Clostridium botulinum occurrence in Kosovo honey samples

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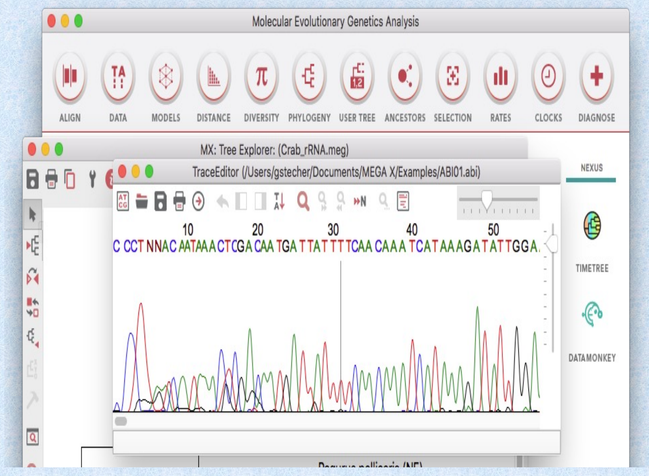
Introduction

- The aim of this study was an examination of 30 polyfloral honey samples collected directly from small Kosovo beekeepers (artisanal producers) to determine the presence of *Clostridium botulinum*.
- The Kosovo territory was divided into 30 equal surface units spanning the entire region of Kosovo.
- Samples were collected from July to September 2018 and dark stored at 8° C until further analysis.

Materials and methods

- The direct centrifugation method was used for culturing the *C. Botulinum* and to confirm the presence of C. Botulinum, cultured bacterial colonies were subjected to Gram's stain, catalase, and oxidase tests.
- From identified colonies, enrichment culture was prepared, DNA extracted and the amplification of the 16S rRNA gene with two sets of universal 16S rRNA bacterial primers was performed.
- After an electrophoresis step to detect specific bands of 1450 bp for 16S RNA, the PCR products were sequenced at Macrogen-Europe and phylogenetic relationships of 16S rRNA sequences were estimated using MEGA X software.



RESULTS

Table 1. Biochemical tests of isolated bacteria

Sample #	Gram's stain	Catalase	Oxidase
K18	+	-	-
K9	+	-	-
K11	+	-	-
K3B	+	-	-
K20S	+	-	-
K17P	+	-	-
K29	+	-	-
K22	+	-	-
K25	+	-	-
K4	+	-	-
K23	+	-	-
K5	+	+	-
K27	+	+	+
K30	+	+	+
K26	+	+	-
K16	+	+	+
K13	+	+	-
K19	+	+	-
K14B	+	+	-
K24	+	+	-
K28	+	+	-
K17S	+	+	-
K15	+	+	-
K14K	+	+	-

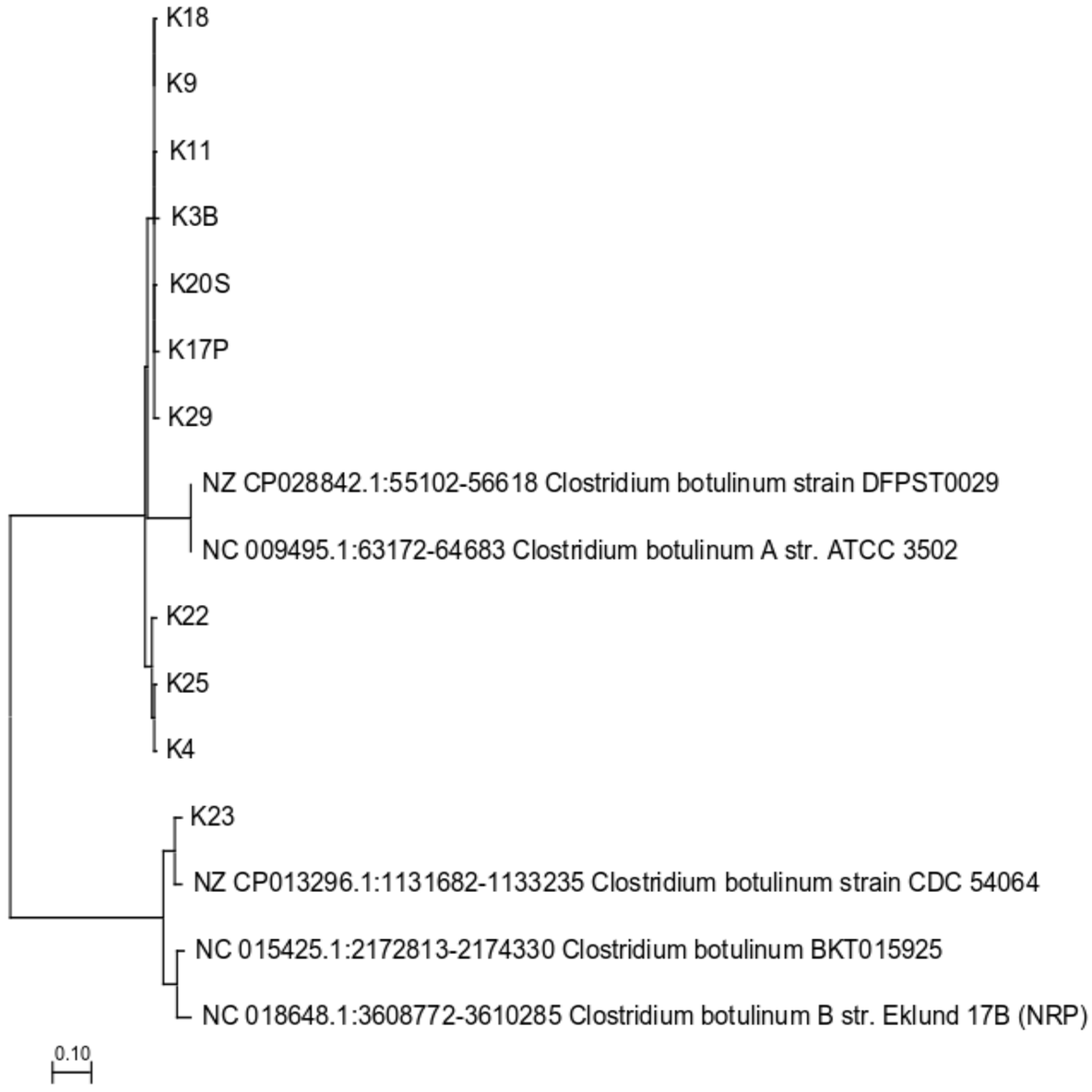


Figure 1. Phylogenetic tree of *Clostridium botulinum* with reference and representative genomes 16S rRNA sequence region. First number indicated the genomes accession numbers (NZ_CP013296.1), and second number showed the 16S rRNA sequence location (1131682-1133235) in the genomes (*C. botulinum* strain CDC 54064).

Conclusions

- ✓ In this first representative study on the occurrence of C. botulinum in honey collected in Kosovo, sequence analyzing results showed that isolates from 11 samples (K18, K9, K11, K3B, K20S, K17P, K29, K22, K25, K4, and K23) were
- ✓ similar to C. botulinum via nearly 96% identities.

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