BEUTH HOCHSCHULE FÜR TECHNIK BERLIN
University of Applied Sciences

Freie Universität Berlin

Characterisation of bacteriocins isolated from *Escherichia coli*

Jennifer Andrack[1], Greta Gölz[2], Tassilo Seidler[1], Thomas Alter[2], Stefanie Orquera[2]

[1] Beuth University of Applied Sciences Berlin; [2] Institute of Food Safety and Hygiene, Freie Universität Berlin

Background and objectives

Increasing bacterial resistance rates against antibiotics and decontaminants lead to an enhanced search for antimicrobial alternatives in the food sector. As natural antimicrobial agents, bacteriocins represent a suitable option. Bacteriocins are proteins that are produced by almost all bacteria to inhibit the growth of similar and closely related bacterial strains. These ubiquitous proteins are able to fulfill the demands of the consumer for food with preferably natural ingredients.

The aim of this study was to isolate suitable bacteriocins with a broad spectrum of action especially against *Escherichia* (*E.*) *coli* strains in food. These bacteriocins are to be implemented in a decontaminant for the application on surfaces and in food.

For this study 82 *E. coli* strains were isolated from food samples and the bacteriocin production was examined through the lytic effect of their overnight cultures as well as their extracts against eight indicator strains. The bacteriocin producing strains were tested by PCR for the presence of known bacteriocin genes. Extracts were analyzed by SDS-PAGE to determine the size of the bacteriocins.

Materials and methods

Spot assay to determine the lysis profile

200 µl overnight culture of each indicator strain was mixed with 3 ml of 0.5 % Luria Bertani (LB) softagar and poured onto LB plates. 5 µl overnight culture of the 82 *E. coli* isolates was spotted onto this overlay agar. The plates were incubated aerobically at 37 °C for 24 h and the lysis profile on eight indicator strains was examined. *E. coli* K12 DH5α, C600, Cremehogs, Φ, P400, 5K, Row and *Shigella sonnei* 17 were used as indicator strains.

Bacteriocin extraction

The overnight cultures of the *E. coli* isolates were centrifuged for 5 min. The pellets were resuspended in 100 µl PBS with 0.5 % Tween 80 and a pH of 2. Samples were heated for 5 min at 99 °C. After cooling, the suspensions were neutralized, centrifuged and the supernatants tested for growth inhibition efficiency in a spot assay on the eight indicator strains.

PCR detection of genes encoding bacteriocins

The DNA of lysing overnight cultures was extracted using the Chelex method and the isolates were screened for the presence of 21 colicins A, B, D, E1-9, Ia, Ib, K, L, M, N, U, V, Y, 5 and 5 microcins C7, Js, J25 and H47 using the PCR protocol described by Smajs et al. (2010). PCR products of the sequentially related colicins E2-E9, Y, U, Ia, Ib, 5 and 10 were sequenced for verification.

SDS-PAGE

To determine the molecular mass of the bacteriocins, SDS-PAGE was performed according to Laemmli (1970). 50 µg protein of the extracts was mixed 1:1 with 2x Laemmli buffer, heated for 5 min at 95 °C and separated on a 7.5 % SDS-polyacrylamide gel. After the run, the gel was washed, placed on a LB plate and overlaid with a mixture of 600 µl of an overnight culture of the indicator strain *E. coli* K12 DH5α and 10 ml soft agar. The plates were incubated at 37 °C for 24 h aerobically.

Results

Spot Assay

An inhibitory effect on the growth of the indicator strains was detected for 40 (49 %) of the overnight cultures. Most of these 40 overnight cultures (68 %) lysed all eight indicator strains (Fig. 1). The highest sensitivity could be determined for the indicator strain *E. coli* K12 DH5α and the lowest for the indicator strain *Shigella sonnei* 17.

