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Biotechnological studies for reusable production of bacteriocin using LAB immobilized cells

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ABSTRACT

Bacteriocins have been described as antimicrobial compounds that are produced by bacteria. Immobilized cell technology has been successfully improved the enzymes thermal stability, to stand the temperature used in food industries and still active. In this work, Lactic Acid Bacteria (LAB) *Leuconostoc mesentroides* was isolated from muscle of the domestic goat from Jeddah-Saudi Arabia, and immobilization on different materials like glass wool, cork, Sodium alginate, Linen fibers and polyurethane foam. The immobilization of the bacteria on alginate has shown the highest antimicrobial activity of the enzyme. Effect of different number of bead as inoculum size on bacteriocin production also tested. The immobilization of LAB on alginate has shown better results for repeated use of bacteria for successive eight times with retention of over 85% of the bacteriocins activity as compared to complete loss of activity and disruption of the control. It concluded that, the immobilization of LAB on alginate has shown better results for enhancement the production of bacteriocin.

Keywords: immobilized, bacteriocin, Lactic Acid Bacteria, isolation, production

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INTRODUCTION

Lactic acid bacteria occur naturally in several fermented foods such as fermented vegetable, fermented fish, fermented meat and fermented milk (Salminen *et al*., 2004). Lactic acid bacteria have been long established as the normal flora in fermented food, because they are believed to be safe; thus, they have senior scope for use in biopreservation (Dubourg et al 2015). The preserve effects of lactic acid bacteria because of the production of bacteriocin or related substances as antimicrobial agents (Cocolin *et al*., 2007).

Most bacteriocins LAB are cationic peptides at a neutral pH, hydrophobic in nature and amphiphilic, containing 20 to 60 amino acids (supplied by the following amino acids, proline, alanine, leucine, valine, isoleucine, methionine, phenylalanine and tryptophan) (Diep and Nes, 2002). The activity of bacteriocin is regarding to these properties when acting on the cytoplasmic membrane (Bertrand et al 2001), where the positive charged of proteins which bind to negative charged of phospholipids that part of the membrane of sensitive cells (Cotter et al., 2005).

Fermentation of Bacterial perishable raw materials have been used for centuries . Adding bacteriocins to food products enhances nutritional properties and organoleptic of processes by lowering the chemical preservatives use (Ross *et al*., 1999 and Dabaand and Saidi 2015). The purification of bacteriocins is often laborious, time-consuming, and difficult needs experience since they are often small and very hydrophobic compounds produced in very low amounts.

Cell immobilization is defined as the fix of fit cells to a limit area of space with the preserve of catalytic activity (Karel et al., 1985). Immobilizing of molecule is one whose movement in space has been restricted either completely or to a small limited region by attachment to a solid structure (Yu-Qung *et al*., 2004).

The technology of Immobilized cell has been successfully appointed in several kinds of lactic acid bacteria fermentation processes using. Immobilized of cell go ahead stabilize the activity of bioreactors in sequential operations, plasmid stability, increasing bacteriophage resistance and lowering the inhibition by antibiotics or salts (Champagne *et al*., 1994).

This study focused on improving the production of bacteriocin by *(L. mesenteroides*) and its immobilization on polymers

MATERIALS AND METHODS

Lactic acid bacteria

Lactic Acid Bacteria (LAB) *Leuconostoc mesentroides* which produces bacteriocin was used in the investigation. This strain was isolated from muscle of the domestic goat from Jeddah-Saudi Arabia. *Leuconostoc mesentroides* routinely grown on (MRS) agar medium ((De Mann *et al.,* 1960) at 35ºC for 72 days then preserved at -80°C in glycerol.

Bacteriocin assay

Agar well diffusion procedure described by Zhang *et al*. (2010), was used to determine the production of bacteriocin in the culture supernatant using, Gram positive bacteria *S. aureus* ATCC25923, while the Gram negative bacteria were *E. coli* ATCC25422, *K. pneumonia* ATCC700603, *P. aeruginosa* ATCC27583. Inhibition of bacterial growth was measured as inhibition zone diameters in (mm).

