Correct morphological identification of Alternaria is important and demands a combination of modern standardised methods and up-to-date literature. The production of secondary metabolites has previously been used as a means of identification and classification. In this study, 153 fungal isolates belonging to the genus Alternaria were examined. They were grown under standardised conditions and subjected to morphological and chemical examination. All isolates were grouped according to their three-dimensional sporulation pattern on potato carrot agar and their colony colour on dichloran rose bengal yeast extract sucrose agar (DRYES). After extraction, all isolates were analysed by a high performance liquid chromatograph equipped with a diode array detector and the resulting metabolite profiles were subjected to multivariate statistic analyses. The analyses of metabolite profiles showed that the isolates could be divided into three major species-groups that were morphologically identifiable as the A. infectoria species-group, the A. arborescens species-group and the A. tenuissima species-group. The A. infectoria species-group is chemically very different from both the A. arborescens and the A. tenuissima species-groups with only a few metabolites in common. None of the 35 A. infectoria species-group isolates produced any known metabolites and all had white or greyish white colonies on DRYES. The A. arborescens species-group and the A. tenuissima species-group, shared most of the known metabolites and had colonies of various shades of green on DRYES. One cluster of isolates belonging to the A. tenuissima species-group was able to produce tentoxin, which has not been reported previously from any A. tenuissima isolate. The results suggest that each species-group contains several taxa and these taxa need to be formally described before species specific metabolite profiles can be established.