 1B). The IC<sub>50</sub> for E. coli BL21 was found to be 56 ± 8 µM (results not shown).

Effect of curcumin on the morphology of B. subtilis 168

Curcumin induced filamentation in B. subtilis 168 cells (see Supplementary Figure 2 at http://www.BiochemJ.org/bj/410/bj4100147add.htm). The average length of B. subtilis 168 was determined to be 3.3 ± 1 µm. The average cell length increased by 2.5-fold from 3.3 ± 1 to 8.6 ± 5.3 µm after 4 h of incubation with 25 µM curcumin. Also, the cell growth was inhibited by 81 ± 11% in the presence of 25 µM curcumin (Figure 2B). In the presence of 50 µM curcumin, the average cell length of B. subtilis 168 was increased by 6-fold from 3.3 ± 1 to 20 ± 16 µm (Figure 2C) and the growth was inhibited by 90 ± 9% (Figure 2B).

Most of the curcumin-treated cells had strikingly larger cell length than the control (vehicle-treated) cells (Figure 2C). In the case of control, 3.3, 66 and 1% of the cells had mean length in the range of 1–3, 3–5 and 5–7 µm respectively (Figure 2C). In the presence of 50 µM curcumin, 12, 4, 8 and 76% of the cells had mean length in the range of 3–5, 5–7, 7–9 and > 9 µm respectively. The results together indicate that curcumin inhibits bacterial proliferation by inhibiting cytokinesis. Further, curcumin did not perturb the membrane structure of B. subtilis 168.

Effect of curcumin on Z-ring formation and nucleoid segregation in B. subtilis 168

B. subtilis 168 cells formed large filaments in the presence of curcumin. Therefore we sought to find the effects of curcumin on the nucleoids and as well as on the Z-ring in B. subtilis 168. In the absence of curcumin, most of the B. subtilis 168 cells had a well-defined Z-ring in between the two nucleoids (Figure 3). Curcumin strongly inhibited the formation of Z-rings (Figures 3D, 3F, 3G and 3I). For example, 63% of the control cells had well-defined Z-rings (Figures 3A and 3C), whereas only 20 and 7% of the curcumin-treated cells had Z-rings in the presence of 25 and 50 µM curcumin respectively (Table 1). Further, the frequency of occurrence of Z-rings/µm of the bacterial cell length was reduced strongly. For instance, the frequency of occurrence of the Z-ring/µm of the bacterial cell length was found to be 0.18 ± 0.15, 0.03 ± 0.07 and 0.01 ± 0.03 in the absence and presence of 25 and 50 µM curcumin respectively.

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Curcumin increased the number of nucleoids per cell, but the frequency of occurrence of nucleoids/µm of cell length was similar to that of the control cells. For example, the percentage of control cells having 1, 2 and 4 nucleoids per cell was 21, 75 and 4% respectively. In the case of 25 and 50 µM curcumin-treated cells, the number of cells with 1 or 2 nucleoids reduced considerably and the number of cells with 4 or 8 or more nucleoids increased significantly. For example, 5, 37, 33 and 25% of the 25 µM curcumin-treated cells had 1, 2, 4 and 8 or more nucleoids respectively. Further, in the case of 50 µM curcumin-treated cells, the percentage of cells with 1, 2, 4 and 8 or more nucleoids was found to be 4, 14, 18 and 64% respectively. The frequency of occurrence of nucleoids/µm of cell length was found to be similar without or with curcumin. For example, the frequency of occurrence of nucleoids/µm of cell length was determined to be 0.58 ± 0.19, 0.52 ± 0.16 and 0.53 ± 0.11 in the absence and presence of 25 and 50 µM curcumin respectively. The results showed that curcumin had no detectable effect on the nucleoid segregation.

Out of the total control cells, 21% of cells had a single nucleoid with no Z-ring having a cell length of 2.5 ± 0.5 µm. This population might reflect the neonate cells. The percentage of cells with a single nucleoid decreased strongly in curcumin-treated cells, indicating that curcumin inhibited cell division. Further, the percentage of cells with two nucleoids reduced from 75 to 14% in the case of 50 µM curcumin. Although approx. 80% of the control cells containing two nucleoids had a Z-ring, only a small population (27%) of the cells with two nucleoids had a Z-ring in the presence of 25 µM curcumin. None of the 50 µM curcumin-treated cells with two nucleoids had a Z-ring, indicating that curcumin strongly inhibited the Z-ring formation. Taken together, the results showed that curcumin inhibited the formation of the Z-ring without affecting the nucleoid segregation detectably.

