Mannose Inhibition as a Significant Marker for Differentiating among Novobiocin-resistant Staphylococci of Relevance in Clinical Microbiology

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A specific method for the identification of *Staphylococcus saprophyticus* among novobiocin-resistant species isolated from man is described. The test is based on novobiocin resistance, non-fermentation of D-mannose and early inhibition with late secondary growth on glucose/mannose + novobiocin agar plates. On this medium novobiocin-resistant *Staphylococcus cohnii* showed a confluent, continuous and homogeneous growth after 24 h which remained unchanged at 48 h whether or not it fermented D-mannose, whereas novobiocin-resistant *Staphylococcus xylosus* fermented D-mannose. These results are discussed in relation to a possible causal role of PTS enzymes and phosphomannose isomerase deficiency in mannose inhibition.

INTRODUCTION

Staphylococcus saprophyticus is a coagulase-negative novobiocin-resistant species responsible for a common urinary tract infection in young females. Current identification is based on the simplified Kloos and Schleifer scheme containing 13 key characters (Kloos & Schleifer, 1975). Mannose inhibition of *S. saprophyticus* has been previously described (Pereira & Cristino, 1985). Here we report a specific method of identification of *S. saprophyticus* among novobiocin-resistant species based on mannose inhibition, and also the demonstration of antigen 51 on strains responsible for urinary infection.

METHODS

Staphylococcus strains. The novobiocin-resistant species *S. cohnii* (119 strains), *S. xylosus* (14 strains) and *S. saprophyticus* (27 strains) were isolated from human skin and mucosal membranes. In addition, 50 strains of *S. saprophyticus* isolated from urine, 5 strains of *S. aureus* and 10 strains of *S. epidermidis* were included in the tests for comparison. A total of 225 strains was tested.

Identification of species. Susceptibility to lysostaphin (100 µg ml⁻¹) was tested on plates. Purple agar plates with different substrates were used as described by Kloos & Schleifer (1975). Gelatinase production tubes (Lab-Lemco, peptone, nutrient gelatine) were incubated at 22 °C and observed for 15 d. Most strains were also studied with API system galleries containing 19 substrates.

Glucose/mannose + novobiocin agar medium. Plates were prepared with Columbia Agar Base (Oxoid), glucose (1%, w/v) and bromocresol purple (0.002%, w/v). One hour later half the solidified medium was removed and replaced by the same base with bromocresol purple (0.002%, w/v), D-mannose (1%, w/v) and novobiocin (5 µg ml⁻¹). Glucose and D-mannose stock solutions were filter-sterilized.

Inoculation of the medium. A saline suspension of each strain was prepared with an opacity equivalent to tube 4 of the McFarland scale. A loopful of the suspension was lightly streaked perpendicularly to the separation line of the two carbohydrates, starting on the half of the plate containing D-mannose and novobiocin. Four strains were inoculated on each plate. Cultures were incubated at 37 °C and examined after 24 and 48 h.

Slide agglutination. Slide agglutinations were performed as previously described (Pereira, 1962). Rabbits were inoculated with the *S. saprophyticus* reference strain no. 51877 (Pereira, 1962), and sera were absorbed with standard strain 18 from overnight plates, for 4 h in a water-bath at 50 °C.

Phosphomannose isomerase (EC 5.3.1.8) determination. The assays were done as described by Bergmeyer *et al.* (1974).

Fig. 1. Mannose inhibition phenomenon on glucose/mannose + novobiocin agar. Top, growth in 24 h; bottom, growth in 48 h. 1, *S. epidermidis*; 2, *S. saprophyticus*; 3, *S. cohnii* subsp. 1; 4, *S. aureus*; 5, *S. saprophyticus*; 6, *S. cohnii* subsp. 2; 7, *S. saprophyticus*; 8, *S. cohnii* subsp. 1.

RESULTS

Identification of S. saprophyticus in glucose/mannose + novobiocin agar medium. On this medium the *S. aureus* and *S. epidermidis* strains could be excluded because they did not grow on the half of the plate containing novobiocin. Novobiocin-resistant strains grew on both halves of the plate (Fig. 1).

S. xylosus and mannose-positive *S. cohnii* (subsp. 2) could be excluded because D-mannose fermentation produced a colour change. This reaction and glucose fermentation were responsible for the colour change extending from the culture streak in the surrounding medium along the whole streak.

S. saprophyticus and mannose-negative *S. cohnii* (subsp. 1) produced a colour change only on the glucose half of the plate. On the D-mannose half there was a striking difference between the two species: *S. cohnii* showed confluent, homogeneous growth after 24 h which remained unchanged after 48 h, while *S. saprophyticus* showed inhibition after 24 h and after 48 h an irregular secondary growth with colonies of many different sizes (Fig. 1).

Mannose inhibition was shown by all 77 strains of *S. saprophyticus*, by one out of 14 strains of *S. xylosus* and by none of the 119 *S. cohnii* strains.

Identification of antigen 51 on S. saprophyticus strains. Of the strains isolated from urine and identified as *S. saprophyticus* by the above method, 94% revealed antigen 51, whereas it was present only on 38% of the strains isolated from skin and mucosal membranes.

Phosphomannose isomerase assays. Phosphomannose isomerase activity was detected in all 10 strains of *S. cohnii* subsp. 2 and in both strains of *S. xylosus* that were tested, whereas the 14 strains of *S. saprophyticus* and 5 of *S. cohnii* subsp. 1 tested revealed no enzyme activity.

DISCUSSION

Among novobiocin-resistant coagulase-negative *Staphylococcus* species, only *S. saprophyticus* is responsible for urinary infections in young females. The non-fermentation of D-mannose and the carbohydrate inhibition in *S. saprophyticus* seem to provide a specific method for its identification. On glucose/mannose + novobiocin agar medium, novobiocin selected the three species that occur in man: *S. saprophyticus*, *S. cohnii* and *S. xylosus*. Only *S. saprophyticus* and *S. cohnii* subsp. 1 do not ferment mannose, but only the former showed early inhibition with late secondary growth in the presence of D-mannose.

The lethal influence of D-mannose in honeybees was explained by a phosphomannose isomerase deficiency (Saunders *et al.*, 1969; Sols *et al.*, 1960). A teratogenic effect of mannose in early rat embryos has also been reported (Freinkel *et al.*, 1984), where decreasing levels of glycolysis were not yet counterbalanced by the oxidative Krebs-cycle metabolism. Mannose inhibition in *S. saprophyticus* may be due to a direct effect of D-mannose or to an effect of some metabolite(s) of mannose. A phosphotransferase system (PTS), which has been demonstrated in various species of bacteria (Postna & Lengeler, 1985) may be expressed in *S. saprophyticus* with enzymes II^{Glc} and II^{Man}. When the whole system of three or four PTS enzymes functions, phosphorylated mannose may be formed. The absence of phosphomannose isomerase would lead to accumulation of phosphorylated mannose with a toxic effect as seen in the mannose inhibition phenomenon. The absence of enzyme activity in all 14 *S. saprophyticus* strains studied would support this hypothesis.

The presence of antigen 51 appears to be significant, since almost all the *S. saprophyticus* strains isolated from urine specimens, but only 38% of the strains from the skin or mucosal membranes, possessed the antigen. Antigen 51 appears to behave as a virulence factor, since evidently only strains with this antigen are able to colonize bladder mucosal membrane and give rise to disease.

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