Influence of Growth Temperature on the Development of *Escherichia coli* Polysaccharide K Antigens

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The established seventy-one *Escherichia coli* polysaccharide capsular K antigenic test strains were examined for the development of the K antigen after growth at 37 °C and 18 °C. Twenty-eight K antigens were not detectable after growth of bacteria at 18 °C, while the remaining 43 antigens were developed at both temperatures. Most of the temperature-dependent antigens belonged to electrophoretically fast-moving K polysaccharides of rather low molecular weight, characteristically found among the common K antigens from extraintestinal disease isolates. Lipopolysaccharide O antigens were developed at both growth temperatures.

INTRODUCTION

The influence of growth temperature on the development of certain capsular structures in *Enterobacteriaceae* has been known for a long time (Jude & Nicolle, 1952; Felix *et al.*, 1934). Several years ago we found by chance (Ørskov & Ørskov, 1978) that some *Escherichia coli* capsular K antigens were not developed at room temperature, and because of the possible role of the polysaccharide K antigens as virulence factors it was important to examine the influence of growth temperature on the development of the hitherto established K antigens.

METHODS

Strains. Seventy-one *Escherichia coli* antigenic test strains comprising all currently established K antigens were examined; the complete O:K:H serotypes of those strains have been recorded elsewhere (Ørskov *et al.*, 1977; Ørskov & Ørskov, 1984).

Bacterial extracts. These were made from saline suspensions (approx. 10^{10} bacteria ml⁻¹) of organisms harvested from plate cultures grown either at 37 °C overnight or at 18 °C for 2 d on D5 medium (Schlecht & Westphal, 1966) containing 0.05% (w/v) glucose instead of 0.3% glucose. Suspensions were heated to 60 °C for 20 min and centrifuged at 8000 g for 15 min. For some examinations, such 60 °C extracts were subsequently heated for 1 h at 100 °C.

Antisera. These were routine OK and O antisera as prepared and used in the International *Escherichia* and *Klebsiella* Centre (Ørskov & Ørskov, 1978, 1984).

Crossed immunoelectrophoresis. CIE was carried out according to Laurell (1965) and Weeke (1973) on 5 × 5 cm glass plates using agarose (Litex HSA) and barbital buffer, pH 8.6. This technique was used with modifications according to Krøll (1973) and Axelsen (1973). The first run was carried out at 6 V cm⁻¹ for 1 h and the second run at 2 V cm⁻¹ applied overnight. Washing and staining was performed according to Weeke (1973).

Agglutination. Tube agglutinations were carried out in double dilution steps according to standard procedures, using cultures from agar plates incubated at 37 °C overnight or at 18 °C for 2 d. O-inagglutinability or inhibition of O-agglutination was defined as more than a fourfold difference in titre between agglutination of bacteria grown at 37 °C and those grown at 18 °C. For slide agglutination tests, homologous O antisera diluted 1:5 were used for detection of O inagglutinability.

Sensitivity to K1 and K5 phages. Tests to determine sensitivity to K1 phages (Gross *et al.*, 1977) and to K5 phages (Gupta *et al.*, 1982) were carried out by the cross-streak method.

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RESULTS AND DISCUSSION

Fig. 1 shows a typical set of CIE plates. The K antigen of test strain K92 (6181-66) was not developed at 18 °C (Fig. 1*b*), while the typical fast-moving anodic K antigen gave the typical K precipitation line after cultivation at 37 °C (Fig. 1*a*, arrow). The strain has a neutral O antigen (LPS) that does not give an O line in this type of CIE. In other cases, where the K strains have acidic O antigens, these antigens will give an anodic precipitation line close to the application well that will be found on both the 18 °C and the 37 °C plate.

Fig. 1(*c*) shows the pattern obtained after incorporation in the intermediate gel of a culture extract from the same strain grown at 18 °C. In strains in which the K antigen was not developed at 18 °C, the K antibodies were not removed from the upper antibody-containing gel during the second electrophoretic run, and the typical K line was formed (Fig. 1*c*). In cases where the K antigen was developed at 18 °C (Fig. 1*d, e* and *f*) no K lines were found after absorption in situ with 18 °C culture extract (Fig. 1*f*).

Table 1 records the CIE results with all known polysaccharide K antigens. It is apparent that practically all the fast-moving K antigens of low molecular weight (Ørskov *et al.*, 1977) were not, or only weakly, developed at 18 °C, while the slower-moving K antigens of large molecular weight usually found in connection with O groups O8, O9, O20 and O101, many of which were originally termed A antigens (Ørskov *et al.*, 1977), were well developed at both 18 °C and 37 °C; K8, K9, K17, K25, K57, K83, K84, K101, K102 and K103 all belong to the same category even though some were earlier labelled L and some B (Ørskov *et al.*, 1977). Immunochemical investigations have shown that these large molecular weight slow-moving K antigens have characters in common with lipopolysaccharides (Jann & Jann, 1983). K3, K10, K11, K54, K94, K96 and K98 are exceptional among the temperature-independent K antigens by not belonging to O groups O8, O9, O20 or O101. K3, K10, K11 and K54 were originally labelled L antigens as were many of the temperature-dependent low molecular weight K antigens.

The K-specific K1 and K5 phages lysed the respective test strains when these were grown at 37 °C but not when cultures were grown at 18 °C.

