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Optimization of Partial Purification of Bacteriocin by Ammonium Sulphate Precipitation

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Abstract—*The search for an effective alternative to antibiotics like bacteriocin with new mode of action or broad spectrum of activity needs proper purification of bacteriocin. Optimization of partial purification of bacteriocin was determined by 50%, 60%, 70% and 80% of ammonium sulphate saturation. In 80% ammonium sulphate saturation completely all the antimicrobial compound was precipitated. NCDC 114 showed the highest sensitivity to bacteriocin at 80% ammonium sulphate saturation followed by NCDC 112, NCDC 70, NCDC 174.*

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Keywords: *Bacteriocins, Amonium sulphate saturation,, Heavy metals.*

"1. Introduction"

Bacteriocins are mainly isolated from commensal probiotic bacteria (1,2,3). They are being studied for their potential role in food industry (4,5,6) and in combating antibiotic resistance(7,8,9). The method, ammonium sulphate precipitation (ASP) is versatile for concentrating antimicrobial proteins from the crude bacteriocin (10). The method results variation of stages in recovery and sterility of various bacteriocins. Differences in the concentration of mixed proteins lead to variations in the stages of purity amongst defferent bacteriocins. That is why there is not any increase in specific activity of antimicrobial peptide preparations. The present study deals with optimization of partial purification of bacteriocin from lactic acid bacteria by ASP.

."2. Material and Methods"

2.1. Optimization of partial purification of bacteriocin by different ammonium sulphate saturation

Different saturation of ammonium sulphate (50%, 60%, 70%, 80%) was used to get highest precipitation of protein. After dialysis, the activity unit was also determined in every saturation from pellet and supernatant against the indicator strains.

2.2. Indicator strains

Indicator strains were collected from National Collection of Dairy Research (NCDC), India. Indicator strains used were NCDC 114, NCDC 112, NCDC 70 and NCDC 174.

"3. Results and Discussion"

Table 1 shows the antimicrobial action of partially purified bacteriocin inside pellet and supernatant against NCDC 114 in different percentage of ASP. In the case of 50% ASP the activity unit of bacteriocin in pellet was 800 AU/ml where as in supernatant it was 51200 AU/ml. The circumstances indicated that most of the antimicrobial compound was passed through supernatant. The protein was not precipitated properly. In 60% ASP the activity unit were same in both pallet and supernatant. It confirmed that the amount of antimicrobial compound precipitated, the same amount of antimicrobial compound was passed through the supernatant. In 70% ASP it had been seen that the protein content was precipitated more amount than it passed through the supernatant. In 80% ASP completely all the antimicrobial compound was precipitated.

Table 1 : Determination of antimicrobial activity of bacteriocin inside pellet and supernatant of different (%) ASP against NCDC 114.

Treatment (ASS)	Pellet (Activity unit of bacteriocin x 10^5 Au/ml)	Supernatant (Activity unit of bacteriocin x 10^5 Au/ml)
50%	0.008	0.512
60%	0.064	0.064
70%	0.256	0.016
80%	2.048	00

Table 1 shows the antimicrobial action of partially purified bacteriocin inside pellet and supernatant against NCDC 70 in different percentage of ASP. In the case of 50% ASP the activity unit of bacteriocin in pellet was 400 AU/ml where as in supernatant it was 25600 AU/ml. In case of 60% ASP the activity unit was 1600 AU/ml where as in supernatant it was 3200 AU/ml. In 70% ASP it had been seen that most of the protein content was precipitated and very less amount passed through the supernatant. In 80% ASP completely all the antimicrobial compound was precipitated. But the antimicrobial activity of partially purified bacteriocin against NCDC 114 is much higher than the antimicrobial activity against NCDC 70.

Table 2 : Determination of antimicrobial activity of bacteriocin inside pellet and supernatant of different (%) ASP against NCDC 70.

Treatment (ASS)	Pellet (Activity unit of bacteriocin x 10^5 Au/ml)	Supernatant (Activity unit of bacteriocin x 10^5 Au/ml)
50%	0.004	0.256
60%	0.016	0.032
70%	0.064	0.016
80%	0.512	00

Bello et al. (2018) reported that the bacteriocin of lactobacillus plantarum Z1116 was partly purified by 60% ammonium sulphate saturation (11). Feliatra et al. (2018) observed that the bacteriocin partly purified by 80% ASP (12). Cherif et al. (2006) partially characterised a new bacteriocin entomocin with 80% ASP (13). Sure et al. (2016) noticed that bacteriocin from *L. viridescence*was partly purified by 70% ASP (14). on contrary, in early time Muriana and Klaenhammer (1991) discovered that an AMP from *lactobacillus* was almost purified by 40% ASP (15).

Table 3 and Table 4 represents antimicrobial activity of partially purified bacteriocin against NCDC 174 and NCDC 112 respectively in different (%) ASP. In the both case 80% ASP recovered all the proteins. NCDC 114 showed the highest sensitivity to bacteriocin (80% ASP) followed by NCDC 112, NCDC 70, NCDC 174 (Figure 1).

Table 3 : Determination of antimicrobial activity of bacteriocin inside pellet and supernatant of different (%) ASP against NCDC 174.

Treatment (ASS)	Pellet (Activity unit of bacteriocin x 10^5 Au/ml)	Supernatant (Activity unit of bacteriocin x 10^5 Au/ml)
50%	0.00	0.256
60%	0.008	0.016
70%	0.032	0.008
80%	0.128	00

Table 4 : Determination of antimicrobial activity of bacteriocin inside pellet and supernatant of different (%) ASP against NCDC 112.

Treatment (ASS)	Pellet (Activity unit of bacteriocin x 10^5 Au/ml)	Supernatant (Activity unit of bacteriocin x 10^5 Au/ml)
50%	0.004	0.256
60%	0.032	0.016
70%	0.124	0.032
80%	1.024	00

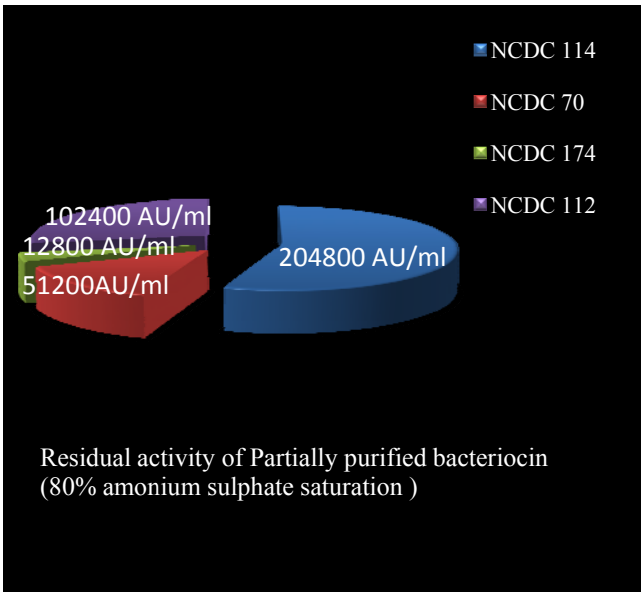

Figure 1 : Antimicrobial activity of partially purified bacteriocin (80% ASP) against different indicator strains.

"4. Conclusion"

The pharmaceutical giants are cautious about the interest in bacteriocins because of the high production and purification expenses this scenario might improve dramatically in the future if bacteriocins are able to translate their therapeutic potential into commercial success. Increased demand and production can then be expected to bring down costs for both manufacturers and consumers.

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