Determination free cells in media.

The viability of cells was counted by dilution plating on MRS agar and after incubation for 24 h at 35° C. The data were expressed as CFU/ml (colony forming unites per ml) (Dabaand and Saidi 2015).

Determination of cell concentration in beads

One ml of calcium alginate beads were suspended in 0.1M phosphate buffer followed by gentle shaking for 30 min for destruction of the beads. The number of obtained cells was determined by plate counting method using MRS agar (Suthasinee 2010)

Immobilization *L. mesentroides* on different materials carriers

Glass wool, cork, Sodium alginate, Linen fibers and polyurethane foam were used for immobilization the cell of *L. mesentroides* according to the methods described by Rao *et al*., (2009). About 2g of all of these materials was sterilized in 250 ml Erlenmeyer flasks containing 50 ml of MRS broth medium inoculated with preculture inoculum of *L. mesentroides*. The flasks were incubated at 35°C with agitation at speed 180 rpm for 24 h. At the end of the growth period the growth of inoculated bacterium and the production of bacteriocin were measured as described before.

Effect of different concentrations of sodium alginate on bacteriocin production were used to investigate the optimum sodium alginate concentration for immobilization, different five concentrations of sodium alginate (2%, 4%, 6%, 8% and 10%) were used for the preparation of beads.

Effects of different numbers of bead as inoculum size affected on bacteriocin production were investigated using the same previous condition.

Evaluation of potential reusability of immobilized cells, MRS broth medium inoculated with immobilized *L. mesentroides* and incubated at 35°C for 24 h, then washed with a physiological saline solution and transferred to 250 ml Erlenmeyer flasks containing 50 ml of MRS broth medium fresh. Eight sequential recycles using immobilized cells in sodium alginate repeated degradation tests were carried out at the same operating conditions as in the first cycle.

RESULTS AND DISCUSSION

Isolation of microorganisms

LAB immobilization method

The application of bacteriocins as bio preservatives for vegetable food matrices started approximately 20 years ago. Many studies have pointed on the inhibition of spoilage and/or human pathogenic bacteria by bacteriocins and their application appeared as a good alternative to chemical compounds and antibiotics. Figure (1) expressed in different materials, glass wool, cork, Linen fibers, alginate and polyurethane foam were used as immobilization supports for the *L. mesentroides*. Data shown that the alginate was gave significant positive results for the immobilization in comparison with other materials (glass wool, cork, Linen fibers, and polyurethane foam) that gave negative results after the immobilization.

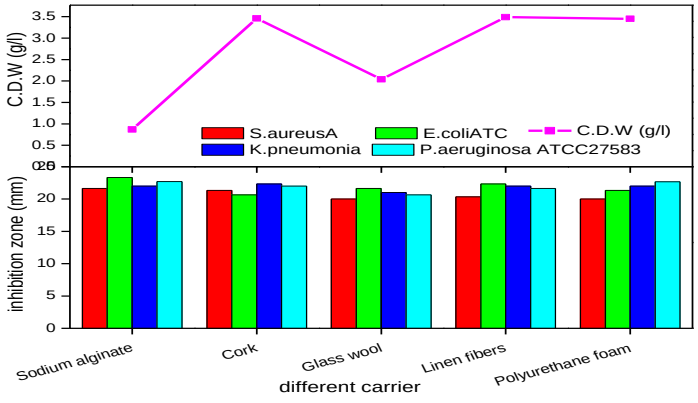

Figure 1: Effect of different concentrations of sodium alginate on bacteriocin production.

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The inhibition zone was ranged from 24.00 to 18.33 mm. The highest inhibition zone diameter was ranged 24mm against *E. coli* ATCC ATCC25422 by sodium alginate and the lowest inhibition zone diameter was 18.33mm against *S. aureus* ATCC25923 by alginate. The bacterial cell survival was minimal by using alginate to be 0.87 g/l. Sodium alginate has eco-friendly nature as it is non toxic and safe for nature, cheap, simply used and mild conditions required for immobilization (Rao *et al*., 2009).