The presence of capsular antigen inhibits to a varying degree the agglutination of live culture (37 °C) in the homologous O antiserum; the phenomenon is called O inagglutinability. This inhibition was abolished or strongly decreased when the temperature-dependent K strains were grown at 18 °C. Likewise temperature-dependent strains did not agglutinate in pure, absorbed K antisera after growth at 18 °C.

A number of K antigens established earlier as B antigens, e.g. K59 (O55:(B5)K59), K58 (O111:(B4)K58), K60 (O26:(B6)K60) etc., which were deleted from the K antigen list (Ørskov *et al.*, 1977) were originally defined by inagglutinability of the living culture in the homologous O antiserum. Special K antigens could neither be detected by agglutination or precipitation methods, nor by immunochemical means (Ørskov *et al.*, 1977). The inagglutinability in O sera of one representative strain (O111) is caused by a special polysaccharide with the same specificity as the polysaccharide side chain of LPS (Goldman *et al.*, 1982). These former K strains and a number of freshly isolated strains of such serotypes were examined for O inagglutinability of cultures grown at 18 °C and 37 °C. Inagglutinability in O sera was well expressed at both growth temperatures, indicating that the regulatory mechanism that controls the development of LPS also controls the inagglutinability phenomenon.

Felix *et al.* (1934) demonstrated that the Vi antigen of *Salmonella typhi*, a typical polysaccharide K antigen, was not developed at low growth temperatures; it is, however, known that the Vi antigen, when found in *Citrobacter*, is developed at both 37 °C and room temperature. Similarly the M (mucoid) antigen found in practically all *Enterobacteriaceae* in some strains is developed at both 37 °C and lower temperatures, but more often is only or best developed at room temperature. Thus it is likely that the development of some of the K antigens described in this paper may show a different dependency of growth temperature when found in other strains. It may be of importance from an evolutionary point of view that the temperature-dependent *E. coli* capsular K antigens are found in strains that are common in extraintestinal diseases, and that some or all such K antigens probably could be looked upon as virulence factors. Thus they have temperature-dependence and association with virulence in common with the Vi antigen

Fig. 1. Crossed immunoelectrophoresis (CIE) of extracts of temperature-independent and temperature-dependent *E. coli* strains. (a, b, c) Extracts of K antigenic test strain K92 (temperature-dependent) against homologous OK antiserum; the wells in (a) and (c) contained extract from bacteria grown at 37 °C; the well in (b) contained extract from bacteria grown at 18 °C. In (c), extract from growth at 18 °C was incorporated in the intermediate gel. (d, e, f) Extract from K antigenic test strain K94 (temperature-independent) against homologous OK antiserum; the wells in (d) and (f) contained extract from bacteria grown at 37 °C; the well in (e) contained extract from bacteria grown at 18 °C. In (f), extract from growth at 18 °C was incorporated in the intermediate gel.

Table 1. *Temperature-dependent expression of Escherichia coli* capsular K antigens (K1 to K103)

K antigen developed at:	
18 °C and 37 °C (temperature-independent)	37 °C only (temperature-dependent)
3, 8, 9, 10, 11, 17, 25, 26*, 27*, 28*, 29*, 30*, 31*, 32*, 33*, 34*, 35*, 36*, 37*, 38*, 39*, 40*, 41*, 42*, 43*, 44*, 45*, 46*, 47*, 48*, 49*, 50*, 54, 55*, 57, 83, 84, 94, 96, 98, 101, 102, 103	1, 2, 4, 5, 6, 7, 12, 13, 14, 15, 16, 18, 19, 20, 22, 23, 24, 51, 52, 53, 56, 62, 74, 92, 93, 95, 97, 100

* Originally labelled as A antigens.

Missing numbers between K1 and K103 were either lost (K21) or could not be confirmed as polysaccharide capsular K antigens independent of the homologous O antigen (Ørskov *et al.*, 1977).

and with many fimbrial, adhesive MR (mannose-resistant) antigens found in pathogenic *E. coli* (Ørskov *et al.*, 1961; Ørskov & Ørskov, 1983). In contrast the temperature-independent K antigens detected in this investigation were found in strains that may play a more doubtful role in invasive diseases of warm-blooded animals.

Recently it was shown that K1 strains grown at 22 °C did not develop the K1 antigen when examined by immunoprecipitation, but also that K1 immunogenicity and the capacity to resist phagocytosis and the bactericidal forces of normal human serum were abolished after growth at 22 °C (Bortolussi *et al.*, 1983). Investigations in progress will show if it is a general phenomenon that temperature-dependent loss of K antigen is followed by complete loss of immunogenicity. For another temperature-dependent surface antigen, the fimbrial, proteinaceous K88 antigen, it is well known that immunogenicity is not completely lost in cultures grown at 18 °C, even though

such cultures have no detectable K88 when examined by usual agglutination or agglutination-absorption tests (Ørskov *et al.*, 1961). A practical aspect of the reported data is that agar plates left at room temperature for some hours after overnight growth at 37 °C may give variable results in agglutination experiments depending on whether bacteria are taken from confluent growth or from single colonies, because well-isolated colonies will continue to grow while multiplication stops in areas with confluent growth.

In this report no details about the development of the LPS-O antigen have been given, except that O antigen was always developed at 18 °C. At least one LPS antigen differs after growth at 37 °C and 18 °C (Ørskov *et al.*, 1961). It remains to be seen if the O specificity in the examined strains is always unchanged after growth at 18 °C.

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