



RESEARCH ARTICLE

ETHANOL EXTRACTION METHOD FOR DNA ISOLATION FROM *MYCOBACTERIUM SMEGMATIS*

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ABSTRACT

Genomic DNA isolation from *Mycobacterium smegmatis* has always been a challenging task due to the tough cell wall of the organism. Published methods for DNA extraction from *M. smegmatis* are tedious that yield only small quantities of DNA. This paper presents a simple and reproducible protocol for obtaining good quality DNA. The method is a part of a procedure used for extraction of the cell-wall-associated iron-chelating compound, mycobactin S from *M. smegmatis*. This method yields significant quantities of DNA as a by-product of mycobactin S extraction.

INTRODUCTION

The necessary steps for genomic DNA (gDNA) preparation from microorganisms are cell-disruption, precipitation of proteins and DNA isolation. Though these steps are easily performed on most microorganisms, *Mycobacterium smegmatis* poses a unique challenge due to its thick waxy cell wall. Techniques used for DNA isolation from mycobacterial cells seem rather complicated. For example, methods include pre-treatment with chloroform and methanol mixture before using guanidine thiocyanate to isolate DNA (Mve-Obiang et al., 2001). Also, lysis of mycobacterial cells with glass beads, treating the lysate with proteinase K, and heating to 95°C for 10 min were used (Via et al. 1995, Izhar et al., 2004). Boiling mycobacterial suspension for 10 to 15 min in distilled water (Tortoli et al., 2001, Svastova et al., 2002) to obtain DNA was also used. Use of enzymes, silicone beads, and ultrasonication (Savic et al., 1992), or use of cetyltrimethylammonium bromide (CTAB) (Bose et al., 1993) are some of the processes for isolating DNA from *M. smegmatis*. The method described here arose accidentally from a published procedure (White and Snow, 1969) used for extraction of a cell-bound siderophore, mycobactin S, from *M. smegmatis*.

For the extraction of mycobactin S, absolute ethanol was added to the cell pellet and the mixture was incubated overnight at 2-8°C. Unexpectedly, we observed hair-like fibres protruding from the cell pellet while decanting the ethanol extract. Their appearance suggested that these fibres could be of DNA. The fibres, when dissolved in TE buffer gave a viscous solution and on agarose gel electrophoresis showed the presence of high molecular weight DNA. So, it was necessary to know the optimum concentration of ethanol, because even though absolute ethanol was added to the cell pellet, effective ethanol concentration would be less than 100% since the cell pellet occupies a finite volume.

MATERIALS AND METHODS

For large-scale extraction of mycobactin S, a dense inoculum of *M. smegmatis* was introduced to five flasks containing 200 mL of iron-deficient minimal medium containing glycerol and asparagine (Ratledge and Hall, 1971) and incubated at 37°C for 5 days on the shaker. The dense culture broth was centrifuged at 10,000 rpm for 5 min. The cell pellets from all the flasks were pooled and 50 mL of absolute ethanol was added to it. After overnight incubation at 2-8°C, the suspension was centrifuged at 10,000 rpm for 5 min, and the extract was processed to obtain mycobactin S. The fibres sticking out from the cell pellet were picked up using a cut tip and dissolved in TE. In this way, a significant amount of gDNA was obtained as

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a by-product of mycobactin S extraction. The optimum concentration of ethanol required to release gDNA was determined by subjecting the *M. smegmatis* cells overnight in the cold to different concentrations of ethanol from 20% to 100%. The DNA fibres released were then harvested. The quality and quantity of the purified DNA was determined by measuring its absorbance at 260 nm and 280 nm.

RESULTS

For the ethanol extraction method, the gDNA released was in large quantities. One litre of *M. smegmatis* broth culture was used for mycobactin S extraction. The weight of the cell pellet obtained was 400 mg wet weight. The yield of gDNA was approximately 2.88 µg DNA/mg cells (Table 1). Determination of the optimum concentration of ethanol for DNA isolation from *M. smegmatis* was carried out using 3 mL of spent broth. The effective concentration of ethanol was found to be 60% (Table 2), where the A_{260} nm was maximum at 60% ethanol concentration used. Figure 1 shows the bands of DNA isolated by ethanol extraction method from three different strains of *M. smegmatis*.

Table 1. DNA yield from *M. smegmatis* using ethanol extraction (each value represents the mean of three independent experiments)

Treatment	$A_{260\text{nm}}$	$A_{280\text{nm}}$	$A_{260\text{nm}} / A_{280\text{nm}}$	DNA (µg)	Wet weight of cells (mg)	DNA (µg/mg)
Ethanol Extraction	0.231	0.145	1.6	1155	400	2.88

*Dilution factor: 100

Table 2. Determination of the optimum concentration of ethanol (each value represents the mean of three independent experiments)

No.	Concentration of ethanol %	$A_{260\text{nm}}$	$A_{280\text{nm}}$
1	20	0.250	0.181
2	40	0.428	0.360
3	60	0.561	0.119
4	70	0.173	0.112
5	80	0.105	0.093
6	100	0.141	0.108

*Dilution factor: 10

DISCUSSION

DNA extraction methods from *M. smegmatis* that are available seem to be complex with low yields of DNA. According to Via and Falkinham (2), DNA yield by glass-bead disruption method was 0.6-1.0 µg DNA/mg cells (wet weight) and by glass-bead, phenol/chloroform method, it was 0.32 µg DNA/mg cells. The combination of these two approaches along with CTAB method had yields of 0.25 µg DNA/mg cells whereas, by the autoclaving process it was 0.04 µg DNA/mg cells. Comparison of three different methods for gDNA extraction from various mycobacterial species, - enzymatic extraction, combined bead-beating with enzymatic extraction, and CTAB extraction have been reported (Amaro et al., 2008). The DNA yield was the highest in the combined method but with shearing of the DNA. Other methods use chloroform-methanol treatment followed by corrosive reagents such as

phenol and guanidine isothiocyanate (Mve-Obiang et al., 2001) making DNA isolation complicated.

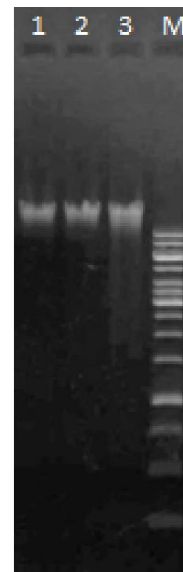


Figure 1. DNA isolated by ethanol extraction method - lanes 1, 2, 3: DNA after ethanol treatment of cells from different strains of *M. smegmatis*, M: 1 kb DNA ladder

The method described in this paper gives high yields of gDNA from *M. smegmatis*. The advantage of DNA isolation from *M. smegmatis* using ethanol is that the process is straightforward and easy to perform, without any protein and RNA contamination. Increasing ethanol concentration decreases the solubility of large molecules such as carbohydrates, proteins and nucleic acids. However, of the three polymers, nucleic acids are the most soluble in ethanol. Use was made of this property to determine the optimum concentration of ethanol at which polymers other than nucleic acids precipitate out and are quickly eliminated by centrifugation. In most methods of DNA isolation, DNA precipitation occurs above 67-70% ethanol concentration. Therefore, subjecting the cells to 60% ethanol would render most proteins and carbohydrates insoluble, while keeping DNA in solution. Some proteins that may remain can be removed using Marmur's method (Marmur, 1961). This simple method of DNA isolation may be readily extended to other cells as well. More work needs to be done to standardise the process for these different types of cells. gDNA isolated from *M. smegmatis* by the ethanol extraction method mentioned here has been successfully used as a template for the PCR amplification of Exochelin MS biosynthesis genes.

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