Figure (2) showed the different concentrations of sodium alginate (2%, 4%, 6%, 8% and 10%) tested to determine the optimum concentration of alginate for the best activity of bacteriocin. The highest activity of *L. mesentroides* was obtained with the beads prepared from 6% sodium alginate. On the other hand, the lowest activity of bacteriocin was observed with concentrations (2 and 4%) of sodium alginate. This may be due to over leakage of cells from the immobilized beads due to the large pore size of the beads and the decrease of tight cross links with adding calcium chloride which resulted in a depressed immobilization. Also, we found that an increase of alginate concentration (8% and 10%) led to reduced porosity of the beads. It was reported that the degree of cross linking of the gelatin agent affects the pore size of the beads (Longo *et al*., 1992).

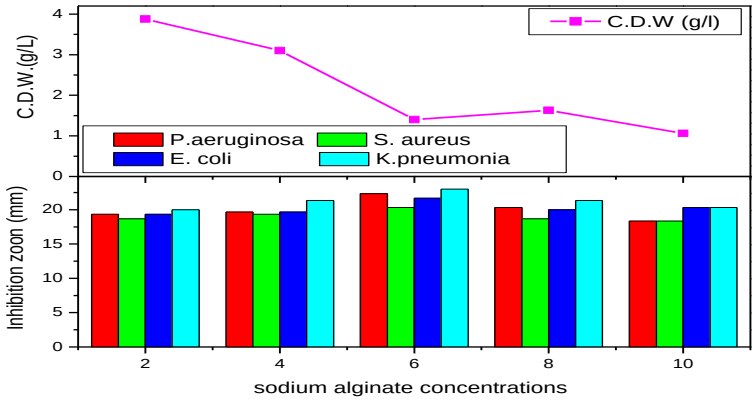

Figure 2: Effect of different inoculum size on bacteriocin production.

Repeated batch cultivation of *L. mesentroides* showed that the escaped cells from concentrations (2%) of sodium alginate were 3.88 g/l while the escaped cells from concentrations (10%) of sodium alginate were 1.06 g/l. These results may be due to the leakage of the substrate and products molecules from the pore of the beads matrix, which demonstrate that the too little concentration of sodium alginate is not recommended as the pore size of the beads lead to increased leakage of the cells from beads. Likewise, the higher sodium alginate concentration leads to smaller pore size of the beads and causes lower immobilization efficiency (Riaz *et al* 2009). Because reduced porosity of the beads limits the nutrient supply and oxygen diffusion to the immobilized cell (Dey *et al*., 2003 and Adinarayana *et al.,* 2004). Figure (3) depicted the effect of different numbers of beads (50, 100 and 150) of 0.6% sodium alginate gel beads added to 50 mL of MRS broth media. Results indicated that the production of bacteriocin reached to 23.66 mm against *K.pneumonia* ATCC700603 at the number of 50 beads, but the production of bacteriocin decreased with increased number of beads inoculum from 100 to 150 beads, reached (19.66-18mm) respectably against *S.aureus* ATCC25923.

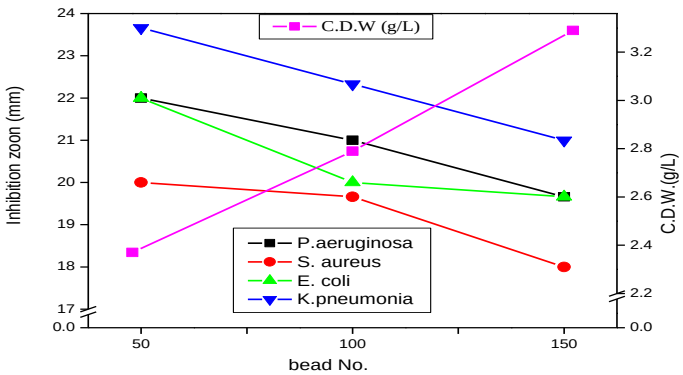

Figure 3: Repeated batch cultivation of isolate M8 cell immobilized on sodium alginate

Hence, the variant with 50 beads inoculum appeared to give the best conditions of inoculums size for production of bacteriocin. Previous studies reported that perhaps the increase of beads number causes high consumption of nutrient in the used media. Also, there was a relation between inoculums concentration and production of bacteriocin by *L. lactis* at different pH (Akinkugbe *et al.* 2013)

Repeated batch cultivation of *L. mesentroides*

Figure (4) showed the possibility of re-using immobilized cells of *L. mesentroides* for bacteriocin production was studied eight cycles of fermentation using immobilized cells in 0.6 concentrations of sodium alginate and 50 bead inoculum sizes.

