

## BACTERICIDAL AND BACTERIOLYTIC ACTIVITY OF LISTERIA

B.SKALKA

Department of Microbiology and Immunology, University of Veterinary Science, 612 42 Brno

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## Abstract

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Strains of all known species of the genus *Listeria* exerted bactericidal and bacteriolytic effects on *Micrococcus luteus* and bacteriolytic effects on *Micrococcus lylae*. The bactericidal effect was tested on living cells and the bacteriolytic effect on heat-killed cells of micrococcal strains. The sample of 141 listeria strains examined comprised 80 *L. monocytogenes* strains, 10 *L. ivanovii* strains, 45 *L. innocua* strains, 2 strains each of *L. seeligeri* and *L. grayi* and 1 strain each of *L. welshimeri* and *L. murrayi*. No effect was observed on the other micrococcal species, namely *M. roseus*, *M. kristinae*, *M. sedentarius*, *M. varians* and *M. nishinomiyaensis*. The bactericidal and bacteriolytic activity observed in the study can be included in the characteristic properties of the genus *Listeria*.

*Listeria* spp., *Micrococcus* spp., bactericidal activity, bacteriolytic activity

The genus *Listeria* now comprises seven species constituting two groups. The first of them comprises *Listeria monocytogenes* and *Listeria ivanovii*, both of them pathogenic for man and animals, and *Listeria innocua*, *Listeria seeligeri* and *Listeria welshimeri*. The second group comprises *Listeria grayi* and *Listeria murrayi* (Rocourt 1988). This recent classification has introduced a change in that *L. grayi* and *L. murrayi* are no longer classified as a separate genus *Murraya* as suggested previously (Stuart and Welshimer 1974) and represents also a change as compared with the recent edition of Bergey's Manual of Systematic Bacteriology (Sneath et al. 1986) where the two species were classified within the genus *Listeria* only as „species incertae sedis“ (Seeliger and Jones 1986). Moreover, *Listeria denitrificans* has been transferred to a new genus *Jonesia* as *J. denitrificans* (Rocourt et al. 1987).

The characteristics of listeria reportedly include the capacity of some strains to produce substances having the properties of bacteriocins. These substances were named „monocins“ (Sword and Pickett 1961) or „listeriocins“ (Tubylewicz 1963) and exerted their effects primarily on *L. monocytogenes* strains. More recently however, these substances have been found to be defective bacteriophages of listeria (Bradley and Devar 1966; Ortel 1989).

Mollerach et al. (1988) obtained a *L. innocua* strain producing an exosubstance referred to as „linnocucin 819“ which showed both bactericidal and bacteriolytic activity. This exosubstance, however, exerted bactericidal and bacteriolytic effects only on one *Listeria* strain, namely *L. ivanovii* 818. Moreover, it had a weak growth-inhibiting effect on *Micrococcus luteus* strain ATCC 9341, one *L. welshimeri* strain and one *L. denitrificans* strain. Another isolated observation was the production of lysozyme by some *L. monocytogenes* strains, virulent for white mice (Seeliger and Jones 1986). This enzyme is produced in varying intensity by some staphylococci (Varaldo et al. 1980; Lämmler 1989) and exerts its effects primarily on *Micrococcus luteus* strains (Kocur 1986).

The present study is concerned in detail with a phenomenon described in our previous studies (Skalka 1986, 1988).

## Materials and Methods

## Media

Brain Heart Infusion Agar CM 375 (Oxoid Ltd.) and Blood Agar Base No. 4 (Imuna) were used.

## Bacterial Strains

### Strains Examined

The sample of strains examined comprised 141 listeria strains, namely 80 *L. monocytogenes* strains including CCM 5 576, CCM 5 879, CCM 5 577, CCM 5 578, CCM 5 880, CCM 5 779, CCM 5 883, Li 17/57 and Li 19/53; 10 *L. ivanovii* strains including CCM 5 884 and Li 72/89; 45 *L. innocua* strains including CCM 5 885 and Li 71/89; 2 *L. seeligeri* strains including Li 55/88; 1 *L. welshimeri* strain (Li 54/88); 2 *L. grayi* strains (Li 31/72 and Li 70/89); and 1 *L. murrayi* strain (Li 69/89).

### Control Strains

Staphylococcal strains *S. aureus* Mau 87/79, *S. hyicus* M 51/88 and *S. simulans* biovar *staphylolyticus* CCM 3 583 were used as controls.