A bactericidal effect could still be detected for 30 (75 %) of the bacteriocin extracts. The lysis profile of the extracts included also mostly (50 %) all eight indicator strains (Fig. 2).The highest sensitivity to the bacteriocins exhibited the indicator strain *E. coli* K12 Row and *Shigella sonnei* 17 showed the lowest sensitivity.

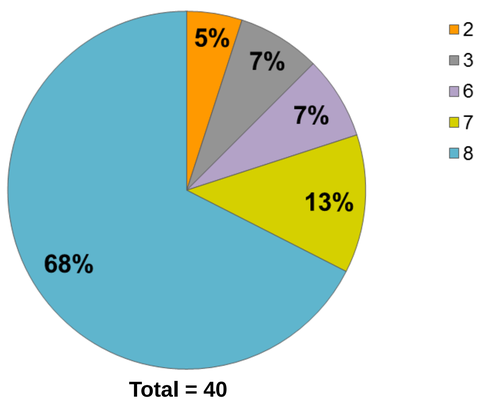

Fig. 1: Number of indicator strains, which were lysed by the bacteriocins of the overnight cultures.

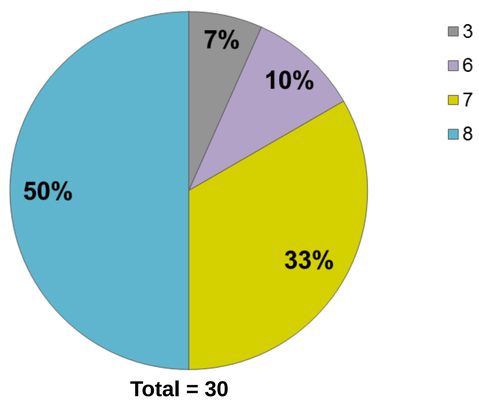

Fig. 2: Number of indicator strains, which were lysed by the bacteriocins of the extracts.

PCR

Of the 38 lysing overnight cultures, 79 % encode for at least one of the bacteriocin genes tested. Most frequently the genes V, Ia, E7 and E8 could be detected in the 38 isolates (Fig. 3). Most commonly one to three bacteriocin genes were detected in one isolate (Fig. 4). Colicin Ia and microcin V as well as colicin E7 and E8 co-occured in 16 % of the isolates. The genes encoding colicins A, D, E3, E4, E5, E6, E9, S4, Y and 5 could not be detected.

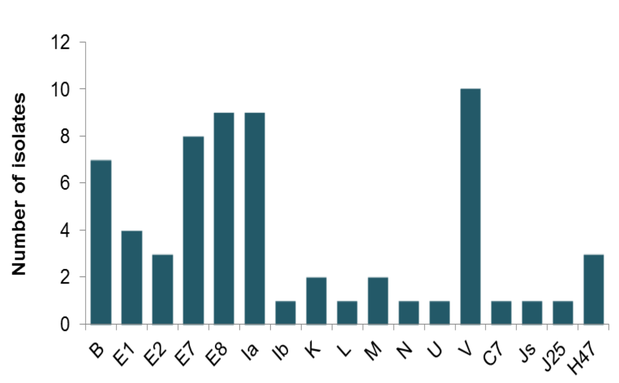

Fig. 3: Number of isolates in which the bacteriocin genes could be detected.

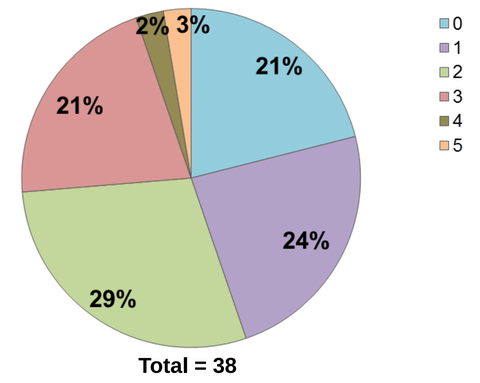

Fig. 4: Number of bacteriocin genes which could be detected by PCR in the bacteriocin producing isolates.

