Microbiome Question 2: Can biological manipulation of gut microbiome, such as administration of prebiotics or probiotics have a role in alleviating the pain of osteoarthritis in preclinical models?

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Response/Recommendation: Yes. Limited preclinical studies demonstrate that biological manipulation of gut microbiome can alleviate the pain of osteoarthritis.

Level of Evidence: Medium

Delegate Vote: Agree: %, Disagree: %, Abstain: %

Rationale:

Osteoarthritis (OA) is modeled in rodents using chemical (Monosodium iodoacetate, MIA) and surgical (Anterior Cruciate Ligament Transection,ACLT; Destabilization of the Medial Meniscus, DMM) approaches that reproduce joint degeneration and measurable pain-related behaviors (1). In these models, OA pain is quantified through validated assays including von Frey testing, paw withdrawal threshold (PWT), paw withdrawal latency (PWL), hot-plate responses, and static weight-bearing measurements (2). Experimental evidence demonstrates that OA-associated nociception is mediated by inflammatory cytokines, activation of dorsal root ganglion (DRG) neurons, and upregulation of nociceptive markers such as TRPV1 and CGRP (3). Emerging preclinical studies indicate that gut dysbiosis can amplify joint inflammation and pain sensitivity by modulating systemic immunity and altering neuroimmune signaling (4, 5). Accordingly, microbiome-targeted interventions—including specific probiotic strains—have been investigated in MIA and ACLT models, demonstrating improvements in mechanical hypersensitivity and weight-bearing deficits (6).

A systematic review by Resta et al (6) of animal studies published recently identified three preclinical studies (7-9) that specifically evaluated OA pain outcomes. Across these studies, probiotics were associated with significant reductions in mechanical allodynia, thermal hyperalgesia, and weight-bearing deficits. Improvements in pain were demonstrated using von Frey testing, paw withdrawal threshold (PWT), paw withdrawal latency (PWL), hot-plate assays, and static weight-bearing measurements. Across studies probiotic administrations were also associated with reduced DRG nociceptive markers including TRPV1 and CGRP, modulation of neurotransmission pathways (GABA and PPAR-γ shifts), Decreased pro-inflammatory cytokines (TNF-α, IL-1β), Increased anti-inflammatory cytokines (IL-10, IL-4), downregulation of NFκB and pain-associated genes (e.g., Ntrk1).

There are also several original studies investigating the administration of different types of probiotics. In a study by So et al.(10) oral *Lactobacillus casei* was administered to female Wistar rats with MIA-induced OA, starting14 days prior to OA induction and continued for 8 weeks. The combination of *L. casei* (2×10^{10} CFU/kg) with collagen II/glucosamine significantly reduced hyperalgesia, decreasing paw withdrawal threshold (PWT) percentage change from ~45% to ~25% by week 2 ($p<0.01$. Oh et al. (11) evaluated administration of oral heat-killed *Bifidobacterium longum* BORI (1×10^{9} CFU, three times weekly) in male Wistar rats with MIA-induced OA. Treatment began three days post-injection and continued for 21 days. Pain-related behavior improved significantly, with increasing to ≈22 g versus ≈15–16 g in vehicle and paw withdrawal latency (PWL) increasing to ≈10.5 s versus ≈7–8 s at day 17. Weight-bearing improved to ≈38% versus ≈32%, with effects comparable in magnitude to celecoxib. Another study assessed administration of oral *Streptococcus thermophilus* (TCI633) in an ACLT-induced OA rat model, at 5×10^{9}–5×10^{11} CFU/kg/day from week 8 to week 20 post-surgery(12). Mechanical allodynia improved in a dose-dependent manner; at week 20, PWT increased from ≈2 g in untreated ACLT animals to ≈6–7 g in the highest-dose group, accompanied by reduced hind limb

loading asymmetry(12). Chang et al. (13) studied administration of live *Clostridium butyricum* GKB7 in ACLT-induced OA, initiated two days after surgery and continued for six weeks. Static weight-bearing force decreased from ≈45–50 g in ACLT + butyricum GKB27 to ≈22–27 g by weeks 3–6 where the ACLT group increased from 50 to 60 g, indicating substantial reduction in pain. Collectively, these studies show consistent improvements in mechanical sensitivity and weight-bearing deficits across both chemical (MIA) and surgical (ACLT) OA models following microbiome-targeted interventions. In comparative studies, certain probiotics failed to replicate the analgesic effects of *L. acidophilus*, indicating strain specificity (7). It is important to note that not all strains of the same microbial species can have beneficial effects on the host. The observed beneficial effects in these studies may be directly caused by the beneficial microbial strain, or may be secondary to changes in the abundance of other gut microbiota caused by competition with the probiotic strain.

Prebiotics are nutrients or other factors that promote the growth of beneficial microbes within the gut. Dosing the prebiotic oligofructose has been found to reduce OA severity and systemic inflammation in experimental models (14), but direct evidence for OA pain reduction is limited. In a randomized, prospective, double blind study, Shine et al. investigated the effect of *Lactobacillus sakei* LB-P12 in patients with OA of knee. Patients receiving the probiotic experienced significantly higher degree of pain relief and improvement in functional outcomes than those receiving placebo (16). No adverse events of clinically meaningful abnormalities in laboratory values were noted in patients receiving the live bacteria.

Study / Year	Population	Intervention	Comparators	Pain Outcome
O'Sullivan 2022	Female C57BL/6 mice; meniscectomy OA	*Lactobacillus acidophilus* (3×10^9 CFU, oral, 12 wks)	Sham, OA+PBS, OA+*B. breve*, OA+*L. reuteri*, OA+*B. subtilis*	Von Frey ↑ (0.44g→0.92g, $p<0.001$); hot plate ↑ (~3s→~5s, $p<0.05$); reduced weight-bearing deficits
Cho 2022	Male Wistar rats (n=6/group); MIA OA	LA-1 probiotic; sodium butyrate	Normal, MIA+vehicle, MIA+celecoxib	PWL and weight-bearing improved ($p<0.05$–0.001)
So 2011	Female Wistar rats (n=5–7/group); MIA OA	*L. casei* (2×10^{10} CFU/kg) ± CII/Gln	Normal, MIA+PBS, MIA+Gln, MIA+CII/Gln	Hyperalgesia reduced ~45%→~25% (P:0.01)
Jhun 2021	Male Wistar rats (n=6/group); MIA OA	Heat-killed *L. rhamnosus* (500 mg/kg)	Normal, MIA+vehicle	PWT & PWL ↑ ($p<0.05$–0.001); improved weight-bearing
Resta 2025 (SR)	Animal studies review	Gut microbiota-targeted interventions	Various controls	Concluded consistent reduction in mechanical/thermal hypersensitivity in included studies
Oh 2023	Male Wistar rats (n=6/group); MIA OA	Heat-killed *B. longum* BORI (1×10^9 CFU)	Normal, MIA+vehicle, MIA+celecoxib	PWT ≈22g vs 15–16g; PWL ≈10.5s vs 7–8s; WB ↑
Lin 2021	Rats; ACLT OA	*S. thermophilus* (5×10^9–10^{11} CFU/kg/day)	Sham, ACLT, ACLT+glucosamine	Dose-dependent PWT ↑ (≈2g→6–7g); reduced asymmetry
Chang 2022	Rats; ACLT OA	Live *C. butyricum* GKB7 ($\approx5.5\times10^6$ CFU/kg/day)	Sham, ACLT	Weight-bearing force ↓ ≈45-50g→22–27g

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