**Effect of curcumin on FtsZ assembly and bundling in vitro**

Since curcumin strongly induced filamentation in *B. subtilis* 168, and inhibited the formation of the Z-ring in the bacteria, we wanted to find out whether these effects are due to its interaction with FtsZ. Therefore we polymerized FtsZ (12.5 µM) in 25 mM Pipes buffer containing 50 mM KCl, 10 mM MgCl₂ and 1 mM GTP without or with different concentrations of curcumin *in vitro* (Figure 4). FtsZ monomers assembled at a much higher rate in the absence of curcumin compared with that of the control, indicating that curcumin inhibited the assembly of FtsZ (Figure 4). The IC₅₀ of FtsZ assembly occurred in the presence of 30 µM curcumin.

The effect of curcumin on the assembly of FtsZ was also analysed by transmission electron microscopy (Figure 5). The control showed thick bundles of FtsZ polymers with an average width of 71 ± 36 nm (Figure 5A). The size and thickness of FtsZ polymers reduced extensively with increasing concentrations...
Curcumin inhibits bacterial cytokinesis by inhibiting FtsZ assembly

Curcumin inhibited Z-ring formation without affecting nucleoid segregation in B. subtilis 168

Table 1 Effect of curcumin on the Z-ring and nucleoids of B. subtilis 168

Effect of curcumin on the GTPase activity of FtsZ

Effect of curcumin on the GTPase activity of FtsZ

GTP-bound FtsZ polymers are thought to be more stable than the GDP-bound polymers [2,4,6,37,38]. Under challenged GTP conditions, FtsZ polymers are expected to disassemble once all available GTP molecules are exhausted. Curcumin increased the
Curcumin reduced the rate and extent of FtsZ polymerization

FtsZ (12.5 µM) was polymerized in 25 mM Pipes buffer (pH 6.5) containing 50 mM KCl, 10 mM MgCl₂ and 1 mM GTP without (*) and with 10 µM (■), 20 µM (▲), 30 µM (△) and 40 µM (□) of curcumin as described in the Materials and methods section. Appropriate blanks were subtracted from all the traces. The experiment was performed four times.

GTP hydrolysis rate of FtsZ (Figure 6A). Therefore we argued that under the challenged GTP conditions, FtsZ protofilaments would disassemble at a higher rate in the presence of curcumin than in its absence. To examine this possibility, the effects of different concentrations of curcumin on the assembly of FtsZ (12.5 µM) were analysed in the presence of a limited amount (100 µM) of GTP. The light scattering signal of FtsZ assembly (control) decreased sharply after ∼300 s of assembly, indicating the disassembly of FtsZ protofilaments (Figure 6B). As expected, curcumin not only reduced the extent of FtsZ assembly but also strongly reduced the steady-state duration of the assembly (Figure 6B). Under the experimental conditions used, FtsZ protofilaments disassembled more quickly (within 200 s of assembly) in the presence of curcumin than in the absence of curcumin (Figure 6B). The results indicated that curcumin-induced increase in the GTP hydrolysis rate might be the cause of the destabilization of FtsZ protofilaments.

Curcumin binds to FtsZ in vitro

Curcumin (1 µM) was incubated in the absence and presence of different concentrations (1–12 µM) of FtsZ for 30 min. The fluorescence intensity of curcumin increased with increase in concentration of FtsZ, indicating that it binds to FtsZ (Figure 7A). The increase in the curcumin fluorescence at 495 nm upon binding to FtsZ was used to determine the binding of curcumin to FtsZ. FtsZ (1 µM) was incubated with different concentrations of curcumin for 30 min. The fluorescence intensity of the curcumin–FtsZ complex increased with increase in concentration of curcumin (Figure 7B). The dissociation constant (K_d) of the curcumin–FtsZ interaction was determined to be 7.3 ± 1.8 µM (Figure 7C). The number of binding sites of curcumin on FtsZ was calculated to be 0.7 ± 0.2 per FtsZ molecule.