### Indicator Strains

The sample of indicator strains comprised 22 micrococcal strains, namely 10 *M. luteus* strains including CCM 169 and M 15/65; 5 *M. lylae* strains including CCM 2693 and CCM 2695; 3 *M. roseus* strains including CCM 839; 1 strain each of *M. kristinae* (CCM 1690), *M. nishinomiyaensis* (CCM 2 140), *M. sedentarius* (CCM 2 697) and *M. varians* (CCM 884).

The CCM strains were obtained from the Czechoslovak Collection of Microorganisms, Brno, and the Li, Mau and M strains, from the Czechoslovak National Collection of Type Cultures (CNCTC), Prague. The remaining strains were isolated in the author's laboratory.

### Demonstration of Bactericidal Activity

A suspension of each micrococcal strain was prepared in saline in a density corresponding to the 1st degree of McFarland's turbidity standard. The suspensions were added in 0.1 ml volumes to 10 ml volumes of melted agar medium cooled to 48 °C. After mixing, the media with the incorporated suspensions were poured into Petri dishes and allowed to solidify. Afterwards the listeria strains to be examined and the staphylococci used as controls were cultured on the surface of the media using the „spot-on-lawn” technique. The results were evaluated after 24-h incubation at 37 °C.

### Demonstration of Bacteriolytic Activity

A suspension of each micrococcal strain was prepared in saline in a density corresponding to the 4th degree of McFarland's turbidity standard. Prior to use the suspensions were autoclaved at 121 °C for 20 minutes. The devitalized suspensions were added in 1 ml volumes to 9 ml volumes of melted agar medium. After mixing they were poured into Petri dishes. The subsequent procedure and evaluation of the results were as described above.

## Results

All the listeria strains examined exerted bactericidal effects on the strains of *M. luteus*. On the media into which suspensions of living *M. luteus* strains were incorporated an entirely transparent zone was observed round the listeria cultivation "spots". The width of the zone depended on the potency of the respective listeria strain rather than on its species. Nevertheless, this activity showed least fluctuation and was almost equally intense in *L. monocytogenes*, *L. ivanovii*, *L. grayi* and *L. murrayi* strains and was least pronounced in *L. seeligeri* strains. A remarkably intense activity was shown by *L. innocua* strain bearing the laboratory designation Lin 23 which exerted bactericidal effects also on *M. lylae*, *M. roseus*, *M. kristinae* and *M. varians*. Of the 3 staphylococci used as controls *S. hyicus* had marked bactericidal effects on all the micrococcal strains, whereas the other two staphylococci showed a negative activity in this respect (Fig. 1).

All the listeria strains showed bacteriolytic effects on the media with incorporated heat-killed suspensions of the cells of *M. luteus* and *M. lylae* strains and did not exert this effect on the remaining indicator micrococci. The manifesta-

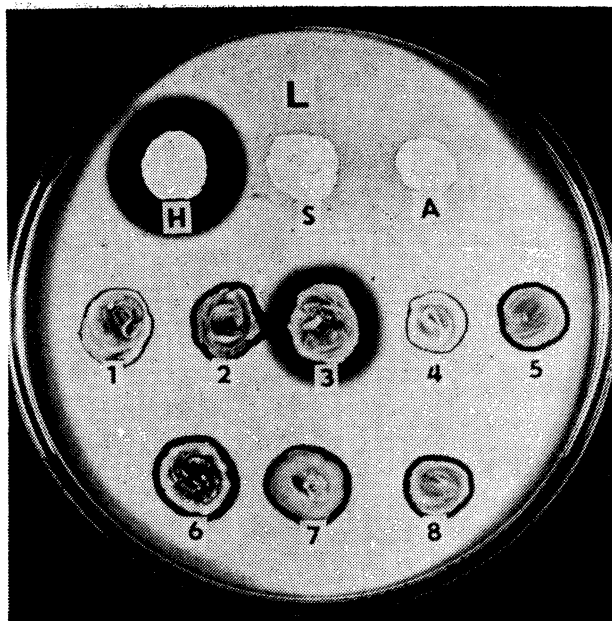


Fig. 1. L, agar medium with living *M. luteus* strain CCM 169; H, *S. hyicus* M 51/88; S, *S. simulans* biovar *staphylolyticus* CCM 3 583; A, *S. aureus* Mau 87/79; 1, *L. welshimeri*; 2, *L. innocua*; 3, *L. innocua* Lin 23; 4, *L. seeligeri*; 5, *L. ivanovii*; 6, *L. monocytogenes*; 7, *L. grayi*; 8, *L. murrayi*. Result after 24-h incubation.

