Zagazig J. Pharm. Sci., December 1996
Vol. 2, No. 2, pp. 32-40

ISSN 1110-5089

EXPRESSION OF MOUSE ANTICREATINE KINASE-M (MAK 33), FAB FRAGMENT OF IgG ANTIBODY IN THE METHYLOTROPIC YEAST *HANSENULA POLYMORPHA*

1- Kappa Chain

Hassan A. Abdel-Salam[1], Fathy M. E. Serry[1], Ralf Gatzke, Hemmet K. Abdel-Latif[1] and Cornelis P. Hollenberg[2]

[1]Department of Microbiology, Faculty of Pharmacy, Zagazig University, Egypt.
[2]Institut für Mikrobiologie, Heinrich-Heine Universität, Düsseldorf, Universitätsstr. Düsseldorf, Germany.

ABSTRACT

An expression plasmid was constructed and transformed to *Hansenula polymorpha* cells to produce kappa chain peptide of Fab fragment of the MAK33, IgG antibody. The yeast *Hansenula polymorpha* was shown to be one of the most efficient host cells for expression of genetically engineered antibody genes. Although the rate of transformation of this yeast by polyethylene glycol was low, 3-5 cells/plasmid DNA, yet the transformants showed high mitotic stability for more than 100 generations. The expression plasmid was integrated within the yeast genome in one or more integration site (s) with low and multicopy-number plasmid via non-homologous integration mechanism. A N-terminal glucoamylase gene fragment was linked to the light chain (kappa) gene of the F_{ab} derivative of the MAK 33 antibody. Prepro-alpha factor of the yeast *S. cerevisiae* was inserted into the plasmid between glucoamylase and light chain genes as a secretion signal sequence for the light chain peptide. The kappa chain was produced as 50 mg/L free protein in the culture medium and 500 mg/L entrapped within the cells, amounting 550 mg/L, representing about 10% of the total cell protein. The prepro-factor was shown to be incompletely processed in the yeast *Hansenula polymorpha* and the pro-segment was accompanied with the light chain peptide.

INTRODUCTION

In 1975, the hybridoma method was described for making cell lines that can secrete a single species of antibody called monoclonal antibody (MCA) with the desired specificity to antigen (1).

However, these mammalian expression systems are highly expensive and have low productivity(2), and the cells can grow and prodnce limited number of generations and do not survive in suspension culture for a long time (3).

Sacchromyces cerevisiae was considered as one of the most efficient and well characterized host organism for expression and secretion of heterologous proteins (4). However, the limitations encountered with *S. cerevisae*, including their genetic instability (5), hyperglycosylation (6) and poor secretion effeciency(7), necessitate the search for anather candidate expression host .

Hansenula polymorpha, a methylotrophic yeast has emerged as potentially useful expression host (8). Gellissen et al (9), were able to construct an expression vector for *H.Polymorpha*, liberating the formate dehydrogenase (FMD) and methanol oxidase (MOX) terminator as regulatory elements. This vector would direct experssion and secretion of the recombinant protein if a secretory signal sequence is included (10,11). A variety of secrection signals sequences can be used for protein export; including that of *S. cerevisiae* mating factor (MF) and that of *Schwanniomyces occidentalis* glucoamylase gene (12).

H.Polymorpha, has high frequency of integration of plasmid DNA and produces mitotically stable transformants (12). Recently, *H.Polymorpha*, has been shown to be an ideal host for expression of different heterologous proteins (12,13) and simultaneous expression and secretions of others (9,10,14)

In the present study, *H.Polymorpha* was employed for expression of kappa chain of F_{ab} fragment of Ig G, MAK 33.

MATERIAL AND METHODS

Plasmids :

1- The pBR322 plasmid carrying cDNA of kappa chain was a gift from Boehringer Mannheim, Germany . The cDNA of kappa chain is released as 1.0 Kb fregment folowing *B am* H1 -*Eco* R1 treatment .

2- The *H. polymorpha*, expression vector, pFPMT130(9) plasmed, was a gift from Rhein Biotec, Düsseldorf, Germany .

3- PUC19 GA1 plasmid carrying 1.9 Kb N-terminal fragment of glucoamylase gene of *Schwanniomyces occidentalis*, was also a gift from Rhein Biotec .

4- pGRT 1.16 plasmid, carrying prepro-alpha mating factor (MF) secretion signal of *S. cerevisiae* as a 0.25 Kb segment was a gift from Professor Reipen, Institute für Mikrobiologie, Universität Düsseldorf, Germany.

