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The cell surface of Candida albicans is enriched with highly glycosylated mannoproteins that are involved in the interaction with host tissues. N- and O-glycosylation are post-translational modifications that initiate in the endoplasmic reticulum, and finalize in the Golgi. The KRE2/MNT1 family encode a set of multifunctional mannosyltransferases that participate in O-, N- and phosphomannosylation. In order to gain insights into the substrate specificities of these enzymes, recombinant forms of Mnt1, Mnt2, and Mnt5 were expressed in Pichia pastoris and the enzyme activities characterized. Mnt1 and Mnt2 showed a high specificity for α-methylmannoside and α1,2-mannobiose as acceptor substrates. Notably, they also used Saccharomyces cerevisiaeO-mannans as acceptors and generated products with more than three mannose residues, suggesting than Mnt1 and Mnt2 could be the mannosyltransferases adding the fourth and fifth mannose residue to the O-mannans in C. albicans. Mnt5 only recognized α-methylmannoside as acceptor, suggesting that participates in the addition of the second mannose residues to the N-glycan outer chain.