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An actomyosin-like cytoskeleton in the cyanobiont (*Nosctoc* sp.) of *Peltigera canina*

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ABSTRACT

Lichenized *Nostoc* cells isolated from the lichen *Peltigera canina* develop chemotaxis towards a lectin purified from the lichen thallus. Similar movements in unicellular eukaryotes require actin and myosin to generate contraction and relaxation along the chemotactic axis. We provide evidences for prokaryotic actin-like and myosin-like proteins in the cyanobiont *Nostoc* sp using cross-reacting antibodies against α- and β-actin and non-muscle myosin II light and heavy chains, and two-dimensional gel electrophoresis to determine the isoelectric point (IP) of the actin-like protein. Actin antibodies bound to a single reactive *Nostoc* polypeptide of an approximate molecular mass of 50 kD with IP values between 4 and 7 pH, similar to eukaryotic actin. The myosin light chain antibody reacted with a *Nostoc* protein with an apparent molecular weight of 20 kDa and another of 48 kDa. Immunoprecipitation of cell free extracts using anti-heavy chain myosin separately yielded only one signal corresponding to a protein of a molecular weight around 200 kDa. A bioinformatics analysis indicated that (cyano)bacterial actins are rare but do exist. Our results are consistent with the possible existence of protein homologues of actins and myosins in cyanobionts of *P. canina*, suggesting the existence of an actin-like apparatus that supports chemotactic swimming.

1. Introduction

Cyanobacteria are one of the oldest known groups present on Earth. They are characterized by their ability to fix nitrogen and photosynthetic oxygen. Cyanobacteria are also highly resistant to extremely environmental stresses (Guven and Howard, 2006). Cyanobacteria must be one of the first groups to have acquired directional motility between morphologically diverse groups of phototrophic prokaryotes (Hoiczyk, 2000). The mechanism of motility has been considered as primitive and simple; however, only limited progress has been made in understanding the process (Okamoto and Ohmori, 2002).

During the last 40 years, many models have been proposed to explain the mechanism of bacterial motility, including surfactant effects, moving chains of adhesions, rotating membrane-embedded rotors, and, more recently, slime extrusion through nozzles (Wolgemuth et al., 2002; Mignot et al., 2007). However, clear-cut evidence for any of these models has been lacking. Several findings suggested that this motility involves distributed motors and focal adhesion complexes (Sliusarenko et al., 2007). This proposed motility mechanism has similarities to eukaryotic focal adhesion complexes, in which cell-surface ligands, that provide anchor points with the extracellular matrix, are connected to the actin–myosin network in the interior of the cell (Wozniak et al., 2004).

Some filamentous cyanobacteria, such as *Nostoc* sp., produce specialized gliding filaments in cells known as hormogonia, which constitute a brief, dispersive stage of their life cycles. Hormogonia are the infectious agents in the establishment of cyanobacteria–

Abbreviations: BSA, bovine serum albumin; CHAPS, 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate; DTT, dithiothreitol; HEPES, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid; IPG, immobilized pH gradient; PBS, phosphate buffered saline; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TBS, Tris-buffered saline; Tris, tris(hydroxymethyl)aminomethane.

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plant symbioses and they are attracted to the plant structures that aggregate in symbiotic colonies by chemotaxis towards chemicals released by the plant (Adams et al., 1999). Experiments performed with several types of lichen symbioses, in particular those that have *Nostoc* sp. as a photobiont, have demonstrated the presence of hormogonia when cyanobionts are attracted by lectins produced and secreted by a potential mycobiont (Diaz et al., 2009; Vivas et al., 2010; Díaz et al., 2011). The absence of superficial elements related to cell movement in the hormogonia of cyanobiont of *Peltigera canina*, and the appearance of sunken cells during or after movement verified by scanning electron microscopy support the hypothesis that the motility of these cyanobacteria could be achieved by contraction-relaxation episodes of the actin-cytoskeleton induced by fungal lectin acting as a chemoattractant (Díaz et al., 2011).

Whereas the eukaryotic cytoskeleton was first described centuries ago (Frixione, 2000), the notion that bacteria also may contain cytoskeletal elements is relatively recent (Bi and Lutkenhaus, 1991; Jones et al., 2001). Actin has been described in all eukaryotic cells as the major cytoskeletal protein forming microfilament. Although in prokaryotic cells a cytoskeleton has not been described so far, different researchers report the presence of several structures and proteins associated with such a structure (Eda et al., 1997; Guerrero-Barrera et al., 1996; Korolev et al., 1994; van den Ent et al., 2014). Functions proposed for prokaryotic actin-like proteins include motility in the absence of locomotion appendages as well as the maintenance of the cell shape in absence of a cell wall (Neimark, 1977). Labbe et al. (1996) also describe and characterize an actin-like protein in another prokaryote: *Synechocystis* sp., a motile unicellular strain of cyanobacteria. This prokaryotic 56-kDa protein can polymerize into filaments, activate myosin ATPase, inhibits DNase-I activity and displays antigenic cross-reactivity with actin from rabbit skeletal muscle.