Strains

Hansenula polymorpha LR9 strains, *ura 3*, uracil auxotroph, as host strain for the integration and expression of the plasmid pGAGU (Fig. 1). *Escherichia coli* strain DH5Fα (Hanahan et al)[16] was obtained from Gibco, BRL, USA, and used for vector construction and amplification. It was grown in LB medium supplemented with 150 (g/ml ampicillin).

Transformation

E.coli was transformed as described by Glove et al[17] and *H. polymorpha* was transformed by polyethylene glycol (PEG) as described by Klebe et al[18] and Dobmen et al[19].

Molecular methods

Southern and Northernblot analyses, as well as *in vitro* DNA manipulation were carried out according to the standard methods of Sambrook et al.[20]. Western blot was carried out according to Towbin et al[21].

DNA analysis

For Southern analysis, the cells were grown on YPD for 16-24 hrs to heavy growth, until the cells flocculate in the culture. After centrifugation and cell collection, the total DNA was isolated according to Davis[22]. About 0.5 µg DNA was digested with the appropriate restriction enzyme (s), electrophoresed in 0.8% agarose gel and transferred to Nylon membrane filter (Amersham) to be hybridized by radioactive probe as described by he Manufacturer (Boehringer Mannheim, Germany)[18].

RNA analysis

For Northern analysis, yeast cells were grown in YNBMG medium (induction condition) to OD of 0.8-1.0 at $\lambda_{600\ nm}$. Cells were collected by centrifugation and lysed using Glass beads (0.45 Ø mm). Total RNA was extracted from the lysed cells, collected from about 20 ml culture according to Ausubel[23]. About 10 µg per quantities of total RNA, wer loaded into RNA-formaldehyde, agarose 1.2% gel, prepared and run according to Manniatis et al.[24]. Electrophoresed, RNA was transferred into nitrocellulose membrane filter (Schleidcher Schwell, Dassel Germany), to be hydridized with glucoamylase cDNA fragment, radioactive probe according to Feinberg and Vogelstein[25].

Cultures media

Hansenula polymorpha was grown in non-selective medium, 1% (w/v) . Difco yeast-extract, 2% (w/v) Difco Bacto-peptone (YP) supplemented with 2% w/v Dextrose (YPD medium). The selective medium, yeast nitrogen base (YNB) consisted of 0.17% w/v yeast nitrogen base (Difco), 0.5% (w/v) ammonium sulfate (Merck) plus 2% (w/v) glucose in YNBG medium or 0.5% (v/v) glycerol in YNBMG medium.

Protein analysis

Cell-culture supernatant : Cultures were centrifuged at 10,000 rpm for 10 min., and proteins in the supernatant were precipitated by 4% sodium deoxycholate and 10%, trichloroacetic acid TCA. The precipitate was washed once with cold acetone and dissolved in phosphate-buffered saline (PBS). About 20 (g proteins in about 20 µg, representing 10 ml culture were analysed by SDS-polyacrylamide gel according to Laemmli[26].

Cell-free extract : The cells were collected by centrifugation, disintegrated by Glass beads according to Ausubel et al.[23]. The clear supernatant proteins were analysed by electrophoresis in 12% SDS-polyacrylamide gel and either directly stained with coomassie brillient blue (CBB) or alternatively transferred into nitrocellulose membrane filter for carrying out western blot analysis. The expressed kappa chain peptide was visualized by anti-kappa, antibodies obtained form Boehringer Mannheim .

Quantitative analysis of protein : Was carried out according to Bradford method[27].

RESULTS

The ability of *Hansenula polymorpha* to produce and secrete genetically engineered forms of antibodies was tested by expressing derivatives of the mouse anti-creatine kinase IgG antibody. The $F(ab')_2$ fragment of this monoclonal antibody has been previously expressed in non-lymphoid hybridoma cell lines and the yield from the culture medium was too low [28].

Plasmid construction

Hansenula polymorpha strain was transformed with a shuttle plasmid vector constructed to carry the light chain (k) gene and glucoamylase fragment F (Fig. 1). The light chain cDNA and the first N-terminal, 1.9 kb of the glucoamylase, containing its secretion signal sequence were linked to the expression cassette which consists of the FMD promoter, 1.2 kb, and the MOX

terminator, 0.350 kb in the *H. polymorpha* expression vector (pFMD plasmid)[12]. The prepro-alpha factor was inserted in between the glucoamylase fragment and the light chain gene.

