Heterologous Expression of the Mycobacterium tuberculosis Gene Encoding Antigen 85A in Corynebacterium glutamicum

K. SALIM,1 V. HAEDENS,1 J. CONTENT,2 G. LEBLON,1 AND K. HUYGEN2*
Laboratoire de Biologie Moléculaire des Corynétiques, Institut de Génétique et de Microbiologie, URA 2225, Université Paris-XI, F-91405 Orsay cedex, France,1 and Department of Virology, Pasteur Institute of Brussels, 1180 Brussels, Belgium2

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By using appropriate Corynebacterium glutamicum-Escherichia coli shuttle plasmids, the gene encoding the fibronectin-binding protein 85A (85A) from Mycobacterium tuberculosis was expressed in C. glutamicum, also an actinomycete and nonsporulating gram-positive rod bacterium, which is widely used in industrial amino acid production. The 85A gene was weakly expressed in C. glutamicum under the control of the ptac promoter from E. coli, but it was produced efficiently under the control of the promoter of the cspB gene encoding PS2, one of the two major secreted proteins from C. glutamicum. The 85A protein was produced in various forms, with or without its own signal sequence and with or without the signal sequence and the NH2-terminal (18-amino-acid) mature sequence of PS2. Western blot analysis with monoclonal antibodies raised against the M. tuberculosis antigen 85 complex showed that recombinant 85A protein was present in the corynebacterial cell wall extract and also released in extracellular culture medium. NH2-terminal microsequencing of recombinant 85A secreted by C. glutamicum showed that signal peptide was effectively cleaved off at the predicted site. The recombinant 85A protein was biologically active in vitro, inducing significant secretion of Th1 T-cell cytokines, particularly interleukin-2 and gamma interferon, in spleen cell cultures from mice vaccinated with live Mycobacterium bovis BCG. Heterologous expression of mycobacterial antigens in C. glutamicum now offers a potent tool for further immunological characterization and large scale preparation of these recombinant proteins.

Acquired protective immunity against pathogenic mycobacteria is considered to be the result of interactions between phagocytic host cells harboring the intracellular pathogen and specifically sensitized CD4+ and CD8+ T lymphocytes. Activation of infected macrophages by the pivotal Th1 cytokine interferon gamma (IFN-γ), secretion of tumor necrosis factor alpha, and destruction of infected macrophages by cytotoxic T cells are thought to be the essential components involved in restricting mycobacterial infection in the immunocompetent host (18, 29).

Improved strategies for the control of tuberculosis and leprosy depend on the definition of immunodominant and immunoprotective mycobacterial antigens (26, 56). It has been known for over four decades that killed mycobacteria have a lower protective potential than living bacteria (6), and it has therefore been argued that secreted proteins, produced only by actively metabolizing organisms and present in early mycobacterial culture filtrates, may be critical for the development of protective immunity (40). This hypothesis was recently substantiated in experimental vaccination studies of mice and guinea pigs with crude mycobacterial culture filtrate (2, 21, 22, 45).

The antigen 85 (Ag85) complex is the major 30- to 32-kDa protein component in culture filtrates from surface-grown Mycobacterium tuberculosis and Mycobacterium bovis BCG (55). Antigens cross-reacting with the Ag85 complex from M. bovis BCG have been found in all mycobacterial species tested so far (55). We and others have previously shown that Ag85 from M. bovis BCG (and from M. tuberculosis, which displays an identical amino acid sequence) is a potent inducer of interleukin-2 (IL-2) and IFN-γ and of cytotoxic T lymphocytes in most healthy individuals with primary infection by M. tuberculosis or M. leprae (32, 38, 46, 51) and in mice infected with live M. bovis BCG (24). In contrast, T cells from only a minority of tuberculosis patients (those with minimal lung lesions) and from paucibacillary (but not multibacillary) leprosy patients were found to proliferate and secrete IL-2 and IFN-γ to the Ag85 complex. Ag85 has also been used for the serodiagnosis of tuberculosis and leprosy (47, 53). Recently we have vaccinated mice with plasmid DNA encoding Ag85 and have found that such DNA vaccine has powerful immunogenic properties, resulting in (i) robust Ag85-specific Th1 and cytotoxic T lymphocyte responses and (ii) protective efficacy against a subsequent aerosol challenge with M. tuberculosis, demonstrating that Ag85 is indeed a protective antigen for tuberculosis (25, 34).

