Elastolytic Activity of Bacteroides nodosus Isolated from Sheep and Goats with Foot Rot

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The elastolytic activities of 82 Bacteroides nodosus strains were studied. Two substrates, insoluble elastin and soluble elastin, were used for this purpose. Roughly 15% of the strains which did not digest insoluble elastin were elastolytic with soluble elastin, the latter providing greater sensitivity, speed, and objectivity than its insoluble counterpart.

Foot rot is a disease, or more properly, an infectious syndrome, which is caused by the synergistic action of certain microbial species and which is specific to sheep and goats, although it is also reported in cattle. It is characteristic by an exudative inflammation with a strongly characteristic odor, followed by necrosis of the epidermal tissues of the hoof, leading in some cases to the complete separation of the horn (11).

Bacteroides nodosus and Fusobacterium necrophorum, gram-negative nonsporulated anaerobic bacilli, have been reported as etiological agents of foot rot in sheep (3, 6, 7, 16), cattle (2), and goats (5).

A classification of B. nodosus organisms into virulent, intermediate, and benign pathovarieties has recently been carried out in Australia, with a view to eliminating chronic carriers of virulent pathovarieties. This classification is based on the ability of B. nodosus strains to degrade elastin, an ability traditionally determined by the method described by Murphy (12) on the basis of bacterial digestion of insoluble elastin. Williams et al. (22) recently used a soluble elastin substrate for the detection of elastase produced during the growth of different aerobic and anaerobic bacteria.

In the present study, we analyzed the elastolytic activity of B. nodosus isolated from sheep and goats with foot rot and compared the efficiency and sensitivity of insoluble and soluble elastin substrates.

A total of 82 B. nodosus isolates were analyzed. Samples were obtained over the period from October 1987 to November 1988 (14, 15). Sheep were drawn from 15 flocks and goats were drawn from 13 flocks in the province of Cáceres (Spain).

Strains were identified by the methods described by Holdeman et al. (8, 9) and Sutter et al. (20). In addition, Clostridium histolyticum NCTC 503 was from the National Collection of Type Cultures (London, United Kingdom) and was included as a positive control in the elastase tests.

Insoluble elastin agar medium testing was done by the procedure outlined by Murphy (12), using Trypticase-arginine-serine medium (17) with 1.5% agar (Difco Laboratories, Detroit, Mich.) supplemented with 0.3% bovine neck ligament elastin (Sigma Chemical Co., St. Louis, Mo.). Each of the B. nodosus strains analyzed was classified into three pathovarieties on the basis of its ability to degrade elastin. Those bacteria which had completely digested elastin particles around the growth area after 7 or 14 days of incubation were classified as virulent; bacteria which had digested elastin particles by 21 days were classified as intermediate, and those which failed to do so were classified as benign (19).

The insoluble elastin method proposed by Claxton (4) and Stewart et al. (19) was also used. The only difference between this and the previous method was the addition of CaCl₂ at a final concentration of 0.15%. Incubation times and the interpretation of results were as described previously (4, 19).

The soluble elastin agar medium described by Williams et al. (22) was used. A nutrient elastin agar medium was prepared by adding 1% (wt/vol) solubilized elastin (Fluka AG, Buchs, Switzerland) to Columbia agar base (Oxoid Ltd., Basingstoke, United Kingdom). Plates were dried at 37°C for 30 min before use. Four strains were inoculated onto each plate and were incubated at 37°C for 4 days in anaerobic jars (BBL Microbiology Systems, Cockeysville, Md.) by using Gas-Pak sachets (Oxoid Ltd.). The reaction was catalyzed with aluminum-palladium pellets (1). Elastase activity was detected by flooding the elastin plate cultures with an aqueous solution of 30% (wt/vol) trichloroacetic acid. Elastase activity was indicated by a clearing of the agar around the growth area.

The elastolytic activities of the 82 B. nodosus strains were also tested, with soluble elastin used as the substrate by using the original technique reported by Williams et al. (22) and three modified versions of that technique. In the first of these techniques, Columbia agar base medium (Oxoid Ltd.) was replaced with Trypticase-arginine-serine medium with 1.5% agar, as described by Skerman (17). The second modified version supplemented the medium used in the technique reported by Williams et al. (22) with 0.15% CaCl₂. The third version used soluble elastin agar medium (22) with Trypticase-arginine-serine medium (17) and added 0.15% CaCl₂. Incubation times and reading and interpretation of results for all three techniques were the same as those reported by Williams et al. (22).

The soluble elastin method proved to be a more sensitive indicator of the elastolytic activity in the 82 B. nodosus strains analyzed than its insoluble counterpart was. As Table 1 shows, at least 50 of the 82 strains showed elastolytic activity in a medium containing soluble elastin, while only 38
and 36 strains by methods $A_1$ and $A_2$, respectively, digested insoluble elastin. These results indicate that at least 14.6% of strains classified as benign (no elastolytic activity) by the insoluble elastin method subsequently proved to be able to degrade elastin when they were inoculated into a soluble elastin medium. All strains showing elastolytic activity with insoluble elastin showed similar activity with the soluble form.