Electron micrographs of FtsZ polymers

FtsZ (12 µM) was polymerized without or with different concentrations of curcumin at 37°C as described in the Materials and methods section. The FtsZ polymers were transferred to Formvar-carbon-coated copper grids (300 meshes), negatively stained with 1% uranyl acetate and observed under a transmission electron microscope (FEI TECNAI G² 12) at ×20500 magnification. Shown are the electron micrographs of FtsZ polymers in the absence (A) and presence of 10 µM (B), 25 µM (C) and 50 µM (D) curcumin. The aggregates are shown by arrows (B–D). The thickness of a minimum of 100 polymers was measured in all cases. Scale bar, 2 µm (A–D).
Curcumin inhibits bacterial cytokinesis by inhibiting FtsZ assembly

In the present study, we have found that curcumin induced filamentation in B. subtilis 168, showing that it inhibits bacterial cytokinesis. Curcumin was found to bind to FtsZ in vitro; it increased the GTPase activity of FtsZ and inhibited the assembly and bundling of FtsZ protofilaments. Curcumin had no detectable effect on the segregation and organization of nucleoids in B. subtilis 168, indicating that it does not inhibit bacterial division by targeting DNA. We present evidence suggesting that curcumin inhibited cytokinesis in B. subtilis 168 by inhibiting the Z-ring formation through a direct interaction with FtsZ.

The assembly dynamics of FtsZ is considered to be regulated by the GTPase activity of FtsZ [5,34,37–41]. Curcumin decreased the steady-state duration of FtsZ polymers (Figure 6B) and curcumin was also found to increase the GTPase activity of FtsZ (Figure 6A). GDP-bound FtsZ polymers are thought to be less stable than the GTP-bound polymers and they readily disassemble into FtsZ monomers [39,40]. In addition, GDP-FtsZ monomers do not assemble as effectively as GTP-FtsZ monomers [38]. The results indicated that curcumin decreases the stability of FtsZ protofilaments by increasing the GTP hydrolysis rate of FtsZ, which in turn inhibits FtsZ assembly. In addition, curcumin reduced the α-helix content, while it increased the random coil content of FtsZ, suggesting that the perturbation in the secondary structure of FtsZ might also be the cause of destabilization of FtsZ protofilaments. Curcumin is the first small molecule inhibitor of FtsZ assembly known to date that increases the GTPase activity of FtsZ. Most of the inhibitors of FtsZ assembly have been reported to suppress the rate of GTP hydrolysis [7,9,10,12,13]. For example, 100 μM SRI-3072 and SRI-7614 inhibited the GTPase activity by 20 and 25% respectively [7]. Similarly, PC58538 and its analogues inhibited the GTPase activity of FtsZ with an IC_{50} value of 136 μg/ml [12]. Zantrins inhibited GTP hydrolysis with IC_{50} values ranging from 4 to 100 μM [9]. Tarotrol suppressed the GTPase activity of M. tuberculosis FtsZ [13]. For example, 24% inhibition and 67% inhibition of GTP hydrolysis were found in the presence of 10 and 75 μM tarotrol respectively. Therefore curcumin perturbed the assembly dynamics of FtsZ by a mechanism that is different from the other known assembly inhibitors.

Curcumin decreased the light scattering intensity of FtsZ protofilaments, indicating that it reduced the polymerized mass of FtsZ protofilaments and/or decreased the bundling of FtsZ protofilaments (Figure 4). Electron microscopic analysis of the polymerization reaction showed that curcumin strongly reduced the length of FtsZ protofilaments and the extent of bundling of the protofilaments (Figure 5). The decrease in polymer length and bundling of FtsZ protofilaments may be due to the binding of bulky curcumin molecules on FtsZ. The presence of bulky curcumin in the protofilaments may cause steric hindrance that inhibits the assembly and bundling of FtsZ. Curcumin perturbed the secondary structures of FtsZ (Figure 8). The decrease in the polymerization and bundling of FtsZ may be due to the conformational changes induced by curcumin in the FtsZ monomers. The IC_{50} of FtsZ assembly occurred in the presence of 30 μM curcumin. Thus the inhibitory effects of curcumin on FtsZ polymerization were comparable with that of other known inhibitors of FtsZ assembly [7,9,11,13]. For example, the polymerization ID_{50} (infectious dose to 50% of exposed individuals) values of SRI-3072 and SRI-7614 are shown to be 52 ± 12 and 60 ± 0 μM respectively [7].