Ag85 is actually a protein family with three members: 85A, 85B, and 85C, with respective molecular masses of 32, 30, and 32 kDa (55). These three components have different isoelectric points and can be separated by isoelectric focusing (16). They are encoded by three distinct but highly homologous (about 75% similarity) genes, which probably arose by duplication and subsequent mutation, and these genes have now been cloned from different mycobacterial species (9, 11, 13–15, 36).

The use of native mycobacterial proteins in serodiagnosis, fundamental immunological studies, and subunit vaccination is hindered by difficulties in growing the organism and purifying the antigens. Antigen aggregation, degradation in cell extracts, and particularly slow and fastidious growth are the main problems associated with purification of antigens from mycobacterial cultures (17). The development of recombinant DNA systems for efficient expression of mycobacterial genes in...
**Escherichia coli** has previously allowed the efficient production of a number of mycobacterial intracellular stress proteins and cell wall-associated lipopolysaccharides (for a review see reference 56). Heterologous expression of extracellular, secreted mycobacterial proteins, however, has proven to be more difficult. Thus, the major secreted Ag85 complex from *M. tuberculosis* and *M. bovis* BCG can be expressed in *E. coli* under the control of the tac promoter, but the product is unsuitable for T- and B-cell studies, due to its insolubility and accumulation in the inner cell membrane (11a).

This report describes for the first time the use of *Corynebacterium glutamicum* as an alternative host for functional heterologous expression of the *M. tuberculosis* 85A gene. This approach was chosen for the following reasons. (i) *C. glutamicum* is a gram-positive organism that has been engineered to produce large quantities of amino acids such as lysine and glutamate (for a review see reference 33). It has been used for industrial amino acid synthesis for several decades (50), and thus there is much accumulated experience concerning appropriate fermentation conditions. (ii) *C. glutamicum* is an organism that produces no hazardous toxins. (iii) Techniques have been developed for genetic manipulation of *C. glutamicum* and produces no hazardous toxins. (iv) There is no broad-spectrum antibiotic resistance. (v) *C. glutamicum* has previously allowed the efficient production of heterologous proteins (for a review see reference 33) in *E. coli* lac-proAB strains by alkaline extraction (5). The plasmids used in this work are listed in Table 1. Construction of plasmids containing the various 85A gene fusions is described in Fig. 1 and 2. Details of the various gene fusions are shown in Fig. 3.

### MATERIALS AND METHODS

**Bacterial strains, plasmids, and culture media.** Two strains of *C. glutamicum* were used in this work: *C. glutamicum* B155 (previously named Brevibacterium lactoferrum) 15 [5]; restrictionless, rifampin-resistant, derivative of ATCC 21086; accepting plasmids from *E. coli*) and *C. glutamicum* CGL1017 (previously named *Corynebacterium melassecola*) [7]; a derivative of ATCC 17965 that does not accept plasmids from *E. coli* but can grow in minimal synthetic medium. *C. glutamicum* was grown in 3.7% Difco brain heart infusion medium at 34°C with aeration such that the culture reached stationary phase after 10 h. A synthetic basal medium for *Corynebacterium* growth (BMCG) (19) was used with 2% sodium lactate as a substitute for the 2% glucose as carbon source. *C. glutamicum* was grown in Luria broth (LB) and used as an intermediate host for various constructions. Antibiotics were added to final concentrations of 100 μg/ml for ampicillin, 25 μg/ml for kanamycin, 10 μg/ml for tetracycline (*E. coli*), and 15 (C. glutamicum) or 30 (E. coli) μg/ml for chloramphenicol. *C. glutamicum* was transformed by electroporation as described previously (7). The *Mycobacterium* strain was used in *M. bovis* BCG GL2 (Pasteur Institute of Brussels), which was grown for 2 weeks as a surface pellicle on synthetic Sauton culture medium at 37.5°C (10).

### PCR amplification.

PCR with Vent polymerase, in Vent reaction buffer (New England Biosite) containing 150 μM deoxynucleoside triphosphates (Boehringer Mannheim), was performed in 50-μl reaction mixtures for 30 cycles of 1 min at 95°C, 1 min at 55°C, and 2 min at 72°C followed by a 5-min final extension at 72°C.

### DNA sequence analysis.

DNA sequences were determined by using double-stranded DNA with the primer extension dideoxy chain termination method (49).