The result depicted that 100 % of activates was retained after second cycle, the highest inhibition zone reached 23.33 mm against *K.pneumonia* ATCC700603 and the lowest was 20.66 mm against *S.aureus* ATCC25923. In third cycle, the inhibition zone reached 21.33mm against *K.pneumonia* ATCC700603 but the lowest inhibition zone was 19.66mm against *S.aureus* ATCC25923. The begging of the fifth to sixth cycle a sharp fall in the antimicrobial activity, the highest inhibition zone reached 19.66 mm against *K.pneumonia* ATCC700603 and *P.aeruginosa* ATCC27583 while the lowest inhibition zone was 17.33 mm against *S.aureus* ATCC25923. During the seventh and eighth cycles the highest loss of activity was observed.

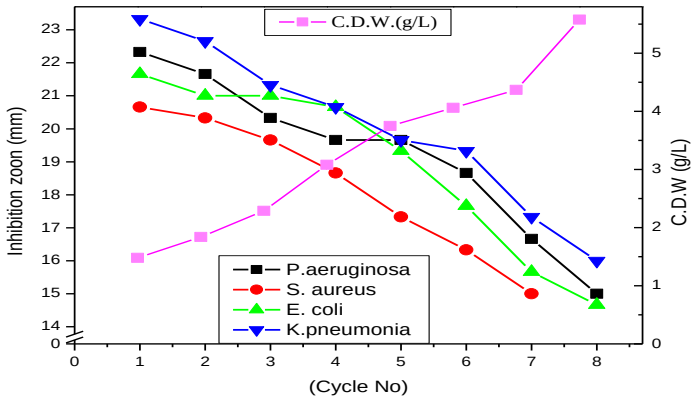

Figure 4: Repeated batch cultivation of isolate M8 cell immobilized on sodium alginate

The reusability of the immobilized cell is important because it proves the efficiency of the product and certify the financial feasibility of bioprocess fixed in immobilized system. The decrease in the activity by the cycle and time was due to the leakage of cell from the beads. The beads lost their tensile strength because of repeated washings at the end of each cycle (Goradia *et al*., 2006).

CONCLUSION

It is concluded that the immobilization of LAB on alginate has been succesed for enhancement the production of bacteriocin.

REFERENCES

[1] Adinarayana, K. Bapi, K.V.V.S.N. and Ellaiah, P. (2004) Investigations on alkaline protease production with *B.subtilis* PE-11 immobilized in calcium alginate gel beads, Process Biochemistry Journal, 39(11): 1331–1339.

[2] Akinkugbe A. O., Onilude A. A., 2013 Selective comparability and physiological studies of lactic acid bacteria protease and Calotropis procera (linn) extracts. Peer J PrePrints 1:e4v1 http://dx.doi.org/10.7287/peerj.preprints.4v1.

[3] Al-Zahrani, S. and Al-Zahrani, F.(2006) Production of bacteriocin by four lactic acid bacteria isolated from raw milk on organic waste, World Applied Science Journal, 1 (20):135-143.

[4] Bertrand, D.C., Lacroix, C. and Champagne, C.P. (2001) High nisin Z production during repeated cycle batch cultures in supplemented whey permeate using immobilized Lactococcus lactis UL719, Journal of Dairy Science, 11: 953-960.

