Supplementary files

Supplementary tables

Table S1 Genes commonly deregulated in Δncr1 and ΔN-Ncr1 sporidia 2 h after transfer from ammonium minimal medium to nitrogen starvation medium.

Table S2 Classification of genes regulated in both Δncr1 and ΔN-Ncr1 on nitrogen starvation medium into functional categories (FunCats).

Table S3 Genes commonly deregulated in both Δncr1 and Δton1 sporidia 2 h after transfer from ammonium minimal medium to nitrogen starvation medium.

Table S4 Classification of genes regulated in both Δncr1 and Δton1 on nitrogen starvation medium into functional categories (FunCats).

Supplementary figures

Figure S1 Full protein sequence alignment of NiT2 homologs. Multiple sequence alignment was executed using the MUSCLE algorithm (Edgar, 2004).

Figure S2 Filament formation on Δton1 and Δncr1 on charcoal plates. Sporidia were grown in $YEPS_{light}$ medium over night, washed with ddH_2O and diluted to an OD_{600} of 0.1 and 5 µL of each suspension were spotted twice on PD charcoal plates. Photos were taken after 4 d incubation at 28°C.

Figure S3 Thin layer chromatography of glycolipids isolated from SG200 and ΔN-Nit2 sporidia grown for 3 d on YNB nitrogen starvation medium. Approximately 10 µg of glycolipids from two independent cultures per genotype were loaded. The glycolipid composition of ΔN-Nit2 (left two lanes) did not differ from the one of SG200 sporidia (right two lanes).

Supplementary material and methods

Glycolipid isolation and determination
Glycolipids were extracted using a protocol adapted from (Josephs, 2004).
Ustilago maydis sporidia were cultivated on AMM over night and transferred to 1 L YNB (yeast nitrogen base) medium, which contains no nitrogen source, supplemented with 5% glucose for 3 days. Cell were harvested by centrifugation (20 min at room temperature and 4000 rpm) and the pellet was resuspended in 200 mL methanol and heated to 30°C. After centrifugation (20min, room

temperature, 4000 rpm), the cell pellet was discarded and the supernatant mixed with 1.8 L 50°C hot ddH_2O. The suspension was cool to room temperature over night. Cristallized glycolipids were harvested by centrifugation and washed subsequently with 100 mL ddH_2O and 20 mL diethyl ether and dried at room temperature. Separation of glycolipids occurred by TLC as described (Hewald *et al.*, 2005).

Supplementary literature cited

Edgar, R. C., (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucl. Acids Res.* 32: 1792-1797.

Hewald, S., K. Josephs, and M. Bolker, (2005) Genetic analysis of biosurfactant production in Ustilago maydis. *Applied and Environmental Microbiology* 71: 3033-3040.

Josephs, K. A., (2004) Untersuchungen zur Bildung von Cellobioselipiden (Ustilaginsaure) in Ustilago maydis. In: Fachbereich Biologie. Marburg: Philipps-University Marburg, pp. 118.