### Preparation of cell extracts for Western blotting studies.

### PREPARATION OF CELL EXTRACTS FOR WESTERN BLOTTING STUDIES.

*E. coli* whole-cell extracts were prepared as follows. Two milliliters of bacterial culture (optical density at 570 nm [OD$_{570}$] of 2) was harvested by centrifugation; the cell pellet was resuspended in 200 μl of TE buffer (100 mM Tris-HCl, 10 mM EDTA [pH 8]) containing 2 mg of lysozyme per ml and incubated for 15 min at 37°C. An equal volume of sample buffer (31) was added, and the samples were subjected to Western blotting.

Proteins were extracted from the cell wall of *Corynebacterium* with sodium dodecyl sulfate (SDS), using the procedure described by Peyret et al. (44). Two milliliters of culture (OD$_{570}$ of 7) was centrifuged at 12,000 × *g* for 5 min. The pellet was resuspended in 100 μl of 50 mM Tris-HCl (pH 6.8)−2% SDS and incubated at 100°C for 5 min. The supernatant containing proteins initially associated with the cell wall...
was collected. The cellular pellet was then treated for the preparation of whole-cell extracts. It was washed, centrifuged, resuspended in 100 μl of 50 mM Tris-Cl (pH 8) containing 10 mg of lysozyme per ml, and incubated for 1 h at 37°C. An equal volume of sample buffer (31) was added, and the samples were subjected to Western blotting. The supernatants were precipitated with 5% trichloroacetic acid and washed with acetone solvent.

Preparation of the recombinant 85A-containing culture filtrate from C. glutamicum used in T-cell studies. Recombinant C. glutamicum BI15 and CGL1017 strains were grown overnight in LB medium (OD<sub>530</sub> of 7). The culture supernatants were collected after centrifugation and precipitated with 80% (wt/vol) ammonium sulfate. The resulting pellet was dissolved in water (100 μl); 70 μl of this sample was denatured, and applied to SDS-polyacrylamide gel electrophoresis (PAGE), electrotransferred onto a Problot membrane, and visualized with Coomassie blue staining and Western blotting (Fig. 3B and C).

Fractions containing the purified protein 85A (14 ml) were pooled, dialyzed against water, and lyophilized. The pellet was resuspended in water (100 μl); 70 μl of this sample was added to 100 μl of 50 mM Tris-Cl (pH 7.5) containing 10 mg of lysozyme per ml, and incubated for 1 h at 37°C. Tritiated thymidine (specific activity, 8.3 Ci/mmol; 37). To test whether the M. tuberculosis 85A protein from the M. bovis BCG culture filtrate, Ag85 complex (30 to 32 kDa) and its 85A (32-kDa) component were purified from a 2-week-old culture filtrate of surface pellicle-grown M. bovis BCG (strain GL2 derived from the Paris 117/32 strain) cultures grown on synthetic Sauturon medium by sequential hydrophilic chromatography on phenyl-Sepharose, ion-exchange on DEAE-Sephaloc, and molecular sieving on Sephadex G-75 (12).

**SAIDS-PAGE and Western blot analysis.** SAIDS-PAGE was done as described by Laemmli (31). Samples were denatured in the presence of 2% SDS in 50 mM Tris-HCl (pH 6.8) and layered on a polyacrylamide slab gel containing SDS. The gel was stained with 0.1% Coomassie blue or used in Western blot analysis. The 32-kDa 85A protein was excised, and amino-terminal amino acid sequencing was applied by automated Edman degradation with a 48-h cycle on a Beckman sequencer (Amersham) to determine the amino acid sequence of the recombinant 85A protein.

**RESULTS AND DISCUSSION**

**Expression of the M. tuberculosis 85A gene from the ptac promoter in E. coli and C. glutamicum.** The M. tuberculosis 85A gene including the sequence encoding its 43-amino-acid (aa) signal peptide (9) was inserted as a 1.04-kb FokI-BamHI fragment at the ECoRI site of pKK223-3 (Table 1) to give pKK223-3/85A (Fig. 1 and 3). E. coli TG1 cell extracts were prepared and subjected to Western blot analysis with Ag85-specific MAb. A faint band migrating to the same position as the native 32-kDa protein in M. bovis BCG culture filtrate was obtained, indicating that the signal peptide was cleaved off (Fig. 4, lane pKK-A).

