The crucial part of the biosynthesis of lincosamide antibiotics lincomycin and celesticetin is the condensation of amino sugar and amino acid moieties. This reaction is catalysed by the oligomeric enzyme lincosamide synthetase (LS). One of the most important components of LS is adenylation domain recognizing and activating amino acid precursor. The substrate specificity of adenylation domain is determined by “nonribosomal code”, 10 amino acids residues which side chains are in close contact with the activated substrate. The homologous adenylation domains LmbC from biosynthesis of lincomycin and CcbC from biosynthesis of celesticetin exhibit strong substrate specificity for their natural substrates (2S,4R)-4-propyl-L-proline (PPL) and L-proline, respectively.

At first the effect of selected amino acid residues of LmbC nonribosomal code on the substrate specificity of the whole domain was tested. The amino acids residues, most important for preference of PPL substrate over L proline, were determined: G308, A207 and L246.

Then the effect of double mutations in nonribosomal codes of both LmbC and CcbC on their substrate specificity was evaluated. The double mutants LmbC G308V + A207F and CcbC V306G + F205A were prepared and tested biochemically. The results brought new evidence of validity of homologous models of substrate binding pockets of LmbC and CcbC, especially the evidence of existence of hydrophobic channel in the substrate binding pocket of LmbC.