Genomic Analysis of the Lysogeny Module of Temperate *Streptococcus thermophilus* Bacteriophage TP-J34

M.Sc. Mazhar Desouki Ali Mohamed **1. Berichterstatter: Professor Dr. K. Heller**

Analysis of the DNA sequence of TP-J34 suggested that all except four of the phage genes are transcribed in one direction. The four genes are separated from the others by a genetic switch region and appear to be transcribed in one operon. They encode the following functions – in the order of transcription: repressor (*crh*), potential metalloproteinase (*orf3*), lipoprotein mediating superinfection exclusion (*ltp*), and integrase (*int*). To elucidate the roles of Orf3 and Int in induction, I performed gene knock out and transcriptional analysis by Northern blot and RT-PCR. In addition, a non-inducible TP-J34 derivative (TP-J34-12) and an isolated mutant was analysed, which harbored just one mutation within the lysogeny module: a frame-shift mutation in *orf3*, causing premature termination of translation TP-J34-Cu50 harbored a missense mutation causing replacement of an aspartic acid for an alanine in orf3. Knock out of *int* and *orf3* in TP-J34, respectively, resulted in prevention of induction by mitomycin C. Transcription of *int* was detected in both lytic-and lysogenic cells as a monocistronic mRNA. RT-PCR analysis showed that *int* was exclusively transcribed from its own promoter, located in the intergenic region between *ltp* and *int*, and sequence analysis detected this promoter as well as it revealed some polar or activation effect of *orf3* in transcription of *int*. For *ltp*, Northern blots revealed two transcripts: i) a few polycistronic transcripts comprising genes *crh*, *orf3* and *ltp* (also detected by an *orf3* probe), and ii) a majority of monocistronic transcripts comprising just *ltp* The latter transcript was not affected by addition of mitomycin C. In *S.thermophilus* J34 cu50, only one relatively weak monocistronic mRNA was detected, revealing that Orf3 affects the transcription of *ltp*. Sequence analysis revealed that a weak promoter, $P_{ltp,}$ in the *ltp/int* intergenic region. A very stable stem-loop structures were detected downstream of *ltp* and seems to act as a transcription terminator. *Crh* was detected in both lytic and lysogenic growth cycles. *Cro* is transcribed only in the lytic cycle as a ploycistronic mRNA comprising *ant*-encoding the antirepressor-*, orf7,* and *orf8*.and a very stable stem-loop structure about 200-nt downstream of *orf8* . From these results the following conclusions are drawn: i) the integrase is also involved in excision of prophage DNA; ii) Orf3 plays a role in induction, possibly by binding to Cro for initiation of lytic development, since Cro alone does not appear to carry out this function; iii) Crh is able to block transcription of the *cro* operon, while Cro alone cannot prevent of *crh*.