[5] Champagne, C.P., Gaudy, C., Poncelet, D. and Neufeld, R.J. (1994) *Lactococcus Lactis* Release from Calcium Alginate Beads, Journal of Applied & Environmental Microbiology, 58: 1429-1434

[6] Cocolin, L. R., Foschino, G. and Fortina, M.G. (2007) Description of the bacteriocins produced by two strains of *Enterococcus faecium* isolated from Italian goat milk, International Journal of Food Microbiology, 31: 753-758.

[7] Dabaand H. Saidi S. (2015). Detection of bacteriocin-producing lactic acid bacteria from milk in various farms in north-east Algeria by a new procedure. Agronomy Research 13(4), 907–918

[8] Cotter, P.D., Hill, C. and Ross, R.P. (2005) Bacteriocins: developing innate immunity for food, Nature Microbiology Reviews, 3: 777-788.

[9] De Mann, J.C., Rogosa, M. and Sharpe, M.E. (1960) Amedium for the cultivation of *lactobacilli*, Journal of applied bacteriology, 23(1): 130-135.

[10] Dey, G., Singh, B. and Banerjee, R. (2003) Immobilization of □-amylase produced by *Bacillus circulans* GRS 313, Brazilian Archives of Biology and Technology, 46(2): 167-176.

[11] Diep, D.B. and Nes, I.F. (2002) Ribososmally synthesized antibacterial peptides in gram positive bacteria, Current Drug Targets, 3: 107-122.

[12] Dubourg G.; Elsawi, Z.and Raoult, D.(2015) Assessment of the in vitro antimicrobial activity of Lactobacillus species for identifying new potential antibiotics. International journal of antimicrobial agents 46 (5): 590-593

[13] Goradia, D., Cooney, J., Hodnett, B.K. and Magner, E. (2006) Characteristics of amesoporous silicate immobilized trypsin bioreactor in organic media, Biotechnology Progress, 22: 1125-1131.

[14] Karel, S.F., Libicki, S.B. and Robertson, C.R. (1985) The immobilization of whole cells: Engineering Principles, Chemical Engineering Science, 40: 1321-1354

[15] Longo, M.A., Novella, I., Garcia, L.A. and Diaz, M.(1992) Diffusion of proteases in calcium alginate beads, Enzyme and Microbial Technology - Journal, 14 (7): 586-590.

[16] Rao, C.S., Prakasham, R.S., Lakshmi, C.S. and Rao, A.B. (2009) Effect of various immobilization matrices on *Lactobacillus delbrucekii* cells for optically pure L+ lactic acid production, Current Trends in Biotechnology and Pharmacy, 3(3): 311-319.

[17] Riaz, A., UlQader, S.A., Anwar, A. and Iqbal, S. (2009) Immobilization of a thermostable A-amylase on calcium alginate beads from *Bacillus subtilis* KIBGE-HAR, Australian Journal of Basic and Applied Sciences, 3(3): 2883-2887.

[18] Ross, R.P., Galvin, M., McAuliffe, O., Morgan, S.M., Ryan, M.P., Twomey, D.P., Meaney, W.J. and Hill, C. (1999) Developing applications for *lactococcal* bacteriocins. Antonie van Leeuwenhoek, 76: 337-346.

[19] Salminen, A., von Wright, A. and Ouwehand, A. (2004a) Lactic Acid Bacteria: Microbiological and Functional Aspects, New York, CRC Press.

[20] Suthasinee N. (2010) Bacteriocin Production by Lactic Acid Bacteria Encapsulated in Calcium Alginate Beads. KKU Res J 15 (9)

[21] Tampion, J. and Tampion, M. D. (1987) Immobilized cells: Principles and Application. Australia: University of Cambridge Press.

[22] Yu-Qung, Z.T., Mei-Lin, S., Wei-De, Z., Yu-Zhen, D., Yue, M. Wen-Ling, Z. (2004) Immobilization of L-asparaginase of the microparticles of the natural silk serum protein and its characters, Biomaterials Journal, 25: 31-51

[23] Zhang, H., Cui, Y., Zhu, S., Feng, F., and Zheng, X. (2010) Characterization and antimicrobial activity of a pharmaceutical microemulsion, International Journal of Pharmaceutics, 395: 154-160.