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A ß-galactosidase negative *E. coli* isolated from a raw milk sample leading to atypical colonies on RAPID'*E.coli* 2 agar

Atypische Kolonien auf RAPID'E.coli 2 Agar bedingt durch einen ß-Galactosidase negativen E. coli isoliert aus einer Rohmilchprobe

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Summary

In recent years, the use of chromogenic media for detection of coliforms and *E. coli* increases as they allow fast identification of colonies directly on the plate without further confirmation steps. We obtained an atypical strain from a raw milk sample forming bright red colonies on RAPID'*E.coli* 2 agar, as opposed to the violet coloration characteristic for *E. coli,* or blue/green coloration characteristic for coliforms. API32E and MALDI-TOF analysis were used to identify isolate 2099 as *E. coli.* While API ID 32 E analysis resulted in a biochemical profile characteristic for *E. coli,* isolate 2099 was found to be phenotypically ß-glucuronidase positive but ß-galactosidase negative, which resulted in atypical red coloration of its colonies on RAPID'*E.coli* 2 agar. In order to elucidate possible causes of the ß-galactosidase negative phenotype, we sequenced the *lacZ* gene encoding ß-galactosidase. A mutation leading to a premature stop codon at amino acid position 774 was identified, rendering the polypeptide abnormally short and most likely not functional. Considering our findings, not only ß-glucuronidase, but also ß-galactosidase negative *E. coli* represent a challenge to routine diagnostic procedures screening for *E. coli* with chromogenic media that rely on detection of ß-glucuronidase and ß-galactosidase activity.

Keywords: ß-galactosidase, *E. coli,* chromogenic agar, atypical colonies

Zusammenfassung

In den letzten Jahren werden für den Nachweis von Coliformen und *E. coli* vermehrt auch chromogene Medien eingesetzt, die eine schnelle Identifizierung der gewachsenen Kolonien direkt auf den Platten und ohne weitere Bestätigungsschritte ermöglichen. Wir beschreiben hier einen atypischen Stamm, isoliert aus einer Rohmilchprobe, der auf dem RAPID'*E. coli* 2-Agar im Gegensatz zum typischen violetten Wachstum von *E. coli* und dem typischen türkisen Wachstum von Coliformen als rote Kolonien wuchsen. Der Stamm 2099 wurde mittels API32E and MALDI-TOF eindeutig als *E. coli* identifiziert, wobei das Isolat phänotypisch ß-Glucuronidase positiv aber ß-Galactosidase negativ war, was zur atypischen Farbe auf RAPID'*E. coli* 2-Agar führte. Um die mögliche Ursache für den ß-Galaktosidase-negativen Phänotyp aufzuklären, wurde das *lacZ Gen,* das die ß-Galactosidase codiert, sequenziert. Die BALST Analyse der Sequenzdaten zeigte eine Mutation, die zu einem vorzeitigen Stopp-Codon an der Aminosäure-Position 774 führt, wodurch das Polypeptid ungewöhnlich kurz und wahrscheinlich nicht funktionsfähig ist. Unsere Ergebnisse zeigen, dass nicht nur ß-Glucuronidase, sondern auch ß-Galactosidase-negative *E. coli* bei der Verwendung von chromogenen Medien, die auf dem Nachweis der ß-Glucuronidase und ß-Galactosidase Aktivität basieren, eine Herausforderung für die Routinediagnostik darstellen.

Schlüsselwörter: ß-Galactosidase, *E. coli,* Chromogen-Agar, atypische Kolonien

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Introduction

Escherichia coli can be used as a process hygiene criterion for various food products and water (Anonymous, 2005). The current ISO/TS 16649-1:2002 specifies the horizontal method for the enumeration of beta-glucuronidase-positive *E. coli* using the colony-count technique and 5-bromo-4-chloro-3-indolyl beta-D-glucuronide.

In recent years, the use of fluorogenic and chromogenic substrates in media for detection of coliforms and *E. coli* has gained popularity as they allow for fast identification of colonies directly on the plate, without requiring further confirmation steps (Caro et al., 2011; Jinneman et al., 2011). RAPID'*E.coli* 2 agar (Bio-Rad Laboratories, Hercules, CA) represents a chromogenic medium able to detect ß-glucuronidase (GLUC) and ß-galactosidase (GAL) activity, thus identifying *E. coli* and other coliform bacteria in food (Lauer et al., 2007). Typical *E. coli* forms characteristic violet colonies (GAL+; GLUC+) on RAPID'*E. coli* 2 agar, in contrast to other coliforms that form blue to green colonies (GAL+; GLUC–). As the RAPID'*E. coli* 2 agar was granted the status of performance tested method by the AOAC, it can replace the ISO method in accredited laboratories.