The constructed plasmid pGAKU (Fig. 1) was used to transform *H. polymorpha* LR9 to construct the light chain producing strain. The transformation resulted in 3-5 colonies per (g plasmid DNA. The expression plasmid pGAKU is a shuttle and integrating vector, and transformation of *H. polymorpha* with such plasmid is based on integrating the plasmid into the yeast chromosome, which demonestrated by:

i) Stability and enrichment of the uracil$^+$ phenotype transformants after growth on YPD medium (non-selective) for more than 100 generations, followed by :

ii) growing the transformants in YNBD medium (selective) and the transformants were grown to high cell density; 10^{10} cells/ml; and by:

iii) Southern analysis.

Southern analysis:

Southern analysis (Fig. 2) revealed that, the plasmid was integrated at different sites within the yeast chromosome, with heterologous rather than homologous integration. The intensity of the bands suggests that the plasmid behaviour was either multiple integration with low copy-number, as in transformants in lane 4, 8, and 10, or multicopy-number integration as in transformants in lane 5, 7, 9, and 12. Chromosomal DNA of the wild type cells showed a faint band representing the original chromosomal genes of formate dehydrogenase and/or uracil genes as the membrane was hybridized by the digested expression plasmid.

Northern analysis:

Northern analysis was carried out to verify the efficiency of transcription elements (promoter and terminator), and to determine the level of transcription of kappa mRNA under the induction conditions.

Figure (3) revealed that cells transformed with plasmid pFMD (lacks kappa, GAM, and MF-(, cDNAs), in lane 1, did not show band of 3.1kb in size, equivalent to the single strand mRNA of light chain, pre-pro-MF-α and glucoamylase. On the other hand, cells transformed with pGAKU plasmid showed bands of 3.1kb with different intensities. Comparing Northern and Southern analyses, there is a relation between the plasmid copy number and the level of the transcribed kappa mRNA. Both transformants No. 6 and 9 showed multicopy-number of the integrated plasmid as well as high level of mRNA transcription. Contrary, transformant No. 1 showed high level of transcription of mRNA transcription despite the low copy-number of the plasmid integrated into the yeast chromosome. These observation shows that the level of transcription may or may not depends on the number of plasmid copy-number but depends mainly on the transcription factor level.

Characterization of light chain (k) and glucoamylase protein:

A transformant of *H. polymorpha* with pGAKU (No.9) was induced by growing in YNBMG medium in parallel with a transformant with the same plasmid but lacks the expression cassette (pFMD plasmid).

Cell culture supernatant: about 20 (g protein in 20 (l, representing 10 ml of the original culture medium were loaded and electrophoresed in 12% SDS- polyacrylamide gel. Figure (4) shows the cell culture supernatant protein after electrophoresis and staining with coomassiee brilliant blue (CBB). The supernatant protein shows 3 bands more than those bands seen in that of the supernatant of control cells, a distinct large band in the approximate molecular weight of 67 kda which is expected to be the band of glucoamylase peptide, (seized 1.9 kb= 630 amino acids).

In addition there are two additional bands; one of about 37 KDa and the second band is approximately 30 KDa. The band of 37 KDa most probably, represents the pro-alpha and kappa in one fusion peptide, however, such band is somehow larger in molecular weight than the calculated one (seized 900 bps= 300 amino acids). The second band of about 29KDa possibly would represent the completely processed kappa peptide but it is also larger than the expected molecular weight (seized 657 bps= 219 amino acids). The larger molecular weight of the expressed proteins may be attributed to the processing and secretion conditions of the foreign host cells of *H. polymorpha* that of the original mouse plasma cells.

Cell-free extract : the cell-free extract of the same induced cells were pooled and about 20 (l volume containing approximately 50 g) proteins were electrophoresed in 12% polyacrylamide gel and stained with CBB as in Fig. (5). There are similar bands to those are seen in the cell culture supernatant, mentioned before, that were missing in the control. However, the intensity and amount of proteins in the bands sized 37 and 29 KDa are several times greater in the cell-free extract than in the cell culture supernatant. This indicate that the expressed proteins within the cell is more than that secreted outside.