The ptac promoter is known to function in C. glutamicum (37). To test whether the M. tuberculosis Ag85A could be directly secreted by its own signal peptide from C. glutamicum, a 1.32-kb BamHI fragment from pKK223-3/85A containing the 85A gene and the ptac promoter was inserted into the single BamHI site of the C. glutamicum shuttle vector pCLG473 to give pCLG496 (constructed in E. coli TG1 [Fig. 1 and 3]). E. coli TG1 cell extracts gave a positive signal at the same position as the native mature 85A protein (Fig. 5A, lanes 1046). A negative control, the C. glutamicum BI15 strain harboring the empty pCLG482 plasmid (Table 1) was used. Despite its substantial sequence homology to the Ag85 complex, PS1 protein present in the C. glutamicum culture filtrate did not react with the specific Ag85 MAb (data not shown).

**Expression of the M. tuberculosis 85A gene under the control of the ptac and cspB promoters in E. coli and C. glutamicum.** In a second phase, we tested the heterologous expression of the M. tuberculosis 85A protein from the cspB gene promoter in E. coli and C. glutamicum. Fragment BamHI-PstI containing the cspB gene promoter was inserted into the shuttle vector pCLG482 (Table 1), giving pCLG486 (Fig. 1), into which was inserted the 1.32-kb BamHI fragment from pKK223-3/85A containing the ptac-85A fusion to give pCLG1052 (Fig. 1). pCLG1052 thus carries a transcriptional fusion between the...
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pCGL1051

(1) SalI digestion

SalI

pcspB

SS-PS2

18 aa

11 aa

SS

SS-PS2

18 aa

11 aa

SalI

1.7 kb SalI fragment

(2) ligation

SalI

pcspB

(circular template)

SalI

(3) PCR amplification with primers a and b:
a: 5'-GCTCTAGAATAACGAAATGCCTGATACCGA CGCC-3'
b: 5'-GCGCTAGACCCGGTTGCTCCGAGTACCTGC-3'

○ = sequence underlined = XbaI site.

XbaI

pcspB

SalI

SalI

SS-PS2

SS

SalI

(4) XbaI fragment was circularised with T4 DNA ligase.

(5) linearised with SalI.

(6) cloned in pUN121 at Smal site.

(7) pCGL1054 EcoRV/BglII fragment cloned at Smal/BglII sites of pCGL482
cspB promoter and the ptac-85A fusion (Fig. 3, pCGL1052). This construction was analyzed in E. coli by Western blotting (Fig. 4, lane 1052) and transferred into C. glutamicum BI15. Production of immunoreactive polypeptide in C. glutamicum BI15 was higher with pCGL1052 than with pCGL1046 (Fig. 5A, lanes 1046 and 1052), which indicates that the cspB promoter is more suitable for the production of significant quantities of the 85A protein in C. glutamicum. Importantly, no degradation of the recombinant 85A protein was observed in C. glutamicum, confirming the lack of protease release in early corynebacterial culture supernatant. However, some degradation of heterologously secreted protein was detected at the end of the stationary phase, and this correlates with lysis of bacteria, accompanied by protease release (41).

Expression of the M. tuberculosis 85A gene under the control of PS2 expression and secretion sequences. The shuttle vector pCGL482 (Fig. 1) is derived from pCGL482 and contains a multiple cloning site facilitating construction of fusion proteins (Table 1).

A 2.46-kb NarI-XbaI fragment from pCGL1046 carrying the N-terminally truncated 85A gene, its downstream region, and a kanamycin resistance gene were inserted between the SalI site of the cspB gene and the upstream EcoRI site. In pCGL847, the BamHI site of the cspB gene is followed by a multiple cloning site facilitating construction of fusion proteins (Table 1).

The production of 85A recombinant protein in E. coli and C. glutamicum harboring pCGL1051 was verified by Western blotting. A positive signal, corresponding to a protein with estimated molecular mass of 35 kDa, was found in whole-cell extract of E. coli (Fig. 4, lane 1051) and C. glutamicum BI15 (Fig. 5A, lanes 1051). The culture supernatant of C. glutamicum BI15 (pCGL1051) also contained significant amounts of an immunoreactive protein with an estimated molecular mass of 35 kDa (the calculated hybrid PS2-85A protein molecular mass being 34,410 Da). In C. glutamicum BI15 cell wall extract, an additional protein band with estimated molecular mass of 28 kDa was detected in similar quantities as the 35-kDa band. It is not clear whether cleaving of this 85A form occurred at the carboxy- or at the amino-terminal end of the recombinant protein.