In this study, we investigated phenotypic and genotypic characteristics of colonies that exhibited an atypical red coloration on RAPID'*E.coli* 2, thus causing problems in routine diagnostics.

FIGURE 1: *E. coli and coliforms streaked on RAPID'E. coli 2 agar. The coliforms (Klebsiella oxytoca) plated on the left form green colonies. The E. coli strain plated on the right exhibits the characteristic violet phenotype on RAPID'E. coli 2 agar, while the atypical ß-galactosidase negative E. coli isolate 2099 forms bright red colonies.*

Materials and methods

Biochemical characteristics and species identification

API 32E test strips (bioMérieux, La Balme les Grottes, France) were used to generate a biochemical profile and to identifiy the strain following the manufacturer's instructions. Additionally, to confirm correct identification, MALDI-TOF (matrix-assisted laser desorption and ionization time-of-flight) analysis was performed in cooperation with Mabritec AG (Riehen, Switzerland).

DNA extraction and *lacZ* sequencing

Kits for DNA isolation and purification were obtained from QIAGEN (Hilden, Germany) and handled following the manufacturer's instructions. Total genomic DNA was extracted using the DNeasy Blood and Tissue kit. DNA purification was performed using the MinElute PCR purification kit. Concentrations of nucleic acids were measured using a Nanodrop ND-1000 UV/Vis spectrophotometer (NanoDrop Technologies, Wilmington, DE) and sequencing was outsourced (Microsynth, Balgach, Switzerland). The whole ß-galactosidase gene *(lacZ)* was amplified with three sets of primers (Tab. 1), using the FastStart High Fidelity PCR system (Roche, Mannheim, Germany) at the following reaction conditions: (i) 5 min at 95 °C, (ii) 30 cycles of 30 s at 95 °C, 30 s at 59 °C, 90 s at 72 °C and (iii) 10 min at 72 °C. PCR products were purified and sequenced.

Results and discussion

The isolate 2099 was obtained during routine microbiological testing of a raw milk sample on RAPID'*E.coli* 2 agar, where it formed bright red colonies, as opposed to the violet coloration characteristic for *E. coli,* or blue to green coloration characteristic for coliforms (see Fig. 1). Interestingly, the RAPID'*E.coli* 2 agar was granted Performance Tested Method status by the AOAC for selected foods, including raw milk (Lauer et al., 2007).

Isolate 2099 was confirmed as *E. coli* by biochemical identification using API ID 32 E test as well as MALDI-TOF. API ID 32 E analysis revealed a biochemical profile characteristic for *E. coli,* isolate 2099 was found to be phenotypically ß-glucuronidase positive but ß-galactosidase negative, which resulted in atypical red coloration of its colonies on RAPID'*E.coli* 2 agar. While ß-glucuronidase negative strains of *E. coli* have been described (Ratnam et al., 1988; Maheux et al., 2008; Fricker et al., 2010), to our knowledge, no ß-galactosidase negative strains have been reported so far in the food matrix. Nevertheless, in a clinical setting non-lactose fermenting *E. coli* strains are known to belong often to pathogenic *E. coli* groups, e. g. enteroinvasive *E. coli.*

In order to elucidate possible causes of the ß-galactosidase negative phenotype in this strain, we amplified and

TABLE 1: *Sequences of oligonucleotide primers used.*

Primers	Oligonucleotide sequence (5'-3')
Bgal_F1:	GCA GGT ATT TGC GCA GCC CGA
Bgal_F2:	CAG CGT TCG ACC CAG GCG TT
Bgal_F3:	CGG CTC CGC CGC CTT CAT AC
Bgal_R1:	GTC CCA CGC CAT CCC GCA TC
Bgal_R2:	CTG GCT ACC GGC GAT GAG CG
Bgal_R3:	CCC TGG CGC CCA ATA CGC AA

sequenced the *lacZ* gene encoding ß-galactosidase in *E. coli.* The *lacZ* nucleotide sequence of strain 2099 was deposited in Genbank (accession number JX072964). Comparison of the *lacZ* nucleotide sequence of the atypical strain 2099 with *lacZ* sequences of *E. coli* strains available at Genbank, revealed a unique point mutation at nucleotide position 2320. This mutation results in the reading of a premature stop codon at amino acid position 774, thus rendering the polypeptide abnormally short and most likely not functional.

Considering our findings, not only ß-glucuronidase, but also ß-galactosidase negative *E. coli* represent a challenge to routine diagnostic procedures screening for *E. coli* with chromogenic media that rely on detection of ß-glucuronidase and ß-galactosidase activity.

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