In contrast, glucoamylase band was more intense in supernatant than in cell-free extract which indicate that glucoamylase protein is efficiently secreted to outside

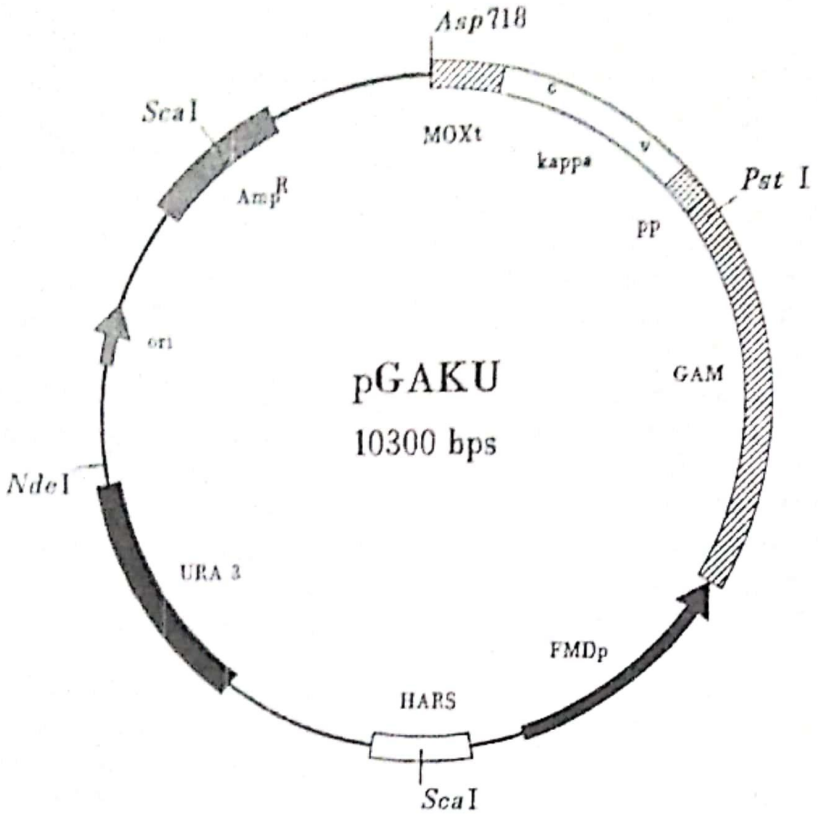

Fig. (1) : Diagram of the expression vector, pGAKU plasmid, containing a kappa coding region. The coding region cDNA of kappa gene was inserted in between FMD promoter and MOX terminator. A 1.9 kb fragment from the N-terminal of the coding region of glucoamylase (*Schwanniomyces occidentalis, GAM1*) with its secretion signal sequence was ligated in between the FMD promoter and kappa gene. Prepro-(was inserted in between kappa and glucoamylase genes to be a secretion signal sequence for kappa chain peptide.

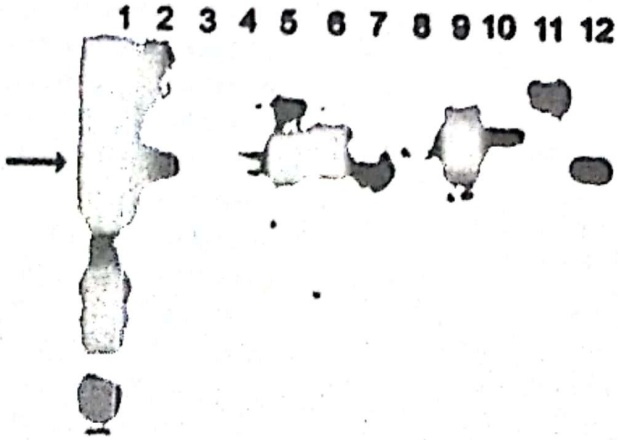

Fig. (2) : Southern analysis of the pGAKU transformed yeast cells of *H. polymorpha* LR9. Lane 1, -DNA. EcoRI/HindIII; lane 2, authentic plasmid pGAKU; lane 3, chromosomal DNA of the wild type; lane 4-12, are the chromosomal DNA of different pGAKU transformants No. 1, 2, 3, 4, 5, 6, 7, 8, and 9 respectively. Arrow indicates the location of the expression cassette. The chromosomal and plasmid DNAs were digested by ScaI before analysis.

the cell. Correlating between Southern, Northern analyses and amount of protein expressed, there is no distinct difference in between cells with multicopy-number plasmid and high transcription level and that of low copy-number plasmid and high transcription level to the amount of protein expressed either secreted or entrapped inside the cells.

On estimating the amount of protein, the amount of the expressed light chain (k) of the MAK33 antibody derivatives in *H. polymorpha* LR9 host cells were 50 and 500 mg/L, secreted and non-secreted, respectively, totaly 550 mg/L and represents about 10% from the total cell proteins under the control of FMD promoter and induction condition.