An in-frame gene fusion between the sequence encoding the PS2 signal peptide (30 aa) and the sequence encoding the complete mature 85A protein was obtained, using oligonucleotides a and b as PCR primers to amplify the 1.7-kb recircularized SalI fragment from plasmid pCGL1051 (Fig. 2). A 1.7-kb PCR product was purified, digested with XbaI, and circularized with T4 DNA ligase. This procedure deleted in vitro the 29-aa region between the PS2 signal peptide and mature 85A protein of pCGL1051 (Fig. 3). Then the circularized product was linearized with SalI and finally subcloned into the Smal site of the E. coli (pUN12) vector to create pCGL1054 (Fig. 2 and 3).

Production of 85A protein from pCGL1054 in E. coli TG1 was verified by Western blotting (Fig. 4, lane 1054). The in-frame gene fusion was confirmed by sequencing both strands of the 98-bp PsrI fragment corresponding to the region overlapping the new XbaI site after subcloning of the specific Psrl fragment from pCGL1054 in the pUC19 vector, leading to pCGL1060 (Table I). DNA sequence analysis indicated that the codon (CCG) corresponding to the fourth amino acid of the mature 85A protein (proline) had been deleted, possibly due to a mistake during the synthesis of the oligonucleotide primers used in PCR amplification.

pCGL1054 is unable to replicate in C. glutamicum. The PS2-85A fusion (SalI/BglII fragment from pCGL1054) was

FIG. 3. Schematic representation of the M. tuberculosis 85A expression constructs pKK223-3/85A, pCGL1046, pCGL1047, pCGL1051, pCGL1054, and pCGL1055. The boxes represent the coding regions of the genes by identification of their corresponding sequence signals; transcription of these fusions is controlled by the tac or cspB promoter. 85A, M. tuberculosis 85A (mature part); SS-85A, 85A signal sequence; SS-PS2, PS2 signal sequence; 18 aa, first 18 aa of PS2; 11 aa, last 11 aa of 85A signal sequence; ↓, cleavage site.

FIG. 4. Western blot analysis of the production, by whole-cell extract corresponding to 200 ml of culture (OD570 of 2) of recombinant 85A from M. tuberculosis in E. coli TG1 probed with a mixture of MAbs 32-15 and 17-4 (24) directed against the M. bovis BCG Ag85 complex. Fractions were prepared as described in Materials and Methods from E. coli TG1 harboring pKK223-3/85A (pKK-A), pCGL1046 (1046), pCGL1052 (1052), pCGL1051 (1051), pCGL1054 (1054), and pCGL1055 (1055). The left lane shows molecular weight standards. BCG, M. bovis BCG culture filtrate; A+C, position of Ag85A and Ag85C (32 kDa); B, position of Ag85B (30 kDa).
C. glutamicum homogeneity from pCGL1051 (1051), and pCGL1055 (1055). (B and C) Coomassie blue-stained gel (B) and Western blot (C) profiles after SDS-PAGE of recombinant 85A purified to homogeneity from C. glutamicum CGL1017(pCGL1055) as described in Materials and Methods. Quantities applied are indicated at the top. The left lane shows molecular weight standards (MPM). For other details, see the legend to Fig. 4.

Expression, as measured by immunoblotting, of pCGL1055 encoding the mature 85A sequence coupled directly to the PS2 signal sequence, was slightly lower in both cell wall extract and culture filtrate than expression of pCGL1051, containing in addition the first 18 aa of the mature PS2 protein and the last 11 aa of the 85A signal peptide. In this respect, several examples have demonstrated the importance of the NH2-terminal region of a mature protein for optimal production and secretion efficiency (3, 43). On the other hand, it cannot be totally excluded that the pCGL1055 derived recombinant protein was, for some reason, less stable.

Production and purification of M. tuberculosis 85A in C. glutamicum CGL1017. All plasmid constructions were independently transferred to C. glutamicum CGL1017, which can grow on minimal BMCG, facilitating subsequent purification of the immunoreactive protein. Western blot analysis of transformed C. glutamicum CGL1017 strains gave results similar to those for C. glutamicum Bl15 (data not shown).