SDS-PAGE

In the SDS-PAGE five of 18 extracts had a lysis band at the height between 50 and 75 kDa (Fig. 5 a, b). One extract showed two lysis bands (Fig. 5 a).

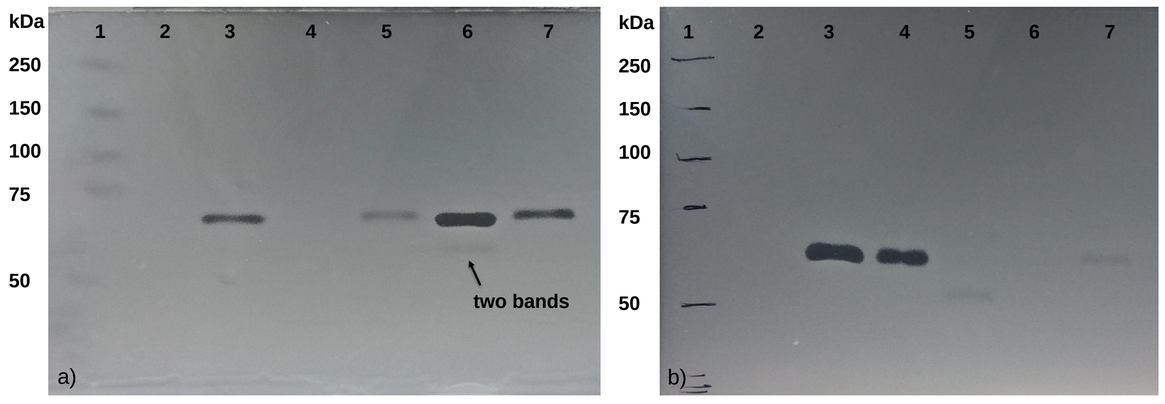

Fig. 5 a) and b): Lytic activity of protein extracts after SDS-PAGE with *E. coli* K12 DH5α overlay (1 = marker (BioRad), 2 = negative control (DH5α), 3-7 extracts of the bacteriocin producing isolates).

Conclusion

Almost half (49 %) of the 82 *E. coli* isolates showed a lytic effect against at least one of the indicator strains. This percentage coincides with the percentage rates (14-64 %) found in *E. coli* isolates from human samples.

After extraction, 75 % of the bacteriocin producing strains still lysed with a similar spectrum. For the remaining 25 %, the bacteriocins were probably inactivated during the extraction method. For further investigation the use of eight indicator strains could be reduced to *Shigella sonnei* 17 (lowest sensitivity) and K12 DH5α, respectively K12 Row (highest sensitivity).

In 79 % of the isolates one to five of the 28 bacteriocin genes were detected. Mainly the genes V, Ia and E8 were found. The gene combinations Ia and V as well as E7 and E8 were most frequently detectable.

The proteins were separated by SDS-PAGE and for five of 18 extracts, a lysis band was detected at the height of 50-75 kDa. Accordingly, these could be colicins which generally have a size between 25 and 80 kDa. The detection of two colicins in one isolate shows that more than one bacteriocin may be responsible for the antimicrobial effect.

The antimicrobial activity of selected bacteriocins on contaminated surfaces and food will be investigated in subsequent experiments.

This project is funded by the Federal Ministry for Economic Affairs and Energy (ZIM No. KF2267305).

References:
Smajs et al. (2010) Bacteriocin synthesis in uropathogenic and commensal Escherichia coli: colicin E1 is a potential virulence factor, BMC Microbiology 2010
Laemmli, U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4, Nature 227 (5259); 680-685

Institute of Food Safety and Hygiene • FU Berlin • Königsweg 69 • 14163 Berlin • Tel. +49 30 - 838 - 62545 • Fax +49 30 - 838 - 462029 • Stefanie.Orquera@fu-berlin.de • www.vetmed.fu-berlin.de