Western blot

Western analysis of the cell culture supernatants and cell-free extracts of both control and transformants was carried out to identify the CBB-stained gel bands. Fig. (6) shows the results of such experiment. In western analysis, the protein bands were transferred to nitrocellulose membrane filter after electrophoresis in 12% SDS-polyacrylamide gel. Rabbit Anti-MAK 33, kappa-specific, antibody were used to probe that the nitrocellulose blots of both culture supernatants and cell-free extracts. While rabbit antiglucoamylase antibody was used to probe that of the culture supernatant. The bands were visualized by anti-rabbit alkaline phosphatase-conjugated antibodies.

The specific antibodies confirmed the identity of bands located before in CBB-stained gel. Glucoamylase band appeared at about 67 KDa and was identified by its specific antibody. Kappa chain (at about 29 KDa) and pro alpha-kappa chain (at about 37 KDa) were identified by their specific antibodies as 2 bands in either cell culture supernatants or cell-free extracts.

DISCUSSION

The methylotrophic yeast *Hansenula polymorpha* like *Saccharomyes cerevisiae* offers several advantages for the expression of recombinant proteins, non of the least, is the lack of detectable endotoxins [29]. It has also a well-characterized secretory pathway and secretes only a little of its own proteins into the medium. The secreted heterologous polypeptides can make up more than 90% of the all secreted protein [12]. In our case, like hirudin, the secreted protein remains stable in the fermentation medium, and exhibit little or no proteolytic degradation [12,30].

Foreign eukaryotic genes, like MAK33 genes, can be expressed in yeast cells if provided with a yeast promoter and terminator. The use of tightly controlled promoter and terminator elements of the methanol metabolic pathway (FMD and MOX genes) enable tight regulation of expression in the yeast *H. polymorpha* [14]. Also, synthesis of the desired product can be regulated to occur at the end of fermentation process when a biomass has been produced and thus the deleterious effects of the heterologous protein to the host cells can be kept minimum.

Gene fusion is used for expression of recombinant protein in heterologous hosts for several reasons. Among these reasons, is to avoid degradation of the foreign protein, to make recovery and purification of the product more easier and to enhance the stability of the expressed protein [31].

H. polymorpha expression system has the unique feature of the possibility of integrating up to 100 copies of an expression cassette via multimeric and non-homologous integration mechanism which reduce the risk of instability of production strain [15]. The stability of phenotype (uracil+) up to 100 generations means the integration of URA 3 gene into the yeast chromosome.

The expression of certain eukaryotic gene is, at least, partially controlled at the level of mRNA transcription[32]. Northern analysis of light chain mRNA showed the abundence under the induction condition.

Our results suggest that the relation between mRNA and plasmid copn-number in the transformants is not linear. This could be explained on the basis that one or more of the integrating expression cassettes are not functional. Another explanation for these finding could be attributed to the low stability of light chain mRNA under the stated conditions. The level of mRNA in cell is determined by the the rate of transcription of the gene and the rate of degradation of a mRNA for maintenance of flixibility and accuracy in the gene expression.

It is well-known that efficient secretion of foreign protein could be better achieved when it is fused to a secretion signal sequence [33]. The pre-pro sequence of the mating factor-(of the yeast *S.cerevisiae* was considered one of the most characterized and used secretion signal sequence for secretion of foreign protein in the yeast *S. cerevisiae* [34]. In the present study, the pre-pro sequence was used for the first time as a leader sequence for secretion of light chain peptide to the secretory pathway of the yeast cells of *H. polymorpha*. Kurjan and Herskowitz, [35]. postulated that the pre-α peptide should be cleaved by signal peptidase in the Endoplasmic Reticulum (ER) and the propeptide should be cleaved by another endopeptidase

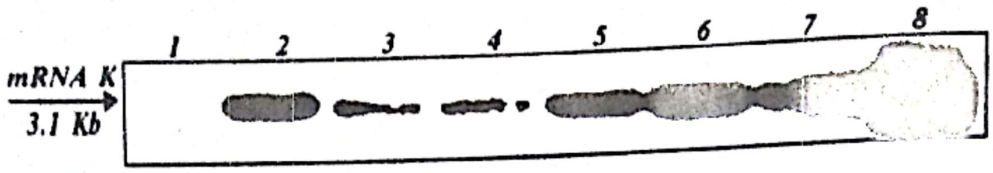

Fig. (3) : Northern analysis of the kappa mRNA of the induced cells of *H. polymorpha* LR9. lane 1, control (pFMD-transformant); lanes 2-8, are pGAKU-transformants No. 1, 2, 3, 4, 6, 7, and 9. A band of about 3.1 kb representing the expected mRNA of kappa, pp-(factor, and glucoamylase coding regions that are missed from the control.