Here, we present biochemical evidence of the existence of actin- and myosin II-like proteins in *Nostoc* sp., the cyanobiont of the lichen *Peltigera canina*. Based on previous studies in which we have shown the chemotactic behaviour of these organisms, we postulate that these proteins could participate in the generation of mechanical force necessary for the chemotactic swimming of these cells.

2. Results

2.1. Actin antibodies bind to proteins from cyanobionts of P. canina

In eukaryotic cells, actin-rich structures produce specific shapes and cellular morphologies that are considered hallmarks of cell migration (Ridley et al., 2003). We hypothesized that if actin-like molecules are present in *Nostoc* cyanobionts, antibodies that recognize eukaryotic actin may detect similar polypeptides from the cyanobacterial cells in this lichen. To address this, we extracted the soluble fractions (~1.0 μg protein μL^{-1}) of cyanobionts, separated them using SDS/PAGE and performed Western blot experiments using a commercial anti-α-actin antibody (Fig. 1A) and anti-β-actin antibody (Fig. 1B).

Blotting of *Nostoc* lysate revealed a single reactive polypeptide of an approximate molecular mass of 50 kDa by Western blotting of total cellular extracts using either anti-α or β-actin antibody. These results indicate that epitopes related to actin exist in this cellular system. We found that the intensity of the band was dose dependent in both of cases. The fraction belonging to the control (MEF) revealed a band with a slightly higher electrophoretic mobility and corresponds to β-actin with a molecular mass between 40- 42 kDa.

2.2. Isoelectrical points of actin-like proteins from cyanobionts of P. canina

2D electrophoresis was performed in triplicate. The amount of cyanobacterial protein used for the first dimension was 100 μg. In the second dimension of the electrophoresis, 20 μg of total cyanobiont protein extract was loaded as a positive control next to the mounted strip of the first dimension. One of the gels was revealed with Coomassie blue (Gel Code Blue stain reagent, Pierce, Rockford, IL, USA) while the other two were transferred to a nitrocellulose membrane and incubated with anti-β-actin or anti-α-actin (Abcam, 40863 ab) antibodies (Sigma A2228)

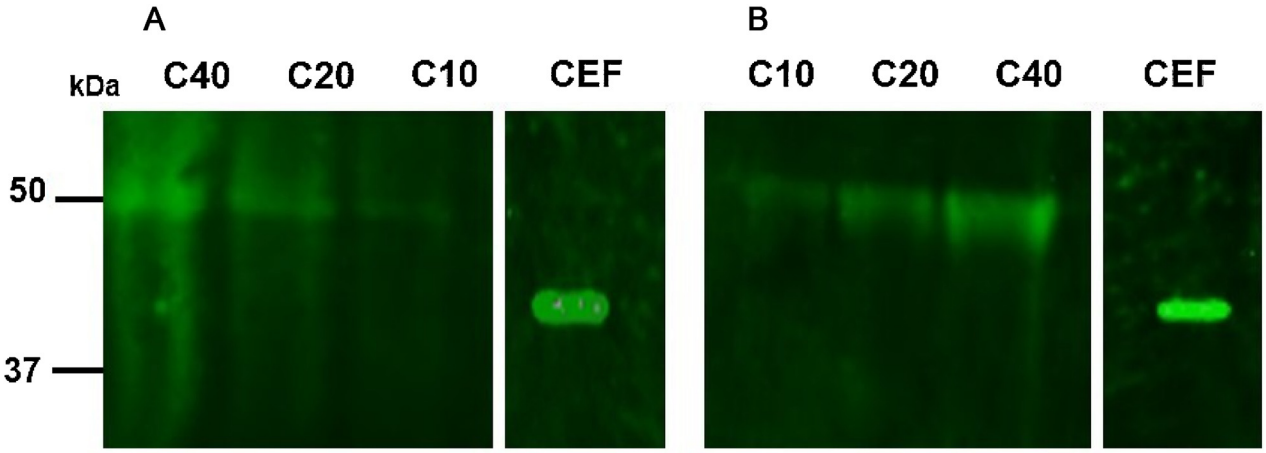

Fig. 1. Western blot with actin isoform antibodies of *Nostoc* protein extract obtained from *P. canina* lichen. The membranes were incubated with (A) anti-α-actin (B) anti-β-actin. The location of the bands of molecular weight marker is indicated (37, 50 kDa). Green bands around 50 kDa correspond to cyanobacterial actin in the lanes labeled with C10, C20, and C40 in which 10, 20 and 40 μg of the total protein lysate was separated, respectively. Control: MEF = embryonic mouse fibroblast cells with beta-actin at 42 kDa.