Table 1  
Effects of *Listeria* spp. and control strains on *Micrococcus* spp.

Listeria	No.	Micrococcus													
		lut		lyl		ros		kri		nis		sed		var	
		10		5		3		1		1		1		1	
		L	D	L	D	L	D	L	D	L	D	L	D	L	D
<i>monocytogenes</i>	80	+	+	-	+	-	-	-	-	-	-	-	-	-	-
<i>ivanovii</i>	10	+	+	-	+	-	-	-	-	-	-	-	-	-	-
<i>innocua</i>	44	+	+	-	+	-	-	-	-	-	-	-	-	-	-
<i>innocua</i> Lin 23	1	+	+	+	+	+	-	+	-	-	-	-	-	+	-
<i>seeligeri</i>	2	+	+	-	+	-	-	-	-	-	-	-	-	-	-
<i>welshimeri</i>	1	+	+	-	+	-	-	-	-	-	-	-	-	-	-
<i>grayi</i>	2	+	+	-	+	-	-	-	-	-	-	-	-	-	-
<i>murrayi</i>	1	+	+	-	+	-	-	-	-	-	-	-	-	-	-
<i>S. hyicus</i> M 51/88	1	+	+	+	+	+	-	+	-	+	-	+	-	+	-
<i>S. simulans</i> CCM 3583	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>S. aureus</i> Mau 87/79	1	-	+	-	+	-	-	-	-	-	-	-	-	-	-

lut = *luteus*; lyl = *lylae*; ros = *roseus*; kri = *kristinae*; nis = *nishinomiyaensis*; sed = *sedentarius*; var = *varians*; No. = No. of strains; + = antagonistic effect; - = without effect.

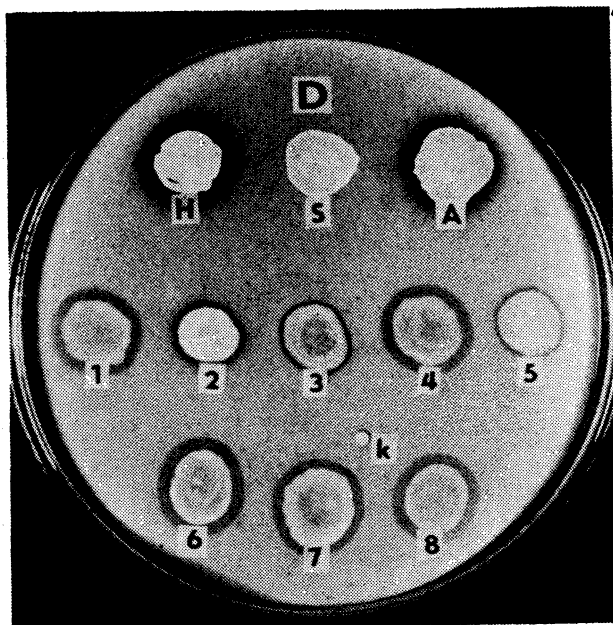


Fig. 2. D, agar medium with a suspension of heat-killed cells of *M. luteus* strain CCM 169; H, *S. hyicus* M 51/88; S, *S. simulans* biovar *staphylolyticus* CCM 3 583; A, *S. aureus* Mau 87/79; 1, *L. welshimeri*; 2, *L. innocua*; 3, *L. innocua* Lin 23; 4, *L. seeligeri*; 5, *L. ivanovii*; 6, *L. monocytogenes*; 7, *L. grayi*; 8, *L. murrayi*; k, accidental contamination. Result after 24-h incubation.

tion of positive bacteriolysis was a zone round the cultivation "spots" generally less transparent than that observed upon the use of the indicator living cells. The bacteriolytic zone in one and the same strain corresponded in size to the bactericidal zone in some cases and was larger or smaller than that in some others. Among the staphylococcal controls bacteriolytic effects on *M. luteus* and *M. lylae* were exerted by *S. aureus* Mau 87/79 and *S. hyicus* M 51/88, the bacteriolytic zone shown by the latter being smaller in size than its bactericidal effect. *S. simulans* biovar *staphylolyticus* had no effects on any of the indicator micrococcal strains (Fig. 2). The results are summarized in Table 1.