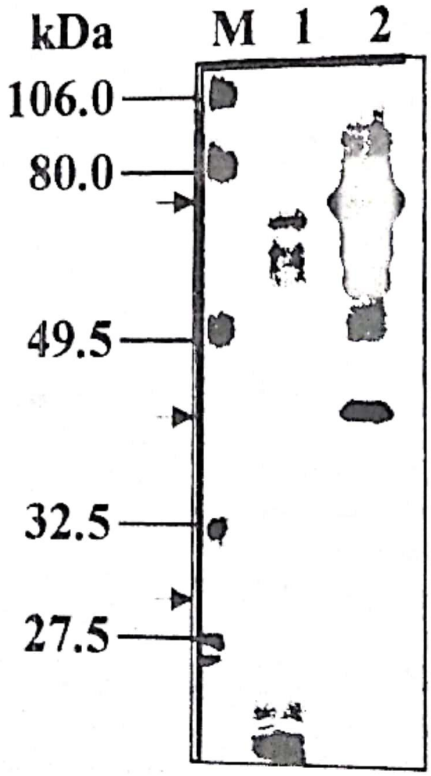

Fig. (4) : 12% SDS-polyacrylamide gel stained with coomassie brillient blue (CBB) of the cell culture supernatant of the induced cells of *H. polymorpha* LR9. Lane M, molecular weight marker (in left side); Lane 1, control (pFMD-transformant); lane 2, pGAKU-transformant No. 9. The pGAKU-transformant showed three distinct bands that are missed from control and indicated by arrows in the left side, the first band is glucoamylase band (67 KDa), pro-(with kappa chain in one peptide (37 KDa) and completely processed kappa chain peptide (30 KDa).

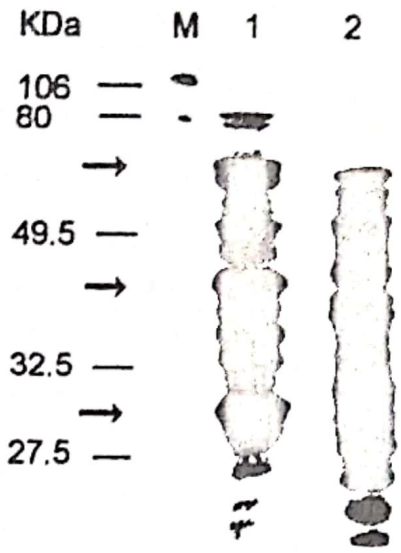

Fig. (5) : 12% SDS-polyacrylamide gel stained with coomassie brillient blue (CBB) of the cell extract of the induced cells of *H. polymorpha* LR9. Lane M, molecular weight marker (in left side), lane 1, pGAKU-transformant No. 9; lane 2, control (pFMD-transformant). Like in the cell culture supernatant, there are 3 distinct bands for glucoamylase at about 67 KDa, pro-(and kappa in one peptide at about 37 KDa and completely processed but unsecreted kappa peptide at about 30 KDa, the bands are missed from control.

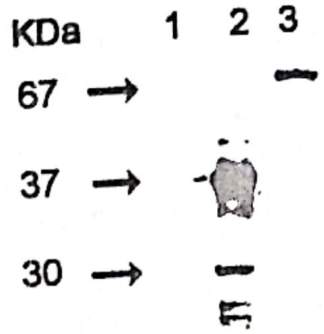

Fig. (6) : Western analysis of the cell culture supernatants (A and C) and cell extract (B) of *H. polymorpha* LR9 transformants No. 3 (lane 1) and 9 (lane 2).

A and B, detected by anti MAK33 kappa specific antibodies.

C detected by antiglucoamylase antibodies.

MW marker in the left side, and a wide range of MCAs which bind to proteins, carbohydrates, nucleic acids and hapten antigens have been produced (2).

(KEX2) in Golgi bodies in the yeast *S. cerevisiae*, after translation and translocation in the ER.

In the expression cassette of light chain, there are both glucoamylase and preprosecretion signal sequences in one peptide i. e. there are two recognition sites for signal peptidase in the ER lumen, which may affect the rate of cleavage and consequently in the rate of secretion.

Also, the propeptide was mostly conjugated to the light chain peptide. This could be due to the presence of insufficient KEX2 enzyme to cleavage the pro-peptide. This assumption conform with the previous report that the signal peptidase is not required for processing of prepro and the prepropeptide is processed by endopeptidase (KEX2) in Golgi bodies [36]. Providing this is the case, KEX2 enzyme would be the rate limiting step in the secretion of proteins when prepro-α is used as secretion signal sequence.