The 32-kDa immunoreactive protein was purified from culture supernatant of C. glutamicum CGL1017(pCGL1055) as described in Materials and Methods and could be visualized after SDS-PAGE by both Coomassie blue staining (Fig. 5B) and Western blotting (Fig. 5C). Microsequencing of the purified 32-kDa protein resulted in the sequence FSRGLPVEYL, confirming DNA sequencing results that the proline in position 4 between Arg-3 and Gly-5 from the mature mycobacterial 85A protein had indeed been deleted in the recombinant corynebacterial fusion protein. This result demonstrates that the immunoreactive protein corresponds to the mature 85A protein and that signal peptide of the fusion protein was effectively cleaved off at the position predicted by the analysis of the cspB gene DNA sequence (43) that is after Ala-30.

Spleen cell cytokine secretion in response to C. glutamicum 85A culture supernatants. Recombinant 85A-containing culture supernatants were prepared from the transformed C. glutamicum CGL1017 strains and tested as inducers of Th1 cytokine production in spleen cell cultures from M. bovis BCG-vaccinated C57BL/6 and BALB.B10 mice (Table 2). Culture supernatants from the recombinant strains containing plasmids pCGL1051 and pCGL1055 induced significant IL-2 and IFN-γ levels. The presence of PS2 amino acids at the NH2 terminus of the 85A hybrid in pCGL1051 apparently did not affect subsequent processing and interaction with the T cell receptor. Filtrates from C. glutamicum CGL1017 harboring pCGL1046 and pCGL1052 were less active, as expected from their lower expression signal in Western blot analysis. Similar results for IL-2 and IFN-γ production were obtained with recombinant culture filtrates from C. glutamicum Bl15 carrying the same plasmids (data not shown).

This analysis shows that the PS2 promoter and signal sequence are efficient for optimal secretion of immunoreactive material in corynebacterial culture filtrate. Moreover, low levels of IL-2 and IFN-γ were also detected in spleen cell cultures from M. bovis BCG-vaccinated mice stimulated with culture filtrate from C. glutamicum CGL1017, the control corynebacterium with the empty vector. This may have reflected a cross-reactive immune response against the corynebacterial PS1 protein, which is highly homologous in its NH2 region with Ag85 from Mycobacterium (27) and which contains pepptide regions that are very similar to regions in the 85A protein from M. tuberculosis with defined T-cell epitope characteristics for H-2d mice (24, 25a).

Heterologous expression of mycobacterial proteins in C. glutamicum may offer a major breakthrough for large-scale fer...
mentation and purification of biologically active recombinant mycobacterial antigens from extracellular culture medium and may be valuable for improved serodiagnosis of tuberculosis and leprosy, for immune characterization, and for experimental subunit vaccination strategies. With respect to the latter, C. glutamicum strains are unfortunately not suitable as live vaccine vectors themselves, because of their sensitivity to 37°C and their immediate in vivo destruction, which hampers all antigenic presentation (25b).

By using the same cspB promoter, expression and secretion of the 85B and 85C genes from M. tuberculosis can now also be envisaged. Large-scale production and purification of these antigens from C. glutamicum CGL1017, adapted to grow on minimal synthetic medium, will be attempted, enabling a rigorous comparison of the three components of the Ag85 complex. Finally, M. leprae, the only mycobacterial species that thus far cannot be cultivated in vitro, also encodes the genes for the three Ag85 homologs (13, 15, 42). Hence, heterologous expression of the genes coding for new antimycobacterial drugs (4). Whether PS1, homologous to mycobacterial cell wall assembly, making them potential targets for new immunocytobacterial drugs (4). Whether PS1, homologous to the gene coding for antigen 85-C of M. tuberculosis, now has become possible and should facilitate further immunological characterization of these antigens.

Ag85 complex binds to fibronectin (1) and was described to interact with the complement receptor type 3 on monocytes (20), which has led to the suggestion that the complex may play a role in macrophage invasion. Furthermore, it may also be involved in bacterial agglutination, as M. bovis BCG grown as a stationary surface pellicle secretes larger amounts of the protein than immersed M. bovis BCG grown in detergents and with shaking (10a, 54). Whereas PS2 from C. glutamicum has been reported to constitute the surface layer organization (43), the role of PS1 is unclear. Among other similarities, mycolic acids are found in the cell walls of both corynebacteria and mycobacteria. Interestingly, a recent report indicates that each of the components of the mycobacterial Ag85 complex can act as a trehalose mycolyltransferase involved in the final stages of mycobacterial cell wall assembly, making their potential targets for new antimycobacterial drugs (4). Whether PS1, homologous in its NH2-terminal part to 85A from M. tuberculosis, also displays such enzymatic activity remains to be determined.

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