In the last few years, several natural and synthetic compounds were shown to inhibit bacterial growth by targeting the assembly dynamics of FtsZ [7–14]. For example, viriditoxin inhibited the
Figure 7  Binding of curcumin with FtsZ monitored by fluorescence spectroscopy

FtsZ (1 μM) was incubated with different concentrations of curcumin (1–10 μM) and the fluorescence spectra were monitored as described in the Materials and methods section. The \( \lambda_{ex} \) and \( \lambda_{em} \) values were 425 and 495 nm respectively. (A) The Figure shows an increase in the fluorescence intensity of FtsZ–curcumin complex in the absence (○) and presence of 1 μM (●), 2 μM (▲), 4 μM (●), 6 μM (●), 8 μM (●), and 12 μM (●) FtsZ at constant curcumin concentration (1 μM). (B) The Figure shows an increase in the fluorescence intensity of the FtsZ–curcumin complex in the absence (○) and presence of 1 μM (●), 2 μM (▲), 3 μM (●), 4 μM (●), 5 μM (●), 7 μM (●), and 10 μM (●) curcumin at constant FtsZ concentration (1 μM). (C) A plot of \( \frac{L_{bound}}{L_{free}} \) against \( L_{bound} \) of the binding of curcumin to FtsZ. The experiment was performed six times.

Figure 8  Effects of curcumin on the far-UV CD spectra of FtsZ

FtsZ (4 μM) was incubated without or with different concentrations (20–50 μM) curcumin at 25°C. The Figure shows the far-UV CD spectra of FtsZ in the absence (○) and presence of 20 μM (●), 30 μM (▲), and 50 μM (▲) curcumin. The experiment was repeated five times.

E. coli wild-type cell division with an MIC of >64 μg/ml and induced filamentation in the bacterial cell [8]. Zantrins are found to inhibit the Z-ring formation in E. coli wild-type followed by cell death with MIC values from 20 to 80 μM or more, and only zantrin Z5 induced filamentation in E. coli [9]. Further, 10 μM sanguinarine is shown to completely inhibit the proliferation of B. subtilis 168 [11]. Sanguinarine induced filamentation in bacteria and inhibited Z-ring formation in B. subtilis 168.

Similarly, in a recent study, totarol has been shown to inhibit the proliferation of B. subtilis 168 cells with an MIC value of 2 μM [13]. Totarol is thought to inhibit the Z-ring formation in bacteria by inhibiting FtsZ assembly. Curcumin inhibited the proliferation of both Gram-positive (B. subtilis 168) and Gram-negative (E. coli K12 MG1655 and E. coli BL21) bacteria with an MIC of 100 μM for B. subtilis 168 and induced filamentation in B. subtilis 168 cells. The high MIC value for curcumin may be due to the poor bioavailability of curcumin [42]. Several bioconjugates of curcumin have been synthesized to enhance the cellular uptake and targeted delivery of curcumin, thereby increasing curcumin’s antibacterial potential, and they have shown improved antibacterial activity [17,43].

Curcumin inhibited Z-ring formation in B. subtilis 168. Curcumin is known to protect DNA against several DNA-damaging agents like UV rays, X-rays, \( \gamma \)-radiation and chemical mutagens [23–25,27]. Further, curcumin is found to inhibit SOS induction in Salmonella serotype Typhimurium and E. coli [24]. In the present study, we found that curcumin did not affect the nucleoid segregation and organization, further supporting the idea that curcumin does not induce DNA damage detectably.

In summary, the results presented in this study suggest that curcumin inhibits bacterial cell division, apparently by perturbing the cytokinetic Z-ring through a direct interaction with FtsZ. These findings may help to design potent curcumin analogues with improved stability and bioavailability.

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