The secretion rate of glucoamylase to the culture medium was found to be high. This means that glucoamylase is rapidly folded and assembled into its secretion form so it is rapidly secreted to outside the cell. Overexpression secretion lethality, missfolding and missassembling are other factors that could lead to malsecretion of light chain peptide.

Pérez-Pérez[37], noticed that the overexpression of foreign protein caused lesions in the secretion system and they were able to increase the secretion efficiency by supplementing the exoression plasmid by one or more secretory cloned genes.

The higher molecular weight of the expressed protein more than to calculated one could be attributed to the glycosylation, the pro-α has 3 N-glycosylation sites[38]. Missfolding and missassembling of the expressed protein could be other reasons responsible for altered mobility on the gel and subsequently appeared high in their molecular weight. Expression of genetically engineered proteins have mostly suffered from such viability and appeared with higher molecular weights.

ACKNOWLEDGEMENTS

We are grateful for the Egyptian Mission Authority, Cairo, Egypt, for financial support of this work. We thank Heinrich-Heine University for the permission to do this work in the laboratory of Institute of Microbiology.

REFERENCES

1. Kohler, G. and Milstein, C.; **Nature**, 256, 495-497 (1975).
2. Mueller, B.M.; Romanelli, C.A.; Gillies, S.D. and Reisfeld, R.A.; **J. Immunol.**, 2, 239-256. (1990).
3. Freshney, R.I.; Culture of animal cells. A manual of basic technique, A.R. Liss, NY., (1983).
4. Kingsman, S.M; Kingsman, A.J.; Dobson, M.J.; Reiker, J. Roberts, N.A.; Heterologous gene expression in *Saccharomyces cerevisiae*. In "Yeast biotechnology" Edited by Russell, GE. pp. 113-152. Intecept LTD, : Winborne, Dorset (1988).
5. Da Silva, N.A., and Baily, J.E.; **Biotechol. Bioeng.** 37, 308-317 (1991).
6. Innis, M.A.; Glycosylation of heterologous proteins in *Saccharomyces cerevisiae* In "Yeast genetic engineering, Edited by Barr, P.J, Brake, A.J, and Valenzuela, P., pp: 233-246 (1989).
7. Ernst, J.F.; Improved secretion of heterologous proteins by *Saccharomyces cerevisiae* : Effects of promoter substitution in α- factor fusions DNA 5, 483-491, (1986).
8. Gleeson, M.A; and Sudbery, P.E.; **Yeast**, 4, 1-15, (1988).
9. Gellisson, G., Janowicz, Z.A., Merckelbach, A., Piontek, M., Keup, P., Weydmann, V.; Hollenberg, C.P. and Strasser, A.M.; Heterologous gene expression in *Hansenula polymorpha* : Efficent secretin of glucoamylase biotechnol 9 291-295, (1991).
10. Janowicz, Z. A., Merkelbach, A., Eckart, M. R. Weydmann, U., Roggemkamp, R. and Hollenberg, C. P. **Yeast**, 48, 155. (1988).
11. Hollenberg, C. P. and Janowicz, Z. A.; DNA molecules coding of FMDH control region, EPA No. 0299108 (1987).
12. Janowicz, Z.A., Melber, K., Merkelbach, A., Jacobs, E., Harford, N., Comberbach, M. and Hollenberg, C. P.; **Yeast**, 7, 431-443. (1991).
13. Shen, S. H., Bastien, L., Nguyen, T., Fung, M. and Slilaty, S. N., **Gene**, 84, 303-309. (1989).
14. Hodgkins, M.A., Mead, B., Ballance, D.J., Goody, A. and Sudbery, P., **Yeast**, 9, 625-635, (1993).
15. Roggemkamp, R., Hansen, H., Eckart, M. Janowicz, Z.A and Hollenberg, C. P., **Mol. Gen. Genet.**, 202, 302-308 (1986).
16. Hanahan, D. (1983). **J. Mol. Biol.**, 166, 557-580.
17. Glover, D.M.; DNA cloning, the practical approach, Vol. I, Edited S., Richwood, D. and Hames, B. D., Oxford Washington, pp. 121-127. (1976).
18. Klebe, R. J., Harriss, J.V., Sharp, Z.P. and Douglas, M.G. **Gene**, 25, 333-341. (1983).
19. Dohmen, J., Strasser, A. M., Hoiser, C. B. and Hollenberg, C.P., **Yeast**, 7, 691-692. (1991).
20. Sambrook, J., Fritsch, E. F. and Maniatis, T. Molecular cloning. A laboratory manual. Cold Spring Harbor press NY. (1989).
21. Towbin, H., Staehelin, T. and Gordon, J., Proc. Natl Acad. Sci. SY., 76, 4350-4354. (1979).
22. Davis, R.W.; Thomas, M. Cameron, J. John, P. Scherlis, S. and Padgett, R., **Meth. Enzymol.**, 65, 404-411. (1980).
23. Aausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D.