### Discussion

The effect of lysozyme, particularly of that produced by staphylococci, on some micrococcal species, is a well-established fact (Satta et al. 1978; Kocur 1986). This enzyme, however, is not produced by all staphylococcal species (Lämmle 1989) and differences in its production may exist even within one and the same species (Kloos and Schleifer 1986). Therefore our choice of control staphylococcal strains was made with respect to the data reported by other investigators (Schindler and Schuhardt 1964; Satta et al. 1978, Valraldo et al. 1980 and Lämmle 1989) as well as in the light of our previous experience (Skalka 1986, 1988).

The fact that strains of all species of the genus *Listeria* exert bactericidal and

bacteriolytic effects on both living and devitalized *M. luteus* strains and bacteriolytic effects on devitalized *M. lylae* strains has not been described before. The differences observed in the intensity of the positive effects can be accounted for by both the individual capacity of the strains and by the fact that the inocula of the examined strain were not invariably of the same size. Although in *L. seeligeri*, *L. welshimeri*, *L. grayi* and *L. murrayi* only one or two strains were tested, it seems reasonable to assume in view of the number of the other listeria species examined in our study that the bactericidal and bacteriolytic activity reported here can be generalized so as to cover the whole genus *Listeria*. Our results exclude the possibility that the activities observed may be in association with the pathogenicity or virulence of listeria. The question whether the effects observed are caused by one or more substances awaits further investigation.

The present knowledge based on our further, hitherto unpublished, results allows us to assume that the antagonism observed is caused by a substance very similar to lysozyme or possibly to *S. hyicus* bacteriolysine (Lämmle 1989). An exception in this respect is *L. innocua* strain Lin 23 producing bactericin with a wide-spectrum effect. This strain the activity of which surpasses that of the other listeria as well as that reported for "linnocuicin" (Möllerach et al. 1988) will be the subject of our further study.

The results reported here differ from the descriptions by other writers (Sword and Pickett 1961; Tubylewicz 1963; Möllerach et al. 1988). Most of these studies were conducted at the time that the present classification of listeria did not exist.

The control strains used in our study corresponded in their effects to the data published previously (Varaldo et al. 1980; Kocur 1986; Skalka 1986, 1988). The observation of Lämmle (1989) who reported the effects of *S. hyicus* on dead *M. luteus* cells was extended by our finding of the effects on dead *M. lylae* cells and on living cells of *M. luteus* and other micrococcal species.

In the light of the present results we suggest that the bacteriolytic and bactericidal activity of listeria described should be included in the characteristic properties of the genus *Listeria*.

### Baktericidní a bakteriolytická aktivita listerií

Kmeny všech známých druhů rodu *Listeria* měly baktericidní a bakteriolytický účinek na *Micrococcus luteus* a bakteriolytický účinek na *Micrococcus lylae*. Baktericidní efekt se testoval na živých buňkách a bakteriolytický na tepelně usmrcených buňkách mikrokokových kmenů. Soubor vyšetřovaných 141 kmenů listerií byl tvořen 80 kmeny *L. monocytogenes*, 10 kmeny *L. ivanovii*, 45 kmeny *L. innocua*, po dvou kmenech *L. seeligeri* a *L. grayi*, po jednom kmeni *L. welshimeri* a *L. murrayi*. Žádný efekt se nepozoroval na jiných druzích mikrokoků, a to *M. roseus*, *M. kristinae*, *M. sedentarius*, *M. varians*, *M. nishinomiyaensis*. Pozorovaná baktericidní a bakteriolytická aktivita může doplnit komplex charakteristických vlastností rodu *Listeria*.

### Бактерицидная и бактериолитическая активности листерий

Штаммы всех известных видов рода *Listeria* отличались бактерицидным и бактериолитическим воздействием на *Micrococcus luteus* и бактериолитическим действием на *Micrococcus lylae*. Бактери-

цидный эффект проверяли на живых клетках и бактериолитический – на теплом умерщвленных клетках микрококковых штаммов. Комплекс исследуемых 141 штамма листерий состоял из 80 штаммов *L. monocytogenes*, 10 штаммов *L. ivanovii*, 45 штаммов *L. innocua*, по два штамма *L. seeligeri* и *L. gray*, по одному штамму *L. welshimeri* и *L. murrayi*. Никакого эффекта не наблюдали на других видах микрококков, а именно *M. roseus*, *M. kristinae*, *M. sedentarius*, *M. varians*, *M. nishinomiysensis*. Наблюдаемые бактерицидная и бактериолитическая активности могут стать дополнением комплекса характерных свойств рода *Listeria*.

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