EFFECT OF CULTURE MEDIUM AND pH OVER THE PRODUCTION OF INCLUSION BODIES IN RECOMBINANT ESCHERICHIA COLI PRODUCER OF SPHINGOMYELINASE-D

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Introduction. Many recombinant proteins are produced in *Escherichia coli.* In this cellular system often happens overexpression causing protein aggregates called inclusion bodies (IBs). The IBs formation represented a bottleneck in protein production because they were considered as deposits of inactive proteins (1). Recent studies showed that IBs in some occasions are composed from properly folded and biologically active recombinant proteins (1). The aim of this work was to evaluate the effect of two culture medium in shake flasks over the recombinant sphingomyelinase-D (rSMD) production in IBs. Moreover, to determined the pH influence in the IBs production and morphology, in bioreactor.

Methods. The *E. coli* strain BL21-SMD producer of rSMD of tick was grown in shake flasks at 200 rpm, 37ºC with 100 µg/mL ampicillin, in Luria Broth (LB) (3) or Super-Broth (SB) by triplicate (4). BL21-SMD also was cultured in batch in 1 L bioreactor controlled at 30% of dissolved oxygen by agitation cascade, 37°C, with controlled pH at 7.5 or without pH control by triplicate. The rSMD expression was induced with 0.1 mM IPTG at the end of the stationary phase in all cultures. Samples were collected after 20 h of induction. Total proteins and those contained in separated IB´s (5) were analyzed by SDS-PAGE. The rSMD was identified by Western Blot and quantified by densitometry. IB´s were observed by transmission electron microscopy.

Results. The kinetic comparison between shake flask using LB and SB medium (Fig.1) shows a similar specific growth rate (Table 1), but an increase of 30% of rSMD productivity in SB cultures. With the better medium, BL21-SMD was cultured in bioreactors with and without pH controlled. Both cultures presented a similar specific growth rate (Table 1). A maximum of 13 D.O. was reached in culture with pH control while in culture without pH control was 18 D.O. (Fig 1). Importantly, pH condition modified the size and structure of the IBs. In cultures without pH control, IBs measured around 0.41 to 0.50 µm, while under controlled pH conditions (7.5) smaller IBs were found. The IBs rSMD productivity was enhanced under without pH control. To evaluate the effect of culture conditions over IB´s, the physiochemical conditions are being evaluated.

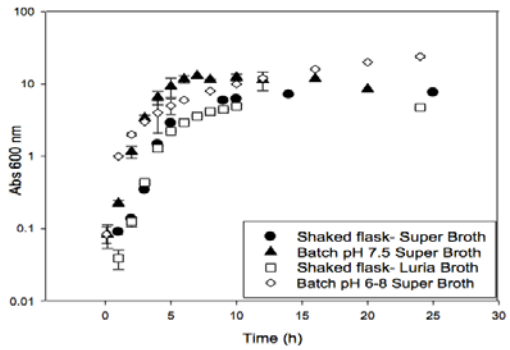

Fig.1 Kinetic characterization of *E.coli* BL21 producing rSMD in different media and pH conditions.

Table 1. Kinetic growth comparison different media and pH conditions of *E.coli* BL21 producer of rSMD.

Culture condition	µ (h^{-1})
Shake flask LB	1.17±0.03
Shake flask SB	1.19±0.04
Reactor SB without pH control	1.37±0.01
Reactor SB pH 7.5	1.36±0.09

Conclusions. The improvement in nutrients in SB seems to favor metabolism to form IBs containing a higher amount of rSMD in shake flasks. Variations in pH affect the size, shape and number of the IBs per cell. Although it has been reported that the *E. coli* cytoplasmic pH does not change drastically (6) under different pH cultivations, those changes affect responses or the environment that modify IBs formation.

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