Seidman, J. G.; Smith, J. A. and Struhl, K.; Current protocols in molecular biology. Vol. I and II Publisher by Wiley, USA. (1989).

24. Manniatis, T., Sambrook, J. and Fritsch, E.F.; Molecular cloning. Cold Spring Harbor Laboratory Press, NY. (1982).

25. Feinberg, A. P. and Vogelstein, B.; **Anal. Biochem.**, 137, 266-267. (1983).

26. Laemmli, U.K.; **Nature**, 227, 680-685, (1970).

27. Bradford, M. M.; **Anal. Biochem.**, 72 : 248-254. (1976).

28. Weidle, U. H., Orgya, A.; Mattes, R., Lenz, H. and Buckel, P.; **Gene**, 51, 21-29. (1987).

29. Novick, P.; Ferro, S. and Schekman, R.; **Cell**, 25, 461-469. (1981).

30. Achstetter, T., Nguyen, J.M., Findeli, A., Merkamm, M. and Lemoine, Y.; **Gene**, 110, 25-31. (1992).

31. Uhlen, M. and Moks, T.; **Methods in Enzymology**, 185, 129-143. (1990).

32. Melefors, Ö. and Hentze, M. W.; **BioEssays**, 15, 85-90, (1993).

33. Kuhn, A. and Wickner, W.; **J. Biol. Chem.**, 260, 15914-15918, (1985).

34. Brake, A.J.; Merryweather, **J.P., Coit**, D.G., Heberlein, U.A.; Masiarz, F.R.; Mullenbach, G.T.; Urdea, M. S.; Valenzuela, P. and Barr, P.J.; **Proc. Natl. Acad. Sci.** NY., 81, 4642-4646, (1984).

35. Kurjan, J. and Herskowitz, I.; **Cell**, 30, 933-943. (1982).

36. Julius, D.; Brake, A.; Blair, L.; Kunisawa, A. and Thorner, J.; **Cell**, 37, 1075-1089. (1984).

37. Pérez-Pérez, J.; Mrquez, G.; Barbero, J.L. and Gutiérrez, A.; **Biotechnol.**, 12, 178-180. (1994).

38. Julius, D.; Schekman, R. and Thorner, J.; **Cell**, 36, 309-318. (1984).

استخدام خميره الهنسنيولابوليمورفا فى تخليق جزئ إميونوجلوبيولين جاما (IgG) الفئران ، الجسم المناعى المضاد لانزيم كيرياتينين كيناز (MAK 33) :

١- السلسلة الخفيفه (كابا)

حسن عبد السلام - فتحى سرى - رالف جاتزك* - همت عبد اللطيف - كورينالز هولينبرج*

قسم الميكروبيولوچى - كلية الصيدلة - جامعة الزقازيق - مصر

* معهد الميكروبيولوچى - جامعة دوسيلدورف - ألمانيا

فى هذا البحث وباستخدام تقنيات الهندسة الوراثية أمكن استخدام خلايا خميرة ***الهنسنيولا بوليمورفا*** فى ترجمة الشفرة الوراثية للمقطع Fab من جزئ بروتين الاميونوجلوبيولين جاما الذى تنتجه الفئران كأجسام مضادة لانزيم كيرياتينين كيناز (الآدمى) والذى يستخدم كأداة تحليليه مفيده فى تشخيص بعض الحالات المرضيه .

وقد تم تصميم وتشييد بلازمين مكوكى حامل للمورثات الخاصة بهذا البروتين ومورثات أخرى خاصة بالتحكم فى انتاجه وافرازه خارج الخلايا وتم نقل هذا البلازميد الى خلايا الخميره الهجين لتفرز البروتين المطلوب .

وقد وجد ان الخلايا الهجين يحكمها انتاج البروتين بكفاءه عاليه حيث يمثل انتاجها من البروتين المطلوب ١٠٪ من الانتاج الكلى للبروتين فى خلايا الخميره وبواقع ٥٥٠ مجم للتر من مزرعه الخميره يفرز منها قرابة ٥٠ مجم خارج الخلية ويبقى حوالى ٥٠٠ مجم بداخل الخلايا ، ومن مميزات هذا الهجين الجديد درجة ثباته العاليه وراثيا حيث أن المورثات الجديدة قد أدخلت فى مواضع عديده داخل صبغيات الخلية .