Table 1 also shows that the addition of 0.15% CaCl$_2$ failed to provide increased sensitivity, although 18 of the 82 strains analyzed digested insoluble elastin with 0.15% CaCl$_2$ after 7 days of incubation (method $A_2$). In comparison, only 14 strains grown in the same medium with no added CaCl$_2$ (method $A_1$) digested insoluble elastin over the same incubation period.

Stewart (18) has reported that the elastolytic activity of B. nodosus is the most important indicator of virulence in the pathogenesis of foot rot, since elastase or other proteolytic enzymes are responsible for the separation of the hoof from the soft tissues. In a study of 82 B. nodosus strains, Stewart (18) found that of the 54 strains which digested elastase, 51 were isolated from animals with virulent cases of foot rot, while 26 of the 28 nonelastolytic strains came from animals with benign foot rot. A number of Australian researchers have used insoluble elastin as a substrate in the analysis of B. nodosus elastolytic activity. In the present study, roughly 15% of strains considered to be unable to degrade insoluble elastin showed elastolytic activity when soluble elastin were used. These results coincide with those reported by Oakley and Banerjee (13), Janda (10), Williams et al. (22), and Valle et al. (21) in suggesting that soluble elastin is a more sensitive medium than insoluble elastin. Using insoluble elastin, Oakley and Banerjee (13) observed no elastolytic activity in strains of Clostridium bifermentans and Clostridium sporogenes. Janda (10) and Valle et al. (21) reported a similar absence of elastolytic activity in Streptococcus strains when the same substrate was used, although some of the bacteria studied did degrade elastase in a soluble elastin substrate.

In addition to its greater sensitivity, the soluble elastin method has other advantages for the detection of B. nodosus elastolytic activity; while the incubation time required in order to obtain results by the method of Murphy (12) may be as long as 21 days, the method reported by Williams et al. (22) requires only 4 days of incubation. This is of particular interest, since a speedy laboratory diagnosis is a valuable aid in the study of any infectious disease. Moreover, as Janda (10) and Valle et al. (21) have shown, the technique outlined by Williams et al. (22) is less subjective and is easier to interpret than that proposed by Murphy (12) is. One of the main drawbacks of the method of Murphy (12) is the need to obtain a homogeneous population of elastin particles.

Claxton (4) and Stewart et al. (19) modified the method of Murphy (12) by adding 0.15% CaCl$_2$ to the medium; they observed that this led to increased stabilization of proteolytic activity, especially in benign pathovarieties. This was not found to be so in the present study, in which the presence of 0.15% CaCl$_2$ failed to make a significant difference to the detection of elastolytic activity.

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REFERENCES


16. Roberts, D. S., and J. R. Egerton. 1969. The aetiology and pathogenesis of ovine foot rot. II. The pathogenic association of

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**TABLE 1. Frequency of elastolytic activity in B. nodosus microorganisms**

| Methods* | No. of isolates tested | No. (%) elastase positive | No. of strains positive by day:
<table>
<thead>
<tr>
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<th></th>
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</thead>
<tbody>
<tr>
<td>$A_1$</td>
<td>38</td>
<td>38 (46.3)</td>
<td>ND</td>
</tr>
<tr>
<td>$A_2$</td>
<td>39</td>
<td>39 (46.2)</td>
<td>14 15 16 17 18 19 20 21</td>
</tr>
<tr>
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<td>30 (81.3)</td>
<td>ND</td>
</tr>
<tr>
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<td>31</td>
<td>31 (45.5)</td>
<td>8 9 10 11 12 13 14 15 16</td>
</tr>
<tr>
<td>$B_3$</td>
<td>32</td>
<td>32 (63.2)</td>
<td>3 4 5 6 7 8 9 10 11 12</td>
</tr>
<tr>
<td>$B_4$</td>
<td>33</td>
<td>33 (69.5)</td>
<td>2 3 4 5 6 7 8 9 10 12</td>
</tr>
</tbody>
</table>

* $A_1$, insoluble elastin agar medium described by Murphy (12), using Trypticase-arginine-serine medium (17); $A_2$, insoluble elastin agar medium described by Claxton (4) and Stewart et al. (19); $B_1$, soluble elastin agar medium described by Williams et al. (22); $B_2$, soluble elastin agar medium (22) with Trypticase-arginine-serine medium (17); $B_3$, soluble elastin agar medium (22) with added 0.15% CaCl$_2$; $B_4$, soluble elastin agar medium (22) with Trypticase-arginine-serine medium (17) and added 0.15% CaCl$_2$.  

# ND, not done.


